Review Article

Application of multi-omics technologies to decipher rabies pathogenesis

Peerut Chienwichai[1] Onrapak Reamtong[2*]

Abstract

Rabies virus infection has affected human and animal health since ancient times and existing treatments are only partially effective. A thorough understanding of rabies virus pathogenesis is required to achieve improved treatment options and the eventual elimination of the disease. In recent years, multi-omics technology has emerged as a powerful tool for understanding viral pathogenesis. However, multi-omics data is lacking for the rabies virus. The aim of this review is to summarize our current understanding of rabies pathogenesis and its study using omics approaches. Several mechanisms have been proposed to contribute to rabies pathogenesis using the results of omics studies. Using data from different omics levels, we highlight the potential role of pathways involved in host immunity and cytoskeletal protein expression in rabies pathogenesis. Future studies should focus on in-depth investigation of these pathways as well as the discovery of potential drug targets. This review will be beneficial to scientists interested in investigating viral pathogenesis with molecular tools, especially in rabies.

Keywords: Rabies, Pathogenesis, Multi-Omics, Genomics, Transcriptomics, Proteomics, Metabolomics

[1]*Faculty of Medicine and Public Health, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok, 10210, Thailand*

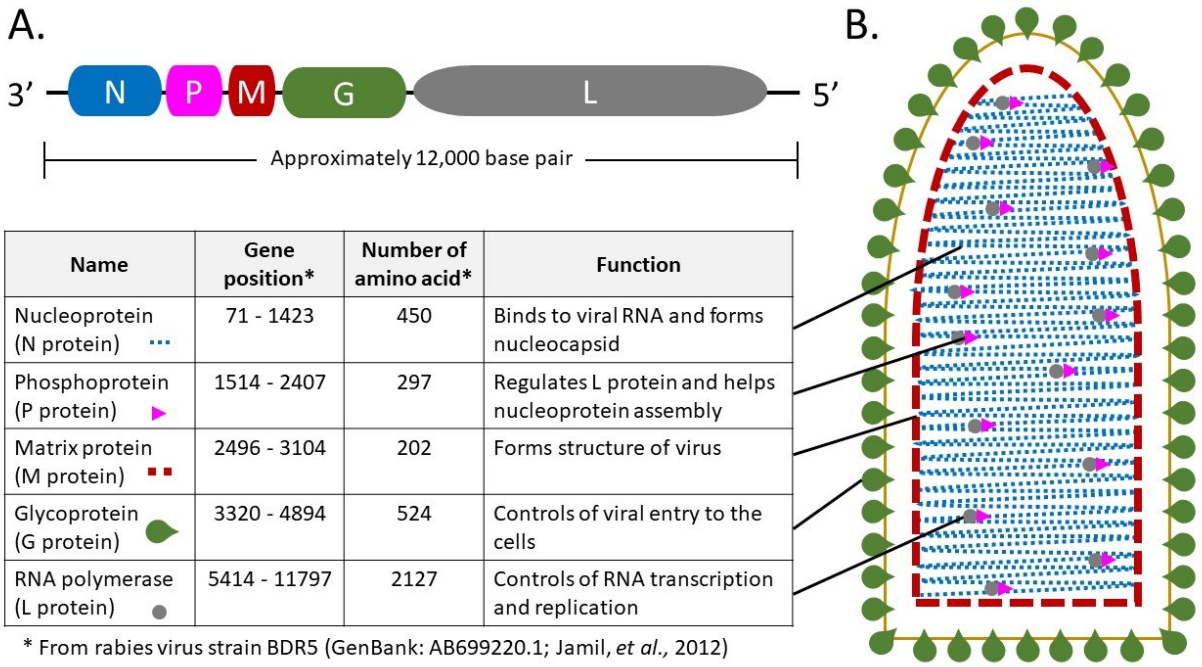
[2]*Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand*

***Correspondence**: onrapak.rea@mahidol.ac.th* (O. Reamtong)

Introduction

Rabies is a zoonotic viral disease that has affected humans since ancient times and has yet to be eliminated. Rabies is a serious public health threat in many developing countries. Human deaths from rabies infection are continuously being reported, and approximately 60,000 cases occur each year worldwide (Fooks, *et al.*, 2017). Human rabies infection mainly occurs following a bite from an infected animal, resulting in the introduction of the saliva-borne rabies virus (RABV) into the wound (Fooks, *et al.*, 2017). Thereafter, RABV infects nearby neurons, moves in a retrograde fashion via axons to adjacent neurons, and eventually reaches the central nervous system (Fooks, *et al.*, 2017; Ugolini and Hemachudha, 2018). RABV replicates inside the brain, causing inflammation and producing the characteristic signs of the disease including hydrophobia, aerophobia, hypersalivation, painful laryngospasm and seizures (Mahadevan, *et al.*, 2016; Fooks, *et al.*, 2017). Patients with rabies encephalitis usually die within a few weeks and no treatment has proven effective for late-stage infection (Mahadevan, *et al.*, 2016). The World Health Organization has initiated a strategic plan to achieve "zero human rabies deaths by 2030" (WHO, 2019). To achieve this ambitious goal, extensive research and development efforts will be required.

Understanding pathogenesis is a prerequisite step toward disease treatment and elimination. The precise mechanisms of RABV-induced clinical manifestations remain elusive. RABV is a bullet-shaped enveloped virus belonging to the Rhabdoviridae family and the *Lyssavirus* genus (Fooks, *et al.*, 2017). RABV has a single-stranded RNA genome approximately 12 kilobases in length (Fooks, *et al.*, 2017). The genome encodes five proteins: the nucleoprotein, phosphoprotein, matrix protein, glycoprotein and viral RNA polymerase (Finke and Conzelmann, 2005; Dietzschold, *et al.*, 2008) (Fig. 1). These proteins are essential for viral replication and pathogenesis. The RABV nucleoprotein interacts with viral RNA to form the nucleocapsid (Wunner, 2007), while the multifunctional phosphoprotein regulates viral RNA polymerase as well as nucleoprotein assembly (Wunner, 2007; Okada, *et al*., 2016). The dimeric matrix protein plays a primary structural role (Wunner, 2007). The RABV glycoprotein is present on the virion surface and plays a vital role in host cell entry; this protein has been extensively studied because of its key roles in viral pathophysiology and immunogenicity (Finke and Conzelmann, 2005; Wunner, 2007). The RNA polymerase is responsible for RNA transcription and replication (Wunner, 2007; Nakagawa, *et al.*, 2017). The viral proteins work in coordination to maintain replication and to produce pathogenesis in mammalian hosts. To gain more insight into RABV pathogenesis, cutting-edge technologies must be combined to yield comprehensive information on RABV infection.



Name	Gene position*	Number of amino acid*	Function
Nucleoprotein (N protein)	71 - 1423	450	Binds to viral RNA and forms nucleocapsid
Phosphoprotein (P protein)	1514 - 2407	297	Regulates L protein and helps nucleoprotein assembly
Matrix protein (M protein)	2496 - 3104	202	Forms structure of virus
Glycoprotein (G protein)	3320 - 4894	524	Controls of viral entry to the cells
RNA polymerase (L protein)	5414 - 11797	2127	Controls of RNA transcription and replication

* From rabies virus strain BDR5 (GenBank: AB699220.1; Jamil, *et al.*, 2012)

Figure 1 RABV genome organization and protein localization in RABV particles. A. Arrangement of RABV genes in the viral genome. B. Localization of RABV proteins on the viral particle. The table describes gene location, the number of amino acids and the function of each protein.

Omics and multi-omics approaches in medical research

Omics is the suffix used to describe the comprehensive or holistic study of a field or molecule (*e.g.*, genomics, transcriptomics, proteomics and metabolomics). In the last few decades, scientists worldwide have used omics technologies to answer biological questions as these technologies have several advantages. Omics studies generate high-throughput data. A genome is considered the most static level of the central dogma. By contrast, the transcriptome, proteome and metabolome are dynamic and can be influenced by environmental factors. To extract meaningful information from multi-omics data, integrative approaches and computer-assisted

methods are required to understand the findings as a whole; such methods are not generally applied in conventional studies (Zhang, *et al.,* 2010). Moreover, the findings of omics studies are derived from data-driven processes, which can help other researchers to target key molecules or pathways (Zhang, *et al.,* 2010; Raja, *et al.,* 2017). Omics studies at all levels have their strengths and challenges. Therefore, data from all omics levels should be combined in multi-omics studies to understand the larger picture of cellular events and to overcome the drawbacks of any individual level (Zhang, *et al.,* 2010; Hasin, *et al.,* 2017; Martins, *et al.,* 2019; Solovev, *et al.,* 2020).

Multi-omics approaches have been applied in diverse fields of medical research including biomarker discovery (Namani, *et al.,* 2019; Kiebish, *et al.,* 2020), drug development (Birrell, *et al.,* 2019; Chen, *et al.,* 2020) and disease pathogenesis (Gao, *et al.,* 2019). Multi-omics approaches have been applied to many viruses including adenovirus (Zhao, *et al.,* 2019), influenza A virus (Söderholm, *et al.,* 2016) and human papilloma virus (Costa, *et al.,* 2018). Unfortunately, no multi-omics data on RABV is currently available. Therefore, this review attempts to integrate the results of prior omics studies, and specifically genomic, transcriptomic, proteomic and metabolomic data, to summarize our current understanding of rabies pathogenesis (Fig. 2). This data also suggests potential pathogenic mechanisms that should be the focus of future studies. This review will be of benefit for researchers interested in RABV pathogenesis and multi-omics technologies.

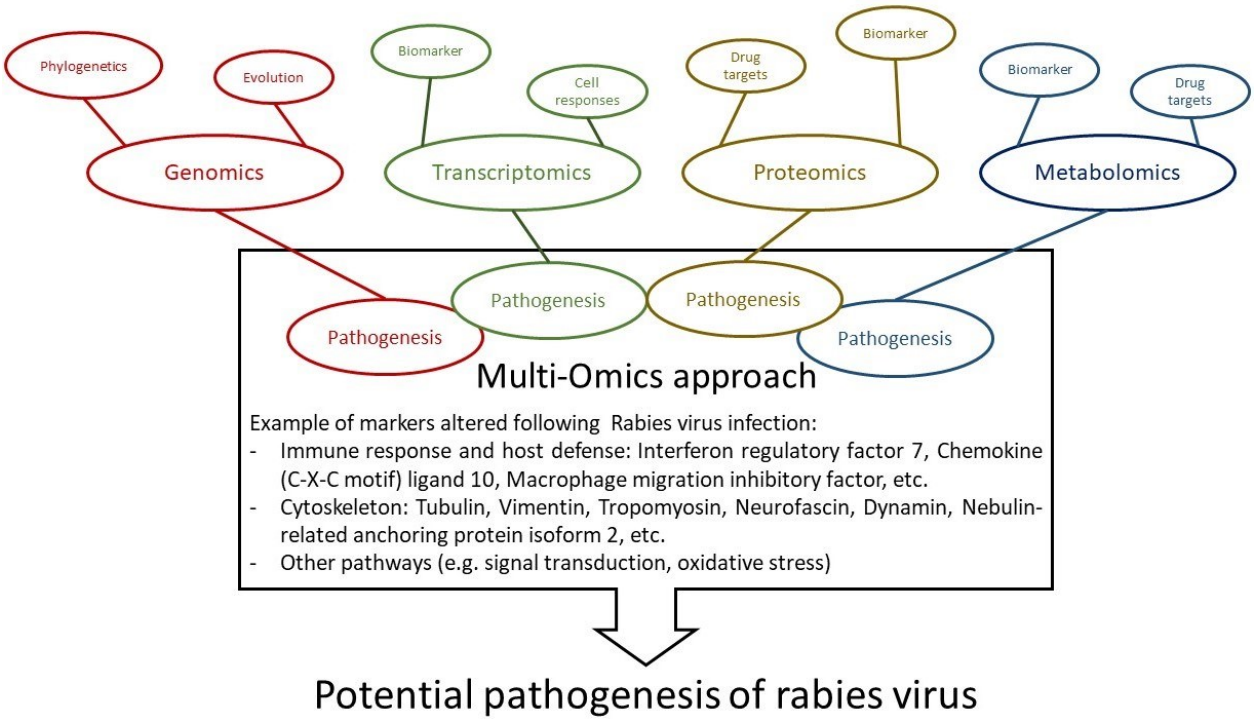


Figure 2 Schematic diagram of multi-omics approaches to understand rabies pathogenesis.

Genomic studies of RABV pathogenesis

Genome sequencing is an excellent tool for studying viral genetic variation. Genomic platforms provide information that can help us understand viral pathogenesis of the virus; they can also be applied to studies of molecular evolution as well as in outbreak investigations (Seto, 2010; Chabria, *et al.,* 2014; Li, *et al.,* 2014; Wohl, *et al.,* 2016; Brunker, *et al.,* 2018). The first step for genomic studies of RNA viruses like RABV is genome fragmentation. Subsequently, the fragmented genome is tagged with virus-specific primers and converted into DNA using the reverse transcription-polymerase chain reaction. Complementary DNA is amplified before starting the sequencing process (Wohl, *et al.,* 2016). Several genomic studies of RABV have been performed but few have focused on disease pathogenesis. Crossing species barriers can affect the pathogenesis of RABV infection. Bonnaud *et al.,* (2019) found that fox-adapted RABV lost its ability to be transmitted to dogs. The authors used a genomic approach to identify mutations in fox-adapted RABV. Although they identified many mutations, these could not explain the reduced pathogenesis of fox-adapted RABV. Borucki *et al.,* (2013) investigated a rabies outbreak in California from 2009–2010 using viral genome sequencing. Their study identified mutations in the viral glycoprotein that resulted in polarity changes in the amino acid residues of this protein. The authors hypothesized that these mutations might have enhanced viral spread in foxes and this contributed to the severity of the outbreak.

Other genomic studies of RABV infection did not focus on investigating pathogenesis. Several studies focused on phylogenetic analyses (Yu, *et al.,* 2014; Troupin, *et al.,* 2016), which are important for understanding the evolution of the virus. Thus, many knowledge gaps in RABV pathogenesis could be addressed by future studies using genomic approaches. For instance, such studies might define associations between viral genome features and clinical manifestations of the disease.

Transcriptomic studies of RABV pathogenesis

Transcriptomic studies involve measurement and comparison of RNA expression levels, including messenger RNA (mRNA), microRNA (miRNA) and long non-coding RNA (lncRNA). Transcriptomic studies begin with isolation of RNA from samples, their conversion into complementary DNA and quantitation of their abundance. Sequences of RNA molecules are studied and aligned with reference genomes to establish RNA expression profiles. Transcriptomics is a promising tool for investigating disease pathogenesis (Lowe, *et al.,* 2017; Van den Esker and Koets, 2019). Transcriptomic approaches have been used extensively to study RABV infection both *in vitro* and *in vivo.* Immune responses are the major mechanisms identified as playing dominant roles in RABV infection (Ubol, *et al.,* 2006; Zhao, *et al.,* 2011; Zhao, *et al.,* 2012a; Zhao, *et al.,* 2012b; Zhao, *et al.,* 2013; Zhang, *et al.,* 2016; Zhao, *et al.,* 2018; Ji, *et al.,* 2019). Following RABV infection, several anti-viral genes, including interferon (IFN)-stimulated genes, IFN-inducible transcription factors, cytokines and their complement, were significantly up-regulated (Zhao, *et al.,* 2011). Moreover, many immune-related pathways were dysregulated including those involved in T-cell receptor signaling, cytokine-cytokine receptor interactions, Fc gamma R-mediated phagocytosis, antigen processing and presentation and the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling (Zhao, *et al.,* 2011; Zhao, *et al.,* 2012a; Zhao, *et al.,* 2012b). Changes in RNA levels of immune-related molecules may influence host defense against RABV in the brain. The role of intracranial immunity in protection against RABV has been shown by Zhang *et al.,* (2016): after infecting mice with a non-pathogenic RABV (HEP-Flury strain), the expression of genes related to innate immunity was dramatically activated. Subsequently, the authors challenged the infected mice with a pathogenic RABV, CVS-11. Their findings indicate that prior infection of mice with HEP-Flury strain increased their survival following challenge with the pathogenic RABV strain CSV-11 (Zhang, *et al.,* 2016).

Alteration of neuronal homeostasis was also highlighted as a potential mechanism contributing to the pathogenesis of RABV. Suppressed expression of genes related to neuronal homeostasis and function has been shown in the brains of rabies patients (Reinke, *et al.,* 2013). Moreover, infection of mouse neurons by RABV caused variation in miRNA expression. The major role of miRNA is in controlling expression of genes. Shi *et al.,* (2014) reported altered miRNA levels following RABV infection of mouse neurons. Most identified miRNA targets were involved in neuronal function and the authors proposed that neuronal dysfunction resulting from RABV infection might be caused by alterations of miRNA levels. Transcriptomic approaches have also been used to explore potential markers of RABV infection (Han, *et al.,* 2011). The versatility of transcriptomic approaches has helped shed light on many biological questions. Hence, transcriptomic studies of viral pathogenesis are of great interest to many researchers.

Proteomic studies of RABV pathogenesis

The field of proteomics studies whole proteins of cells, organs or organisms using mass spectrometry. Gel-based and non-gel-based approaches are used for protein complex separation. Proteins of interest are subjected to tryptic digestion and analyzed using a tandem mass spectrometer. Proteomic data can be used to identify and quantitate proteins, as well as to detect post-translational modifications. The advantages of proteomic methods for medical research are their general applicability for the exploration of disease pathogenesis, biomarker discovery and identification of therapeutic targets (Mehta, *et al.,* 2015). Many researchers have used proteomic methods to study RABV pathogenesis. Several studies have found that proteins involved in cytoskeleton formation (*e.g.,* vimentin, tubulin and tropomyosin) have increased their expression following RABV infection (Thanomsridetchai, *et al.,* 2011; Wang, *et al.,* 2011). Bioinformatic analysis of differentially-expressed proteins has revealed the disturbance of cytoskeleton-related pathways (Zandi, *et al.,* 2009; Thanomsridetchai, *et al.,* 2011; Wang, *et al.,* 2011; Farahtaj, *et al.,* 2013; Zandi, *et al.,* 2013; Sun, *et al.,* 2016). Moreover, when the proteomes of cells infected with pathogenic and non-pathogenic RABV strains have been compared, the findings reveal that pathogenic RABV disrupts the expression of neuronal structural proteins to a greater degree than non-pathogenic RABV (Wang, *et al.,* 2011; Zandi, *et al.,* 2013). The overall findings suggest that RABV infection dysregulates the cytoskeleton and cellular structural proteins leading to impaired integrity and cellular architecture. Specifically, altered cytoskeletal proteins and abnormal morphology of neurons affect axonal arrangement and cell-to-cell communication (Kapitein and Hoogenraad, 2015; Leterrier, *et al.,* 2017). Therefore, pathogenesis of RABV might occur by damaging neuronal networks through the disturbance of cytoskeletal proteins.

Proteomic studies have also highlighted other effects of RABV on host cells. As in transcriptomic studies, proteomic studies have shown disturbance of immune-related proteins following RABV infection. Pathogenic and non-pathogenic RABV infection of mice results in altered protein expression in lymphocytes, which are the major cell type involved in defense against viral infection. Pathways with altered expression include those involved in T and B lymphocyte activation, antiviral activity and cytoskeletal reorganization (Vaziri, *et al.,* 2012). Interestingly, a mutant RABV bearing two glycoprotein-encoding genes was less pathogenic and induced more potent cellular immune responses than wildtype RABV. The mutant virus triggered enhanced IFN signaling and other defenses against viral infection (Yang, *et al.,* 2015). RABV-induced autophagy has been proposed as another mechanism contributing to neuronal pathology. Li *et al.*. (2017) reported that non-pathogenic RABV infection promoted autophagy-associated protein dysregulation in the brains of mice to a greater degree than pathogenic RABV infection. The results of proteomic analyses were confirmed by evaluation of the autophagosome, which supported

the earlier findings. Hence, the induction of cellular immunity and autophagy pathways have been proposed as potential mechanisms to overcome RABV infection.

In addition to investigations of disease pathogenesis, proteomic approaches have been used to discover novel markers of disease. Several studies have been conducted to identify such markers (Venugopal, *et al.,* 2013; Mehta, *et al.,* 2016). No studies have focused on the role of post-translational modifications of the proteome in RABV infection. Post-translational modification is key to understanding protein function as well as cell biology. Therefore, the relationship between post-translational modification of host proteins and RABV pathogenesis merits further study.

Metabolomic studies of RABV pathogenesis

Metabolomic studies are important tools for detecting changes in the levels of small molecules (Noto, *et al.,* 2014). Metabolomic studies start with whole metabolite extraction. The dynamic range of detection is increased by subjecting the metabolite mixture to gas or liquid chromatography. The separation device is usually coupled with a mass spectrometer for metabolite detection. The resulting chromatograms and mass spectra are interpreted to enable metabolite identification and quantification. Only a few metabolic studies of RABV infection have been conducted and few have focused on pathogenesis. Metabolites in brain tissue have been generally altered in RABV-infected patients (Reinke, *et al.,* 2013). Schutsky *et al.,* (2014) found that post-exposure prophylaxis in RABV-infected mice increased the levels of neuroprotective molecules (carnitine and its derivative, acylcarnitine) in the brain, as well as levels of corticosteroids in serum. The authors hypothesized that increased levels of corticosteroid in serum might facilitate viral pathogenesis via suppression of host immunity. Inhibition of corticosteroid synthesis decreased the severity of the disease. In addition, metabolomic approaches were applied to study RABV infection using other biological samples. Cerebrospinal fluid is a rich source of metabolites for studying viral infection and most metabolomic studies of cerebrospinal fluid in RABV infection have focused on biomarker discovery (O'Sullivan, *et al.,* 2013; French, *et al.,* 2018). Other sources of metabolites have included the feces of vaccinated animals; the analysis of volatile metabolites helped determine levels of immune response to the virus (Kimball, *et al.,* 2019) and demonstrated the usefulness of this technology.

Table 1 Pathways potentially involved in rabies pathogenesis identified using multi-omics technologies.

No.	Potential pathways associated with pathogenesis	References
1	Immune response and host defense	Ubol, *et al.*, 2006; Zhao, *et al.*, 2006; Thanomsridetchai, *et al.*, 2011; Zhao, *et al.*, 2011; Vaziri, *et al.*, 2012; Zhao, *et al.*, 2012a; Zhao, *et al.*, 2012b; Farahtaj, *et al.*, 2013; Reinke, *et al.*, 2013; Schutsky, *et al.*, 2014; Yang, *et al.*, 2015; Zhao, *et al.*, 2018; Ji, *et al.*, 2019.
2	Alteration of cytoskeletal proteins	Zandi, *et al.*, 2009; Thanomsridetchai, *et al.*, 2011; Wang, *et al.*, 2011; Vaziri, *et al.*, 2012; Farahtaj, *et al.*, 2013; Zandi, *et al.*, 2013; Sun, *et al.*, 2016.
3	Impairment of signal transduction	Ubol, *et al.*, 2006; Wang, *et al.*, 2011; Zhao, *et al.*, 2012a; Zhao, *et al.*, 2012b; Zandi, *et al.*, 2013; Zhao, *et al.*, 2018; Ji, *et al.*, 2019.
4	Oxidative stress	Zandi, *et al.*, 2009; Thanomsridetchai, *et al.*, 2011; Wang, *et al.*, 2011; Zandi, *et al.*, 2013; Li, *et al.*, 2017.
5	Alteration of cellular metabolism	Ubol, *et al.*, 2006; Wang, *et al.*, 2011; Farahtaj, *et al.*, 2013; Zandi, *et al.*, 2013; Sun, *et al.*, 2016.
6	Disturbance of transcription and translation factors	Ubol, *et al.*, 2006; Zandi, *et al.*, 2009; Thanomsridetchai, *et al.*, 2011; Zandi, *et al.*, 2013; Ji, *et al.*, 2019.
7	Autophagy and death-mediated factors	Ubol, *et al.*, 2006; Thanomsridetchai, *et al.*, 2011; Yang, *et al.*, 2015; Li, *et al.*, 2017.
8	Proteolysis	Ubol, *et al.*, 2006; Thanomsridetchai, *et al.*, 2011; Zhao, *et al.*, 2011; Farahtaj, *et al.*, 2013.
9	Neuronal dysfunction	Thanomsridetchai, *et al.*, 2011; Shi, *et al.*, 2014; Sun, *et al.*, 2016; Zhang, *et al.*, 2016.
10	Increased infectivity conferred by viral genome mutations	Borucki, *et al.*, 2013.

Conclusions and future perspectives

RABV infection has long been a threat to human health, especially in Asian and developing African countries. Despite intensive research, the precise pathogenic mechanisms of RABV are incompletely understood. Multi-omics approaches have shown promise in biomedical research. Here, we have summarized the results of studies aiming to elucidate the pathogenesis of RABV using omics technologies. From the available data, we suggest two potential pathogenic mechanisms as well as areas for future investigation. Firstly, several omics studies have demonstrated that immunity and host defense are altered following RABV infection. Vaccination is highly effective against RABV both for pre-exposure and post-exposure prophylaxis. Moreover, inhibiting the synthesis of immunosuppressive corticosteroids reduces the severity of the disease (Schutsky, *et al.,* 2014). Therefore, we suggest that enhancement of neuronal immune responses, particularly in the brain, might represent a potential mechanism to reduce disease severity. However, significant preliminary data and *in vivo* studies will be required to produce a practical protocol for rabies treatment. Secondly, omics data, and especially the results of proteomic studies, have highlighted the dysregulation of cytoskeletal and

structural proteins following RABV infection. Cytoskeletal proteins are the building blocks of neuron axons and dendrites, and their disturbance may affect cellular organization, morphology and neurotransmission. To date, no studies have attempted to inhibit cytoskeletal damage during RABV infection. Studies in this area would provide greater insight into the relationship between cytoskeletal proteins and rabies pathogenesis.

Acknowledgments

This work was funded by the Chulabhorn Royal Academy. We thank Asst. Prof. Rojjanaporn Pulmanausahakul for her comments and support.

References

Birrell GW, Challis MP, De Paoli A, Anderson D, Devine SM, Heffernan GD, Jacobus DP, Edstein MD, Siddiqui G, Creek DJ. 2019. Multi-omic characterisation of the mode of action of a potent new antimalarial compound, JPC-3210, against *Plasmodium falciparum*. Mol Cell Proteomics. pii: mcp.RA119.001797. doi: 10.1074/mcp.RA119. 001797.

Bonnaud EM, Troupin C, Dacheux L, Holmes EC, Monchatre-Leroy E, Tanguy M, Bouchier C, Cliquet F, Barrat J, Bourhy H. 2019. Comparison of intra- and inter-host genetic diversity in rabies virus during experimental cross-species transmission. PLoS Pathog. 15(6): e1007799. doi: 10.1371/journal.ppat.1007799.

Borucki MK, Chen-Harris H, Lao V, Vanier G, Wadford DA, Messenger S, Allen JE. 2013. Ultra-deep sequencing of intra-host rabies virus populations during cross-species transmission. PLoS Negl Trop Dis. 7(11): e2555. doi: 10.1371/journal.pntd.0002555.

Brunker K, Nadin-Davis S, Biek R. 2018. Genomic sequencing, evolution and molecular epidemiology of rabies virus. Rev Sci Tech Off Int Epiz. 37(2): 401-408. doi: 10.20506/ rst.37.2.2810.

Chabria SB, Gupta S, Kozal MJ. 2014. Deep sequencing of HIV: clinical and research applications. Annu Rev Genomics Hum Genet. 15: 295-325. doi: 10.1146/annurev-genom-091212-153406.

Chen X, Yi Y, You X, Liu J, Shi Q. 2019. High-Throughput Identification of Putative Antimicrobial Peptides from Multi-Omics Data of the Lined Seahorse (*Hippocampus erectus*). Mar Drugs. 18(1): pii: E30. doi: 10.3390/md18010030.

Costa R, Boroni M, Soares MA. 2018. Distinct co-expression networks using multi-omic data reveal novel interventional targets in HPV-positive and negative head-and-neck squamous cell cancer. Sci Rep. 8(1): 15254. doi: 10.1038/s41598-018-33498-5.

Dietzschold B, Li J, Faber M, Schnell M. 2008. Concepts in the pathogenesis of rabies. Future Virol. 3(5): 481-490.

Farahtaj F, Zandi F, Khalaj V, Biglari P, Fayaz A, Vaziri B. 2013. Proteomics analysis of human brain tissue infected by street rabies virus. Mol Biol Rep. 40(11): 6443-50. doi: 10.1007/s11033-013-2759-0.

Finke S, Conzelmann KK. 2005. Replication strategies of rabies virus. Virus Res. 111(2): 120-31.

Fooks AR, Cliquet F, Finke S, Freuling C, Hemachudha T, Mani RS, Müller T, Nadin-Davis S, Picard-Meyer E, Wilde H, Banyard AC. 2017. Rabies. Nat Rev Dis Primers. 3: 17091.

French CD, Willoughby RE, Pan A, Wong SJ, Foley JF, Wheat LJ, Fernandez J, Encarnacion R, Ondrush JM, Fatteh N, Paez A, David D, Javaid W, Amzuta IG, Neilan AM, Robbins GK, Brunner AM, Hu WT, Mishchuk DO, Slupsky CM. 2018. NMR metabolomics of cerebrospinal fluid differentiates inflammatory diseases of the central nervous system. PLoS Negl Trop Dis. 12(12): e0007045. doi: 10.1371/journal. pntd.0007045.

Gao Y, Dasgupta C, Huang L, Song R, Zhang Z, Zhang L. 2019. Multi-Omics Integration Reveals Short and Long-Term Effects of Gestational Hypoxia on the Heart Development. Cells. 8(12): pii: E1608. doi: 10.3390/cells8121608.

Han MG, Park JS, Lee CS, Jeong YE, Ryou JS, Cho JE, Ju YR, Lee KK. 2011. Serum MicroRNA Expression Profiling in Mice Infected with Rabies Virus. Osong Public Health Res Perspect. 2(3): 186-91. doi: 10.1016/j.phrp.2011.11.043.

Hasin Y, Seldin M, Lusis A. 2017. Multi-omics approaches to disease. Genome Biol. 18(1): 83. doi: 10.1186/s13059-017-1215-1.

Jamil KM, Ahmed K, Hossain M, Matsumoto T, Ali MA, Hossain S, Hossain S, Islam A, Nasiruddin M, Nishizono A. 2012. Arctic-like Rabies Virus, Bangladesh Emerging Infect Dis. 18(12): 2021-2024. doi:10.3201/eid1812.120061.

Ji S, Zhu M, Zhang J, Cai Y, Zhai X, Wang D, Li G, Su S, Zhou J. 2019. Microarray analysis of lncRNA expression in rabies virus infected human neuroblastoma cells. Infect Genet Evol 67: 88-100. doi: 10.1016/j.meegid.2018.10.027.

Kapitein LC, Hoogenraad CC. 2015. Building the Neuronal Microtubule Cytoskeleton. Neuron. 87(3): 492-506. doi: 10.1016/j.neuron.2015.05.046.

Kiebish MA, Cullen J, Mishra P, Ali A, Milliman E, Rodrigues LO, Chen EY, Tolstikov V, Zhang L, Panagopoulos K, Shah P, Chen Y, Petrovics G, Rosner IL, Sesterhenn IA, McLeod DG, Granger E, Sarangarajan R, Akmaev V, Srinivasan A, Srivastava S, Narain NR, Dobi A. 2020. Multi-omic serum biomarkers for prognosis of disease progression in prostate cancer. J Transl Med. 18(1): 10. doi: 10.1186/s12967-019-02185-y.

Kimball BA, Volker SF, Griffin DL, Johnson SR, Gilbert AT. 2019. Volatile metabolomic signatures of rabies immunization in two mesocarnivore species. PLoS Negl Trop Dis. 13(12): e0007911. doi: 10.1371/journal.pntd.0007911.

Leterrier C, Dubey P, Roy S. 2017. The nano-architecture of the axonal cytoskeleton. Nat Rev Neurosci. 18(12): 713-726. doi: 10.1038/nrn.2017.129.

Li L, Jin H, Wang H, Cao Z, Feng N, Wang J, Zhao Y, Zheng X, Hou P, Li N, Chi H, Huang P, Jiao C, Li Q, Wang L, Wang T, Sun W, Gao Y, Tu C, Hu G, Yang S, Xia X. Autophagy is highly targeted among host comparative proteomes during infection with

different virulent RABV strains. Oncotarget. 8(13): 21336-21350. doi: 10.18632/oncotarget.15184.

Li, LM, Grassly NC, Fraser C. 2014. Genomic analysis of emerging pathogens: methods, application and future trends. Genome Biol. 15(11): 541. doi: 10.1186/s13059-014-0541-9.

Lowe, R, Shirley N, Bleackley M, Dolan S, Shafee T. 2017. Transcriptomics technologies. PLoS Comput Biol. 13(5): e1005457. doi: 10.1371/journal.pcbi.1005457.

Mahadevan A, Suja MS, Mani RS, Shankar SK. 2016. Perspectives in Diagnosis and Treatment of Rabies Viral Encephalitis: Insights from Pathogenesis. Neurotherapeutics. 13(3): 477-92. doi: 10.1007/s13311-016-0452-4.

Martins C, Dreij K, Costa PM. 2019. The State-of-the Art of Environmental Toxicogenomics: Challenges and Perspectives of "Omics" Approaches Directed to Toxicant Mixtures. Int J Environ Res Public Health. 16(23): pii: E4718. doi: 10.3390/ijerph16234718.

Mehta S, Sreenivasamurthy S, Banerjee S, Mukherjee S, Prasad K, Chowdhary A. Pathway Analysis of Proteomics Profiles in Rabies Infection: Towards Future Biomarkers? OMICS. 20(2): 97-109. doi: 10.1089/omi.2015.0137.

Mehta SM, Banerjee SM, Chowdhary AS. 2015. Postgenomics biomarkers for rabies—the next decade of proteomics. OMICS. 19(2): 67-79. doi: 10.1089/omi.2014.0127.

Nakagawa K, Kobayashi Y, Ito N, Suzuki Y, Okada K, Makino M, Goto H, Takahashi T, Sugiyama M. 2017. Molecular Function Analysis of Rabies Virus RNA Polymerase L Protein by Using an L Gene-Deficient Virus. J Virol. 91(20): pii: e00826-17. doi: 10.1128/JVI.00826-17.

Namani A, Zheng Z, Wang XJ, Tang X. 2019. Systematic Identification of Multi Omics-based Biomarkers in KEAP1 Mutated TCGA Lung Adenocarcinoma. J Cancer. 10(27): 6813-6821. doi: 10.7150/jca.35489.

Noto A, Dessi A, Puddu M, Mussap M, Fanos V. 2014. Metabolomics technology and their application to the study of the viral infection. J Matern Fetal Neonatal Med. 27 Suppl 2: 53-7. doi: 10.3109/14767058.2014.955963.

Okada K, Ito N, Yamaoka S, Masatani T, Ebihara H, Goto H, Nakagawa K, Mitake H, Okadera K, Sugiyama M. 2016. Roles of the Rabies Virus Phosphoprotein Isoforms in Pathogenesis. J Virol. 90(18): 8226-37, doi: 10.1128/JVI.00809-16.

O'Sullivan A, Willoughby RE, Mishchuk D, Alcarraz B, Cabezas-Sanchez C, Condori RE, David D, Encarnacion R, Fatteh N, Fernandez J, Franka R, Hedderwick S, McCaughey C, Ondrush J, Paez-Martinez A, Rupprecht C, Velasco-Villa A, Slupsky CM. 2013. Metabolomics of cerebrospinal fluid from humans treated for rabies. J Proteome Res. 12(1): 481-90. doi: 10.1021/pr3009176.

Raja K, Patrick M, Gao Y, Madu D, Yang Y, Tsoi LC. 2017. A Review of Recent Advancement in Integrating Omics Data with Literature Mining towards Biomedical Discoveries. Int J Genomics. 2017: 6213474. doi: 10.1155/2017/6213474.

Reinke SN, Resch L, Maingat F, Branton W, Jackson AC, Holt R, Slupsky C, Marrie T, Sykes BD, Power C. 2013. Metagenomic and metabolomic characterization of rabies encephalitis: new insights into the treatment of an ancient disease. J Infect Dis. 207(9): 1451-6. doi: 10.1093/infdis/jis479.

Schutsky K, Portocarrero C, Hooper DC, Dietzschold B, Faber M. 2014. Limited brain metabolism changes differentiate between the progression and clearance of rabies virus. PLoS One. 9(4): e87180. doi: 10.1371/journal.pone.0087180.

Seto D. 2010. Viral Genomics and Bioinformatics. Viruses. 2(12): 2587-93. doi: 10.3390/ v2122587.

Shi N, Zhang XY, Dong CY, Hou JL, Zhang ML, Guan ZH, Li ZY, Duan M. 2014. Alterations in microRNA expression profile in rabies virus-infected mouse neurons. Acta Virol. 58(2): 120-7.

Söderholm S, Fu Y, Gaelings L, Belanov S, Yetukuri L, Berlinkov M, Cheltsov AV, Anders S, Aittokallio T, Nyman TA, Matikainen S, Kainov DE. 2016. Multi-Omics Studies towards Novel Modulators of Influenza A Virus-Host Interaction. Viruses. 8(10): pii: E269.

Solovev I, Shaposhnikov M, Moskalev A. 2020. Multi-omics approaches to human biological age estimation. Mech. Ageing. Dev. 185: 111192. doi: 10.1016/j.mad.2019.111192.

Sun X, Shi N, Li Y, Dong C, Zhang M, Guan Z, Duan M. 2016. Quantitative Proteome Profiling of Street Rabies Virus-Infected Mouse Hippocampal Synaptosomes. Curr Microbiol. 73(3): 301-311. doi: 10.1007/s00284-016-1061-5.

Thanomsridetchai N, Singhto N, Tepsumethanon V, Shuangshoti S, Wacharapluesadee S, Sinchaikul S, Chen ST, Hemachudha T, Thongboonkerd V. 2011. Comprehensive proteome analysis of hippocampus, brainstem, and spinal cord from paralytic and furious dogs naturally infected with rabies. J Proteome Res. 10(11): 4911-24. doi: 10. 1021/pr200276u.

Troupin C, Dacheux L, Tanguy M, Sabeta C, Blanc H, Bouchier C, Vignuzzi M, Duchene S, Holmes EC, Bourhy H. 2016. Large-Scale Phylogenomic Analysis Reveals the Complex Evolutionary History of Rabies Virus in Multiple Carnivore Hosts. PLoS Pathog. 12(12): e1006041. doi: 10.1371/journal.ppat.1006041.

Ubol S, Kasisith J, Mitmoonpitak C, Pitidhamabhorn D. 2006. Screening of upregulated genes in suckling mouse central nervous system during the disease stage of rabies virus infection. Microbiol Immunol. 50(12): 51-9.

Ugolini G, Hemachudha T. 2018. Rabies: changing prophylaxis and new insights in pathophysiology. Curr Opin Infect Dis. 31(1): 93-101. doi: 10.1097/QCO.0000000000000420.

Van den Esker MH, Koets AP. 2019. Application of Transcriptomics to Enhance Early Diagnostics of Mycobacterial Infections, with an Emphasis on Mycobacterium avium ssp. paratuberculosis. Vet Sci. 6(3): pii: E59. doi: 10.3390/vetsci6030059.

Vaziri B, Torkashvand F, Eslami N, Fayaz A. 2012. Comparative proteomics analysis of mice lymphocytes in early stages of infection by

different strains of rabies virus. Indian J Virol. 23(3): 311-6. doi: 10.1007/s13337-012-0093-0.

Venugopal, AK, Ghantasala SS, Selvan LD, Mahadevan A, Renuse S, Kumar P, Pawar H, Sahasrabhuddhe NA, Suja MS, Ramachandra YL, Prasad TS, Madhusudhana SN, Harsha HC, Chaerkady R, Satishchandra P, Pandey A, Shankar SK. 2013. Quantitative proteomics for identifying biomarkers for Rabies. Clin Proteomics. 10(1): 3. doi: 10.1186/1559-0275-10-3.

Wang X, Zhang S, Sun C, Yuan ZG, Wu X, Wang D, Ding Z, Hu R. 2011. Proteomic profiles of mouse neuro N2a cells infected with variant virulence of rabies viruses. J Microbiol Biotechnol. 21(4): 366-73.

Wohl S, Schaffner SF, Sabeti PC. 2016. Genomic Analysis of Viral Outbreaks. Annu Rev Virol. 3(1): 173-195.

World Health Organization [Internet]. Rabies. [Updated: May 21st, 2019; Cited July 16th, 2019]. Available from: https://www.who.int/news-room/fact-sheets/detail/rabies.

Wunner WH. 2007. Rabies virus, in: Jackson AC, Wunner WH, Rabies Scientific Basis of the Disease and Its Management. Academic Press: Massachusetts, pp. 23-68.

Yang Y, Liu W, Yan G, Luo Y, Zhao J, Yang X, Mei M, Wu X, Guo X. 2015. iTRAQ protein profile analysis of neuroblastoma (NA) cells infected with the rabies viruses rHep-Flury and Hep-dG. Front Microbiol. 6: 691. doi: 10.3389/fmicb.2015.00691.

Yu F, Zhang G, Zhong X, Han N, Song Y, Zhao L, Cui M, Rayner S, Fu ZF. 2014. Comparison of complete genome sequences of dog rabies viruses isolated from China and Mexico reveals key amino acid changes that may be associated with virus replication and virulence. Arch Virol. 159(7): 1593-601. doi: 10.1007/s00705-013-1966-2.

Zandi F, Eslami N, Soheili M, Fayaz A, Gholami A, Vaziri B. Proteomics analysis of BHK-21 cells infected with a fixed strain of rabies virus. Proteomics. 9(9): 2399-407. doi: 10.1002/pmic.200701007.

Zandi F, Eslami N, Torkashvand F, Fayaz A, Khalaj V, Vaziri B. 2013. Expression changes of cytoskeletal associated proteins in proteomic profiling of neuroblastoma cells infected with different strains of rabies virus. J Med Virol. 85(2): 336-47. doi: 10.1002/ jmv.23458.

Zhang D, He F, Bi S, Guo H, Zhang B, Wu F, Liang J, Yang Y, Tian Q, Ju C, Fan H, Chen J, Guo X, Luo Y. 2016. Genome-Wide Transcriptional Profiling Reveals Two Distinct Outcomes in Central Nervous System Infections of Rabies Virus. Front Microbiol 7: 751. doi: 10.3389/fmicb.2016.00751.

Zhang W, Li F, Nie L. 2010. Integrating multiple 'omics' analysis for microbial biology: application and methodologies. Microbiology. 156(Pt2): 287-301. doi: 10.1099/mic.0. 034793-0.

Zhao H, Punga T, Pettersson U. 2019. Adenovirus in the omics era - a multipronged strategy. FEBS Lett. doi: 10.1002/1873-3468.13710.

Zhao P, Liu S, Zhong Z, Jiang T, Weng R, Xie M, Yang S, Xia X. 2018. Analysis of expression profiles of long noncoding RNAs and mRNAs in brains of mice infected by rabies virus by RNA sequencing. Sci Rep 8(1): 11858. doi: 10.1038/s41598-018-30359-z.

Zhao P, Yang Y, Feng H, Zhao L, Qin J, Zhang T, Wang H, Yang S, Xia X. 2013. Global gene expression changes in BV2 microglial cell line during rabies virus infection. Infect Genet Evol. 20: 257-69. doi: 10.1016/j.meegid.2013.09.016.

Zhao P, Zhao L, Zhang K, Feng H, Wang H, Wang T, Xu T, Feng N, Wang C, Gao Y, Huang G, Qin C, Yang S, Xia X. 2012a. Infection with street strain rabies virus induces modulation of the microRNA profile of the mouse brain. Virol J. 9: 159. doi: 10.1186/ 1743-422X-9-159.

Zhao P, Zhao L, Zhang T, Qi Y, Wang T, Liu K, Wang H, Feng H, Jin H, Qin C, Yang S, Xia X. 2011. Innate immune response gene expression profiles in central nervous system of mice infected with rabies virus. Comp Immunol Microbiol Infect Dis. 34(6): 503-12. doi: 10.1016/j.cimid.2011.09.003.

Zhao P, Zhao L, Zhang T, Wang H, Qin C, Yang S, Xia X. 2012b. Changes in microRNA expression induced by rabies virus infection in mouse brains. Microb Pathog. 52(1): 47-54. doi: 10.1016/j.micpath.2011.10.001.