Progress of Research on Rabies and Rabies Vaccine

Cheng Qi Zhang*

Key Laboratory of Special Animal Epidemic Disease, Ministry of Agriculture, China

Abstract

Rabies is an acute infectious disease caused by the rabies virus and is one of the most common zoonotic diseases in people's lives. Although rabies is a vaccine-preventable viral disease, however, it is almost 100% fatal once clinical symptoms appear. Rabies Virus (RABV) is a single-stranded, non-segmented, negative-stranded RNA virus belonging to the genus Lyssavirus in the family Rhabdoviridae. With the development of molecular virology, many different types of novel rabies vaccines have been developed. Examples include recombinant weak-virus vaccines, recombinant live-vector vaccines, DNA vaccines, inactivated vaccines, subunit vaccines and virus-like particle vaccines. Since most of the rabies vaccines used in developing countries have the disadvantage that they are too expensive to produce and must be repeated. Therefore, there is a need for new vaccines that are cheaper, more efficient and provide continuous immunization in a single dose.

Keywords: Rabies; Rabies virus; Vaccine

*Corresponding author: Cheng-Qi Zhang, Key Laboratory of Special Animal Epidemic Disease, Ministry of Agriculture, Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China

Overview of Rabies

Rabies is an acute infectious disease caused by the rabies virus and is one of the most common zoonotic diseases in people's lives. Rabies susceptible animals include not only humans, but also most mammals, such as dogs, wolves, cats and bats. Rabies exists on all continents except Antarctica. Asia and Africa are the regions with the highest incidence of rabies, where more than 95% of human deaths occur. In Asia and Africa, humans are infected with rabies virus viruses mainly due to scratches or bites from dogs, which are contracted through canine saliva. And in North America the rabies virus comes mainly from bats.

Although rabies is a vaccine-preventable viral disease, however, once clinical symptoms appear, it is almost 100% fatal. According to the World Health Organization, up to 99% of cases are transmitted from domestic dogs to humans. Approximately 55,000 rabies deaths are reported worldwide each year, and the actual number of deaths should be significantly higher than that statistic. Rabies is a neglected tropical disease that primarily affects poor and vulnerable people in remote rural areas. About 80% of human cases occur in rural areas. Although highly effective human rabies vaccines and immunoglobulins exist, they are not always available and accessible to those who need them. Globally, fatal cases of rabies are rarely reported, and children between the ages of 5 and 14 are often the victims. The average cost of rabies post-exposure prophylaxis to treat rabies exposure is $40 in Africa and $49 in Asia, which can be a catastrophic financial burden for affected families, whose average daily income is about $1-2 per person. Each year more than 29 million people around the world are immunized after being bitten or scratched by an animal carrying the rabies virus, a practice that is estimated to save thousands of lives each year. Globally, the economic burden of canine-transmitted rabies is estimated at $8.6 billion per year.

Overview of Rabies Virus

Rabies virus (RABV) is a single-stranded, non-segmented, negative-stranded RNA virus belonging to the genus Lyssavirus in the family Rhabdoviridae, with a particle diameter of 65-80nm and a length of about 130-240nm. The head of RABV is semicircular, often with

a flat end, and the shape of RABV is typically bullet shaped. RABV particles are 65-80nm in diameter and 130nm in length. The head of RABV is semicircular, often with a flat end, and has a typical bullet-like shape with a helically symmetric nucleocapsid, an envelope, and single-stranded RNA. RABV possesses two main antigens: a glycoprotein antigen on the outer membrane of the virus, which binds to the acetylcholine receptor to make the virus neurotoxic and cause the production of neutralizing and hemagglutination inhibitory antibodies, which are protective; and a nucleoprotein antigen on the inner membrane, which produces complement-binding antibodies and precipitant antibodies in the body. The other is the inner nucleoprotein antigen, which causes the production of complement-binding antibodies and precipitin, which are not protective. The rabies vaccine was invented by French scientist Pasteur long before the virus was discovered in 1884. RABV consists of a single-stranded negative-stranded unsegmented RNA of 11928 or 11932 nucleotides, with the genome arranged from the 3′ end to the 5′ end of the N, P, M, G and L genes, which encode five structural proteins, respectively, with reading frame sizes of 1353, 894, 609, 1575, and 6429nt in that order [1].

RABV is easily inactivated by sunlight, ultraviolet light, formaldehyde, Neosporin, 50%~70% alcohol, etc. Viral suspensions are inactivated by 56 ℃ for 30~60min or 100 ℃ for 2min, and the virus can remain viable for several years at -70 ℃ or lyophilized at 0~4 ℃. Infected tissues can be stored in 50% glycerol and sent for testing [2]. RABV is weak to temperature resistance and is completely inactivated by 56 ℃, 30 min or 60 ℃, 10 min or boiling water, 2 min. RABV is inactivated by autolysis in brain tissue at room temperature for 7-10 days, survives for 2-3 weeks at 4 ℃, and survives for several years at -70 ℃ or in 50% glycerol at -30 ℃ without a drop in infection titer of more than a logarithmic amount. RABV is relatively stable at pH 7.2-8.0, and lower pH environments cause a change in the natural conformation of the GP, which leads to viral inactivation [2].

Structural proteins of rabies virus and their functions

NP consists of 450 amino acids (aa) and is highly conserved, with 98-99.6% aa homology between different strains. NP alone induces protective immunity in mice and dogs and resists RABV infection [3]. In addition, the antiviral activity of NP antibodies plays an important role in the protective mechanism of cytotoxic T lymphocytes (CTL) [4]. It has been shown that NP has an important relationship with RABV pathogenicity, that NP helps RABV evade host natural immunity [5], and that NP phosphorylation contributes to RABV transcription and replication [6]. Structural analysis of the NP antigen showed the presence of at least three or four antigenic sites on the NP [7]. NP antigenic sites I and IV are linear epitopes, and antigenic sites II and III are conformational epitopes [8].

PP consists of 297 aa, with high acidic aa content and 92-98% aa homology between different strains. PP peptides contain a hydrophobic region in the middle of the peptide and one at the amino-terminus, and at least 2 phosphorylation sites. PP is composed of several subcomponents originating from varying degrees of phosphorylation, but also from the truncated product

of the AUG, a start codon that is translated from an internal frame by a loophole-scanning mechanism [9]. PP presents at least 2 NP-binding sites, located at the carboxyl terminus and in the 69-177aa region [10], and the combination of the two is important for RNA synthesis. During the period when NP is newly synthesized, or NP and PP are being synthesized, NP-PP interactions occur at the PP amino terminus, whereas interactions between the PP carboxy terminus and NP can occur even after both proteins have been synthesized [10], and the carboxy terminus binds significantly more strongly than the amino terminus [11,12]. PP has also been associated with cytokinetic protein light chains (LCs), suggesting that it is associated with RABV through the neuronal axonal PP binds to the interferon (IFN)-activating transcription factor STAT1, blocking the IFN signaling pathway by preventing its migration to the nucleus, and also binds to the IFN-stimulating gene factor ISGF3 [13,14], and the interaction between PP and IFN signaling pathways has been linked to RABV pathogenicity [15]. 60% were located in 83-172 aa, and most of the antigenic sites showed linear epitopes [16].

MP consists of 202 aa, which is the smallest protein in RABV, but has a high degree of variability.MP has hydrophilic regions at both ends and a membrane-bound site in the middle, which is hydrophobic, and these two regions are conserved among different RABV strains.MP acts as a linker protein to connect the nucleocapsid to the outer shell.MP is completely encapsulated outside the RNP helix and maintains its denseness, and not only the MP-RNP complex but also MP itself are exclusively interacting with GP; MP deletion leads to a decrease in the yield of infectious viruses, suggesting that MP has an important role in the process of viral outgrowth; MP plays an important role in the process of transcriptional replication [17]; the absence of typical sub-bombarded virus particles in the supernatant of cells infected with the MP-deficient RABV suggests that MP deletion has a severe impairment of the process of RABV formation [18].

The GP consists of 524 aa, the first 19 aa constituting a hydrophobic signal peptide, which is excised during transcription to become the mature GP (505 aa), divided into an extramembrane region (antigenic region), a membrane-penetrating region (transmembrane region), and an intramembrane region (attachment region). The GP is the only protein that induces the body to produce viral neutralizing antibodies (VNAs). The GP contains three neutralizing antigenic epitopes, with antigenic epitope III located at 330-357 aa, and 333-338 aa identified as the main VNA binding region, with positions 333, 336, and 338 being critical. GP activates specific helper T cells (Th) and CTLs, and studies have confirmed the presence of linear and spatially-dependent epitopes of T cells on GP. GP plays an important role in the adsorption process of RABV, facilitating rapid adsorption through interactions with cell surface receptors [19]. The mutation of ERA strain GP Arg333 to Gln333 or Asn194 to Lys194 resulted in delayed viral internalization [20]. The ERA strain Gln333 mutant lost pH-dependent cell fusion activity and showed a significant reduction in cell-to-cell transmission [21]. RABV street strains are more pathogenic than tissue culture-adapted strains, with a limited level of GP expression, and RABV

street strains are more pathogenic than tissue culture-adapted strains. RABV pathogenicity is inversely related to GP expression [22]. GP expression above a certain threshold severely affects the cell membrane, leading to the activation of a series of proteins that trigger the apoptotic cascade [23]. GP plays a role in RABV pathogenicity by regulating viral replication, and GP Arg333 and Lys194 have been shown to be intracellular miRNA targets that are recognized to up-regulate or down-regulate RABV replication [24].

LP is the largest protein in RABV, and the open reading frame is not identical between strains, with the presence of 2 hydrophobic regions that may be involved in interactions between NP and PP [25]. LP serves as a multifunctional enzyme with important roles in RABV genome replication, transcription, and post-transcriptional processing. LP encodes more than 2,000 aa and performs RNA-dependent RNA polymerase activity, mRNA capping reaction and mRNA polyadenylation [26]. 600 aa of the carboxyl terminus of LP is a PP binding site [27].

Infection process of rabies virus

The process of rabies virus infection begins when the host is bitten or scratched by an animal carrying the rabies virus and RABV invades the host from the wound. Rabies virus does not normally invade the bloodstream and does not cause viremia. The virus spreads centripetally from the nerve tissue at the site of invasion until it enters the central nervous system [28]. The length of the virus transmission and spread process is related to the site of virus invasion as well as the amount of invasion. The virus moves through the body approximately 15-100mm per day. Vaccination before the virus enters the CNS allows the body to develop antibodies that can prevent the onset of the disease. Once the virus enters the central nervous system, it begins to proliferate, causing nerve cell dysfunction and the onset of clinical symptoms, which are usually untreatable.

The genome of the rabies virus is a negative-stranded RNA, which cannot be directly recognized by intracellular enzymes and translated into proteins. The negative-stranded RNA needs to be transcribed into mRNA, which can then be translated into proteins. The site of entry of the virus into the cell is in the axon, which does not have a suitable environment to synthesize proteins [28], so the rabies virus needs to reach the cytosol of the neuron before transcription and replication can occur.

In the Central Nervous System (CNS), the virus, having replicated in large numbers, can then continue to propagate outward through the efferent nerves and spread to the peripheral nerves and the tissues innervated by these peripheral nerves. And at this stage, the virus can be eliminated from the body through the salivary glands. After the appearance of typical symptoms, the infected person can die within a week [29].

Rabies Vaccine

Rabies is a vaccine-preventable disease. The most cost-effective strategy for preventing human rabies is to eliminate canine rabies through vaccine immunization. In many countries, animal (mostly canine) vaccination has reduced the number of human rabies cases. Therefore, a safe, effective, and affordable rabies vaccine is important. With conventional rabies vaccines, RABV is either cultured and passed on in brain tissue or in cells until the virus is weakened (live virus), or inactivated. To resist RABV, the main thing is VNA, and GP is the only structural protein that induces VNA. With the development of molecular virology, weakened virus vaccines have been generated by genetic engineering techniques, either by purification of viral antigens derived from highly efficient protein expression systems, or by gene insertion to express a single viral antigen in different vaccine vectors. Many different types of novel rabies vaccines have been developed [30].

Recombinant weak virus vaccines for rabies

With the continuous development of reverse genetic technology in RABV research, it is possible to target genomic changes through mutation, deletion or insertion of genes to reduce the pathogenicity of recombinant RABV, while improving immunogenicity and generating many recombinant weak strains for animal immunization. Rescue of recombinant RABV expressing colony-stimulating factor and flagellin, respectively, and immunization of mice by intramuscular and oral administration showed that the recombinant viruses had better immunogenicity, recruited and activated more Dendritic Cells (DCs) and B-cells, produced higher VNA, and protected the mice against RABV infection, as compared with the parental viruses [31,32]. In a follow-up study of recombinant RABV expressing colony-stimulating factor, intracerebral injection of this recombinant RABV after intramuscular tapping in mice triggered high expression of cytokines/chemokines, entry of more inflammatory and immune cells into the CNS, enhancement of blood-brain barrier permeability, increase in VNA titer, protection of mice, and achievement of post-exposure immunotherapy [33]. Recombinant RABV expressing intracellular adhesion molecule 1 showed enhanced ability to infect and activate natural initial murine-derived B-cells in *in vitro* assays, and *in vivo* studies of this recombinant RABV induced faster production of higher VNA upon primary immunization [34]. The mutation of GP Arg333 to Glu333 and Asn194 to Ser194 was named GAS, and recombinant RABV containing three GAS genes was rescued, which was non-pathogenic when injected intracranially in mice, and remained capable of preventing the development of lethal rabies encephalitis after CNS infection with a highly pathogenic RABV [35]. Insertion of MIP-1α between the RABV GP and LP rescued the expression of MIP-1α recombinant RABV, which can be highly expressed locally in the inoculated area after immunization of mice, recruited DC and B cells into the draining lymph nodes and peripheral blood, and induced the production of a higher level of VNA as compared to parental venom [36].

Recombinant live vector vaccines for rabies

Viral vectors, based on different parental viruses carrying the RABV GP gene under appropriate promoters, have been explored as novel vaccines. Newcastle disease virus expressing GP as a vector is safe in a wide range of animals, including dogs and cats, and intramuscular injection induces a VNA response and complete protection against RAB attack in mice, dogs, and cats, and induces

a strong and long-lasting protective VNA in dogs and cats [37]. Recombinant sheep infectious canker virus expressing GP induced a protective immune response in mice with VNA titers significantly higher than 0.5IU/mL for a long period of time, and a single immunization with 107 PFU was sufficient to completely protect against intracranial RABV attack [38]. A replication-competent recombinant canine type 2 adenovirus expressing RABV GP was injected subcutaneously into dogs, and a single immunization produced potent VNA, which was reinforced to produce a strong recalled immune response, with the immunized dogs surviving after the attack [39]. In a further study, this recombinant canine adenovirus type 2 GP-expressing vaccine was made into a bait, and the dogs were immunized by intranasal and buccal passage, respectively; 87.5% of the immunized dogs produced VNA, 90.8% of the seroconverted dogs had *in vivo* VNA responses higher than 0.5IU/mL over a period of more than 24 months, and all of the 10 dogs subjected to takedown experiments survived [40]. To weak Salmonella strain LH430 as a vector expressing GP and *E. coli* heat-resistant enterotoxin B subtype, mice immunized orally produced high levels of VNA and increased the level of IL-2 expression, and successfully protected mice against RABV infection [41].

Rabies DNA vaccine

Advanced recombinant DNA technology has made it possible to produce DNA vaccines against pathogenic infections. DNA vaccines induce a broad-spectrum immune response after delivery to the host, and have the advantages of being stable, mass-producible, inexpensive and effective, and still functional in the presence of maternal antibodies [42]. DNA vaccines provide an effective way to induce cell-mediated lysogenic CD8+ T cells, CD4+ T cells and VNA [43]. DNA vectors expressing GP, 100μg intramuscularly in dogs resulted in stronger and more sustained VNA responses compared to intradermal immunization, and the *in vivo* immunization dose in cats needed to be increased to 300μg to achieve a high seropositive conversion rate [44]. The first report of a rabies DNA vaccine was a recombinant plasmid expressing the RABV ERA strain GP, which induced an immune response that protected the immunized animals after takedown. Numerous studies have demonstrated that DNA-based rabies vaccines are relatively effective in inducing VNA responses, but that pre- and post-exposure immunization fails to protect non-human primates [45]. Rabies DNA vaccine studies, including routes of vaccination, use of delivery systems with greater transfection efficiency, regulatory elements, and the impact of cytokines need to be further explored.

Inactivated rabies vaccine

Inactivated vaccines have a very high level of safety, but traditional inactivated vaccines for rabies have a low efficacy and short duration after immunization, requiring multiple immunizations and relatively cumbersome immunization procedures. The new inactivated rabies vaccine adopts the reverse genetic manipulation technique to modify the strain before preparing the inactivated vaccine, so as to improve the immunogenicity of the vaccine. RABV-G is the only antigen of RABV that induces the body to produce neutralizing antibodies, so increasing the expression amount of RABV-G is one of the strategies to enhance the immunogenicity of the inactivated vaccine. For example, inactivated vaccines prepared from a recombinant virus strain rHEP-dG containing two RABV-G genes (with an additional copy of the G gene inserted between the G/L) induced higher levels of VNA production than inactivated vaccines made from parental viruses after immunization of mice and beagles [46]. In addition, codon optimization is also one of the strategies to increase RABV-G expression.

In addition, codon optimization is also one of the strategies to increase the expression of RABV-G. For example, recombinant RABV inactivated by inserting one additional RABV-G between G/L and optimizing both RABV-Gs with murine codons can produce higher levels of VNA after immunization of mice [47], and it is an excellent rabies inactivated vaccine candidate strain. In another study, immunization of mice immunized after inactivation of recombinant RABV expressing two RABV-G showed that insertion of an additional G gene into the G/L interval was better than the P/M interval in inducing VNA production [48], suggesting that the induction of VNA production is not only related to the expression of RABV-G (protein expression during RABV-G replication is sequential in the order of N-P-M-G-L), but also related to the expression of RNA. G-L in decreasing order), but also other unknown influences. In addition to increasing RABV-G expression, the expression of exogenous immune-activating factors coupled with RABV-G can similarly increase vaccine immunogenicity. For example, a recombinant inactivated RABV vaccine expressing B-cell Activating Factor (BAFF) in fusion with RABV-G and displayed on the surface of viral particles stimulated the production of high titers of VNA in mice for a short period of time [49], which could be developed as a post-exposure immunization vaccine.

In addition, recombinant duplex inactivated vaccines developed by using RABV as a vector to express other pathogen-protective antigens are also one of the directions for the development of novel inactivated vaccines. For example, based on the three-dimensional structure of RABV-G protein, the recombinant inactivated vaccine candidate with RABV and Mokora virus (MOKV) chimeric G protein was designed and constructed, which can provide immunoprotection to the body against a variety of viruses in the genus of RABV and rabies viruses after immunization [50], which has greatly broadened the scope of application of the rabies vaccine. Recombinant inactivated rabies vaccines expressing canine distemper virus (CDV) H (CDV-H) or F protein (CDV-F) can provide immunoprotection against RABV as well as CDV [51], and recombinant inactivated vaccines against RABV and Ebola virus (EBOV) preserved in vaporized form are more thermally stable than inactivated vaccines preserved in refrigerated form and protect against RABV and EBOV simultaneously. RABV and EBOV [52,53]; RABV-CO-VID-19 recombinant inactivated vaccine CORAVAXTM expressing SARS-CoV-2S1 and RABV-G chimera still induced the organism to produce high levels of VNAs against the above two viruses at the same time after 56 d of immunization of mice [54].

Rabies subunit and virus-like particle vaccines

Vaccines developed by directly using artificially expressed RABV-G as an immunogen are called rabies subunit vaccines. Traditionally RABV-G expression was performed using either a prokaryotic expression system or an insect cell expression system [54], whereas RABV-G expressed using Drosophila melanogaster S2 (schneider2) cells has also been shown to have good immunizing effects [55,56]. In contrast, the chimeric protein of RABV-G extracellular domain chimeric protein chimeric with GCN4-PII trimerization domain expressed by mammalian cells HEK-293T can exist in the form of trimer, which is closer to the natural RABV-G and thus has a better immunogenicity and provides better immune protection for mice [57]. In addition, rabies peptide vaccine adjuvanted with canine heat shock protein Gp96 was shown to provide immunoprotection in mice and beagles in immunization and attack protection assays [58], suggesting that synthetic peptides are also promising for the development of rabies vaccines.

RABV-G Virus-Like Particles (VLP), which can be formed from RABV-G (with the assistance of RABV-M), have good potential for vaccine development because they are not infectious or replicative and exhibit antigenic epitopes well. While traditional VLPs are mostly realized with insect cell expression systems, RABV-VLP expressed using mammalian cells HEK-293 also has good immunogenicity [59-61]. In addition, chimerization of GM-CSF on the surface of RABV-VLP resulted in the activation of more dendritic cells, which enhanced the RABV humoral immune response [62]; two Chimeric Virus-Like Particles (cEVLPs) containing adjuvants of either membrane-anchored flagellin (EVLP-F) or *Escherichia coli* thermally unstable enterotoxin B subunit (EVLP-L) molecules induced higher levels of RABV-specific immune responses [62]. Of interest, the Venezuelan Equine Encephalitis Virus (VEEV) vector replicon vaccine expressing RABV-G (VEEV-RABV-G), which is capable of forming virus-like particles containing only a single protein of RABV-G and can be serially passaged in BHK-2 cells, has demonstrated a high level of safety for direct intracerebral injection in both suckling mice and mice with VEEV-RABV-G, and was able to provide immunoprotection comparable to that of a weak rabies vaccine, and could be a safe and efficient vaccine candidate for the next generation [63].

Outlook

Although rabies is a vaccine-preventable viral disease, however, the virus is almost 100% fatal once the incubation period is over and clinical symptoms appear. Therefore, rabies vaccines are of great importance in the prevention and control of rabies. Worldwide, approximately 59,000 people, many of them children, die of rabies each year. Most human cases occur in rural areas of Africa and Asia, where domestic dogs are the primary hosts and up to 99% of rabies is transmitted to humans. The disadvantages of most vaccines used in developing countries are that they are too expensive to produce and must be repeated. Rabies virus requires three doses for pre-exposure prophylaxis and four to five doses for Post-Exposure Prophylaxis (PEP). In addition, for more severe canine bite exposures, a vaccine regimen combined with human or equine rabies immunoglobulin is required to provide passive protection. The high cost of immunoglobulin is difficult for populations in the developing world to afford, and there is a need for a new vaccine that is cheaper, more effective, and can provide sustained immune protection with a single dose.

References

1. Zhang H, Huang J, Song Y, Liu X, Qian M, et al. (2022) Regulation of innate immune responses by rabies virus. Animal Model Exp Med 5(5): 418-429.

2. Gaudin Y, Ruigrok R W, Knossow M, Flamand A (1993) Low-pH conformational changes of rabies virus glycoprotein and their role in membrane fusion. Journal of Virology 67(3): 1365-1372.

3. Hiroyuki F, Sonoda YT, Mifune K, Hirai H, Nishizono A, et al. (1994) Protective efficacy in mice of post-exposure vaccination with vaccinia virus recombinant expressing either rabies virus glycoprotein or nucleoprotein. Journal of General Virology 75(Pt 6): 1339-1344.

4. Sonoda YT, Fujii H, Mifune K, Ito Y, Hiraga M, et al. (1993) Resistance of mice vaccinated with rabies virus internal structural proteins to lethal infection. Archives of Virology 132(1-2): 51-65.

5. Tatsunori M, Naoto I, Kenta S, Yuki I, Keisuke N, et al. (2010) Rabies virus nucleoprotein functions to evade activation of the RIG-I-mediated antiviral response. Journal of Virology 84(8): 4002-4012.

6. Xianfu W, Xiaoming G, Heather DF, Schnell MJ, Zhen FF (2002) Both viral transcription and replication are reduced when the rabies virus nucleoprotein is not phosphorylated. Journal of Virology 76(9): 4153-4161.

7. Lafon M, Wiktor TJ (1985) Antigenic sites on the ERA Rabies virus nucleoprotein and non-structural protein. Journal of General Virology 66(Pt 10): 2125-2133.

8. Minamoto N, Tanaka H, Hishida M, Goto H, Ito H, et al. (1994) Linear and conformation-dependent antigenic sites on the nucleoprotein of rabies virus. Center For Academic Publications Japan 38(6): 449-455.

9. Takamatsu F, Asakawa N, Morimoto K, Takeuchi K, Eriguchi Y, et al. (2008) Studies on the Rabies virus RNA polymerase: 2. possible relationships between the two forms of the non- catalytic subunit (P Protein). Microbiol Immunol 42(11): 761-771.

10. Chenik M, Chebli K, Gaudin Y, Blondel D (1994) *In vivo* interaction of rabies virus Phosphoprotein (P) and Nucleoprotein (N): Existence of two N-binding sites on P protein. Journal of General Virology 75(Pt 11): 2889-2896.

11. Jacob Y, Real E, Tordo N (2001) Functional interaction map of lyssavirus phosphoprotein: identification of the minimal transcription domains. Journal of Virology 75(20): 9613-9622.

12. Raux H, Flamand A, Blondel D (2000) Interaction of the rabies virus P protein with the LC8 dynein light chain. Journal of Virology 74(21): 10212-10216.

13. Moseley GW, Xavier L, Daniela MR, Oksayan S, Richard PF, et al. (2009) Dual modes of rabies P-protein association with microtubules: a novel strategy to suppress the antiviral response. Journal of Cell Science 122(Pt 20): 3652-3662.

14. Vidy Aurore, El Bougrini Jamila, Chelbi-Alix Mounira K, Blondel Danielle (2007) The nucleocytoplasmic rabies virus P protein counteracts interferon signaling by inhibiting both nuclear accumulation and DNA binding of STAT1. Journal of Virology 81(8): 4255-4263.

15. Ito N, Moseley GW, Blondel D, Shimizu K, Caitlin LR, et al. (2010) Role of interferon antagonist activity of rabies virus phosphoprotein in viral pathogenicity. Journal of Virology 84(13): 6699-6710.

16. H Raux, F Iseni, F Lafay, D Blondel (1997) Mapping of monoclonal antibody epitopes of the rabies virus P protein. The Journal of General Virology 78(Pt 1): 119-124.

17. Stefan F, Conzelmann KK (2003) Dissociation of rabies virus matrix protein functions in regulation of viral RNA synthesis and virus assembly. Journal of Virology 77(22): 12074-12082.

18. Mebatsion T, Weiland F, Conzelmann KK (1999) Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. Journal of Virology 73(1): 242-250.

19. Weihe E, Bette M, Preuss MAR, Faber M, Schäfer MKH, et al. (2008) Role of virus-induced neuropeptides in the brain in the pathogenesis of rabies. Developments in Biologicals 131: 73-81.

20. Milosz F, Faber ML, Papaneri A, Michael B, Eberhard W, et al. (2005) A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. Journal of Virology 79(22): 14141-14148.

21. Morimoto K, Ni YJ, Kawai A (1992) Syncytium formation is induced in the murine neuroblastoma cell cultures which produce pathogenic type G proteins of the rabies virus. Virology 189(1): 203-216.

22. Morimoto K, Hooper DC, Spitsin S, Koprowski H, Dietzschold B (1999) Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. Journal of Virology 73(1): 510-518.

23. Faber M, Rojjanaporn P, Suchita SH, Sergei S, McGettigan JP, et al. (2002) Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. Journal of Virology 76(7): 3374-3381.

24. Otsuka M, Jing Q, Georgel P, Liguo N, Jianming C, et al. (2007) Hypersusceptibility to vesicular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR_{24} and miR_{93} expression. Immunity 27(1): 123-134.

25. Tordo N, Poch O, Ermine A, Keith G, Rougeon F (1988) Completion of the rabies virus genome sequence determination: highly conserved domains among the L (polymerase) proteins of unsegmented negative-strand RNA viruses 165(2): 565-576.

26. Euripedes Almeida RE, Leyrat C, Gérard FCA, Albertini AV, Falk C, et al. (2009) Binding of rabies virus polymerase cofactor to recombinant circular nucleoprotein-RNA complexes. Journal of Molecular Biology 394(3): 558-575.

27. Kelly RM, Strick PL (2000) Rabies as a transneuronal tracer of circuits in the central nervous system. Journal of Neuroscience Methods 103(1): 63-71.

28. Malgaroli A, Vallar L, Zimarino V (2006) Protein homeostasis in neurons and its pathological alterations. Current Opinion in Neurobiology 16(3): 270-274.

29. Zhang Ran (2017) Functional study of rabies virus guide RNA and its host interacting proteins. Huazhong Agricultural University, China.

30. Qi Yinglin (2015) Construction, preparation and immune effect evaluation of rabies virus-like particles. Jilin University, China.

31. Zhou M, Zhang G, Ren G, Gnanadurai CW, Li Z, et al. (2013) Recombinant rabies viruses expressing GM-CSF or flagellin are effective vaccines for both intramuscular and oral immunizations. PLoS One 8(5): e63384.

32. Yongjun W, Wang H, Wu Hua, Yang F, Ralph AT, et al. (2011) Rabies virus expressing dendritic cell-activating molecules enhances the innate and adaptive immune response to vaccination. Journal of Virology 85(4): 1634-1644.

33. Hualei W, Guoqing Z, Yongjun W, Songtao Ya, Xianzhu Xia, et al. (2011) Intracerebral administration of recombinant rabies virus expressing GM-CSF prevents the development of rabies after infection with street virus. Plos One 6(9): e25414.

34. Norton JE, Andrew GL, Shixue Shen, Evgeni PT, Dorfmeier CL, et al. (2014) ICAM-1-based rabies virus vaccine shows increased infection and activation of primary murine B cells in vitro and enhanced antibody titers *in-vivo*. Plos One 9(1): e87098.

35. Faber M, Jianwei L, Rhonda BK, Hooper DC, Alugupalli KR, et al. (2009) Effective preexposure and postexposure prophylaxis of rabies with a highly attenuated recombinant rabies virus. Proceedings of the National Academy of Sciences of the United States of America 106(27): 11300-11305.

36. Zhao L, Toriumi H, Wang H, Kuang Y, Guo X, et al. (2010) Expression of MIP-1alpha (CCL3) by a recombinant rabies virus enhances its immunogenicity by inducing innate immunity and recruiting dendritic cells and B cells. Journal of Virology 84(18): 9642-9648.

37. Jinying G, Wang X, Tao L, Zhiyuan W, Feng N, et al. (2011) Newcastle disease virus-vectored rabies vaccine is safe, highly immunogenic, and provides long-lasting protection in dogs and cats. Journal of Virology 85(16): 8241-8252.

38. Amann R, Jörg R, Ulrich W, Douglas C, Rudiger R, et al. (2013) A new rabies vaccine based on a recombinant ORF virus (parapoxvirus) expressing the rabies virus glycoprotein. Journal of Virology 87(3): 1618-1630.

39. Rongliang H, Shoufeng Z, Fooks AR, Huijun Y, Liu Ye, et al. (2006) Prevention of rabies virus infection in dogs by a recombinant canine adenovirus type-2 encoding the rabies virus glycoprotein. Microbes and Iinfection 8(4): 1090-1097.

40. Shoufeng Z, Liu Ye, Fooks AR, Zhang Fei, Rongliang H (2008) Oral vaccination of dogs (*Canis familiaris*) with baits containing the recombinant rabies-canine adenovirus type-2 vaccine confers long-lasting immunity against rabies. Vaccine 26(3): 345-350.

41. Xuelin W, Juan L, Xiuping Wu, Lu Yu, Haiying C, et al. (2012) Oral immunisation of mice with a recombinant rabies virus vaccine incorporating the heat-labile enterotoxin B subunit of *Escherichia coli* in an attenuated Salmonella strain. Research in Veterinary Science 93(2): 675-681.

42. Dong KY, Hyun KH, Kyung WL, Young SJ (2013) The present and future of rabies vaccine in animals. Clinical and Experimental Vaccine Research 2(1): 19-25.

43. Dhama K, Mahesh M, Gupta PK, Rai A (2008) DNA vaccines and their applications in veterinary practice: current perspectives. Veterinary Research Communications 32(5): 341-356.

44. Osorio JE, Tomlinson CC, Frank RS, Haanes EJ, Rushlow K, et al. (1999) Immunization of dogs and cats with a DNA vaccine against rabies virus. Vaccine 17(9-10): 1109-1116.

45. Lodmell DL, Parnell MJ, Bailey JR, Ewalt LC, Hanlon CA (2001) One-time gene gun or intramuscular rabies DNA vaccination of non-human primates: comparison of neutralizing antibody responses and protection against rabies virus 1 year after vaccination. Vaccine 20(5-6): 838-844.

46. Liu X, Yang Y, Sun Z, Chen J, Ai Jun, et al. (2014) A recombinant rabies virus encoding two copies of the glycoprotein gene confers protection in dogs against a virulent challenge. Plos One 9(2): e87105.

47. Jie P, Fei H, Qiong W, Zhaochen L, Zhang YC, et al. (2019) Codon optimization of G protein enhances rabies virus-induced humoral immunity. Journal of General Virology 100(8): 1222-1233.

48. Navid MT, Yingying Li, Ming Z, Cui M, Zhen FF, et al. (2016) Comparison of the immunogenicity of two inactivated recombinant rabies viruses overexpressing the glycoprotein. Archives of Virology 161(10): 2863-2870.

49. Plummer JR, McGettigan JP (2019) Incorporating B cell activating factor (BAFF) into the membrane of rabies virus (RABV) particles improves the speed and magnitude of vaccine-induced antibody responses. Plos Neglected Tropical Diseases 13(11): e0007800.

50. Christine RF, David EL, Todd GS, Yong Y, Christina LH, et al. (2020) Lyssavirus vaccine with a chimeric glycoprotein protects across phylogroups. Cell Reports 32(3): 107920.

51. Budaszewski RF, Hudacek A, Sawatsky B, Krämer B, Xiangping Y, et al. (2017) Inactivated recombinant rabies viruses displaying canine

distemper virus glycoproteins induce protective immunity against both pathogens. Journal of Virology 91(8): e02077-16.

52. Drishya K, Fisher CR, Smith TG, Abreu MT, Yang Y, et al. (2019) Inactivated rabies virus-based ebola vaccine preserved by vaporization is heat-stable and immunogenic against ebola and protects against rabies challenge. The Journal of Infectious Diseases 220(9): 1521-1528.

53. Drishya K, Wirblich C, Ramage H, Schnell MJ (2020) Rabies virus-based COVID-19 vaccine CORAVAX™ induces high levels of neutralizing antibodies against SARS-CoV-2. NJPVaccines 5(1): 98.

54. Zhang Y, Yao W, Kang K, Xue Qi, Li Q, et al. (2020) Research progress on recombinant expression of rabies virus glycoprotein and its genetic engineering vaccine. Chinese Journal of Zoonoses 36(01): 65-69.

55. Astray RM, Ventini DC, Boldorini VLL, Silva FG, Rocca MP, et al. (2014) Rabies virus glycoprotein and immune response pattern using recombinant protein or recombinant RNA viral vectors. Vaccine 32(24): 2829-2832.

56. Monize CD, Diogo PS, Renato MA, Ventini-MDC, Soraia ACJ, et al. (2018) Drosophila S_2 cell culture in a Wave Bioreactor: potential for scaling up the production of the recombinant rabies virus glycoprotein. Applied Microbiology and Biotechnology 102(11): 4773-4783.

57. Koraka P, Berend JB, Cox M, Chubet R, Amerongen GV, et al. (2014) A recombinant rabies vaccine expressing the trimeric form of the glycoprotein confers enhanced immunogenicity and protection in outbred mice. Vaccine 32(36): 4644-4650.

58. Yange N, Ye Liu, Limin Y, Hongren Qu, Jingyi Z, et al. (2016) Immunogenicity of multi-epitope-based vaccine candidates administered with the adjuvant Gp96 against rabies. Virologica Sinica 31(2): 168-175.

59. Fontana D, Kratje R, Etcheverrigaray M, Prieto C (2015) Immunogenic virus-like particles continuously expressed in mammalian cells as a veterinary rabies vaccine candidate. Vaccine 33(35): 4238-4246.

60. Fontana D, Etcheverrigaray M, Kratje R, Prieto C (2016) Development of rabies virus-like particles for vaccine applications: production, characterization, and protection studies. Methods in Molecular Biology 1403: 155-166.

61. Hongtao K, Yinglin Q, Hualei W, Xuexing Z, Yuwei G, et al. (2015) Chimeric rabies virus-like particles containing membrane-anchored gm-csf enhances the immune response against rabies virus. Viruses 7(3): 1134-1152.

62. Yinglin Qi, Hongtao K, Xuexing Z, Hualei W, Yuwei G, et al. (2015) Incorporation of membrane-anchored flagellin or Escherichia coli heat-labile enterotoxin B subunit enhances the immunogenicity of rabies virus-like particles in mice and dogs. Frontiers in Microbiology 6: 169.

63. Ya Nan Z, Chen C, Cheng Lin D, Cheng GZ, Na Li, et al. (2020) A novel rabies vaccine based on infectious propagating particles derived from hybrid VEEV-Rabies replicon. EBioMedicine 56: 102819.