Investigating Gene Expression Dynamics of ZIKV E Protein Interacting Proteins upon ZIKV Infection

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Abstract

Zika virus (ZIKV), a member of the Flavivirus genus, was announced as a Public Health Emergency of International Concern by the World Health Organization (WHO) in 2016 due to its association with congenital malformations, notably microcephaly. Although the emergency was terminated in the same year, global awareness of ZIKV remained significant. This study aimed to explore the interactions between ZIKV and host cells by investigating the gene expression of protein-encoding genes that interact with the ZIKV E protein.

This study examined the expression levels of genes encoding ZIKV E protein-interacting proteins during ZIKV infection through quantitative real-time PCR analysis. The results revealed the significant upregulation of COP9 signalosome subunit 5 (COPS5), ribosomal L24 domain containing 1 (RSL24D1), serine and arginine rich splicing factor 11 (SRSF11), and spectrin repeat containing nuclear envelope protein 1 (SYNE1) genes. Conversely, ATPase Na+/K+ transporting subunit beta 3 (ATP1B3) and centrosomal protein 192 (CEP192) genes exhibited initial upregulation followed by downregulation post-infection. Gaining insights into the molecular interactions between ZIKV and host cells can shed light on potential therapeutic targets and aid in the understanding of ZIKV pathogenesis, thus contributing to the development of effective intervention strategies.

Keywords*: *Zika virus, ZIKV E, Protein, Gene Expression, Host-virus Interaction

1. Introduction

Zika virus (ZIKV), an enveloped icosahedral virus belonging to the *Flavivirus* genus, was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) in 2016 and has since gained significant attention in the global spotlight due to its connection to congenital malformations, specifically microcephaly (Wikan, & Smith, 2016). The WHO terminated the emergency in November of that year. Subsequently, infection rates decreased significantly over the next five years (Yakob, 2022).

ZIKV and other human pathogens transmitted by mosquitoes, such as dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and yellow fever virus (YFV), belong to the *Flaviviridae* family, having a positive-sense RNA genome of approximately 11,000 bases long and enclosed within a lipid bilayer. They also possess numerous copies of the viral capsid protein and 180 copies of both the envelope (E) and membrane (M) glycoproteins, which have lengths of around 495 and 75 amino acids, respectively. Fully developed ZIKV, DENV, WNV, and YFV viruses have a diameter of about 500 Å, while the immature forms have a diameter of approximately 600 Å (Hasan, Sevvana, Kuhn, & Rossmann, 2018).

Different mosquito species of the *Aedes* genus can carry ZIKV, particularly *Aedes aegypti* (Li et al., 2012) and *Aedes albopictus* (Gerard et al., 2014), which are mostly involved in the transmission of the virus. ZIKV can be transmitted through various means, including vertical transmission, sexual contact, and the transfusion of infected blood. Additionally, there is a possibility of transmission through solid organ

transplantation and even contact with infected body fluids, although these modes have not been definitively confirmed (Masmejan et al., 2020).

Distinct clinical features are observed in ZIKV infection depending on demographics. Prenatal exposure to ZIKV can lead to severe malformations, such as Congenital Zika Syndrome (CZS), which is an often fatal condition characterized by neurological anomalies, microcephaly, and developmental issues in fetuses and infants (Pomar et al., 2019). Even children without symptoms at birth can experience long-term developmental consequences, underscoring the importance of ongoing monitoring. The World Health Organization (WHO) and the Pan American Health Organization (PAHO) have established clinical diagnosis criteria for ZIKV in children. These criteria involve the presence of a rash and other associated symptoms such as fever, arthralgias, conjunctivitis, periarticular edema, and myalgia (Burger-Calderon et al., 2020). However, it is worth noting that children often display less severe symptoms than adults, and severe complications are rare.

On the other hand, most adults infected with ZIKV do not experience any noticeable symptoms, though some may show signs of rash, fever, joint pain, or conjunctivitis. However, neurological complications such as meningoencephalitis and Guillain-Barré syndrome can occur, with Guillain-Barré syndrome causing significant morbidity. Furthermore, infected individuals may continue to carry ZIKV RNA for a long time, while renal pathology, if present, typically remains subclinical in immunocompetent individuals (Masmejan et al., 2020).

During infection, ZIKV (and other flaviviruses) remodel the host cell to generate a cellular environment that favors viral replication and dampens the innate immune system. Flaviviruses induce changes in protein expression, gene expression, and lipid composition. To achieve this extensive remodeling, flaviviruses such as ZIKV have a small complement of 10 proteins, comprising three structural proteins (capsid (C), envelope (E) and precursor of the membrane (prM) and seven structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The structural proteins make up the virion (together with the genomic material, an approximately 11kb positive-sense, single-stranded RNA), while the non-structural proteins form the replication complex. One way in which the viral protein can modulate host cell proteins is through protein-protein interactions. This can result in a target cell protein being targeted for degradation, or its activity being lost. Thus, the identification of interacting proteins can shed significant light on how the virus modulates the cell.

In a study conducted by Khongwichit et al. (2020), the interaction between the ZIKV E protein and host proteins was investigated. The focus was on the ZIKV E protein domain III, responsible for receptor binding. The researchers identified 21 proteins that interacted with this domain through a yeast-2-hybrid screen, including the ER-resident chaperone protein GRP78, where co-immunoprecipitation assays confirmed the interaction between GRP78 and ZIKV E, with immunofluorescence staining showing co-localization between the two proteins. In addition, Poonthavee et al. (2023) analyzed the gene expression of 10 out of the 21 ZIKV E protein interacting protein-encoding genes where microtubule-actin crosslinking factor 1 (MACF1), DnaJ heat shock protein family (Hsp40) member B1 (DNAJB1), C-terminal binding protein 2 (CTBP2), cysteine and histidine-rich domain containing 1 (CHORDC1), RNA polymerase II subunit B (POLR2B), and calcyclin binding protein (CACYBP) genes were significantly upregulated upon infection, while Ankyrin 3 (ANK3), ATPase Na+/K+ transporting subunit beta 1 (ATP1B1), and anillin actin-binding protein (ANLN) genes tended to be down-regulated. These identified protein-encoding genes were part of different cellular pathways.

In continuum, this study aimed to investigate some of the remaining ZIKV E protein interacting protein-encoding genes identified in the yeast-2-hybrid screening, namely ATPase Na+/K+ transporting subunit beta 3 (ATP1B3), centrosomal protein 192 (CEP192), COP9 signalosome subunit 5 (COPS5),

ribosomal L24 domain containing 1 (RSL24D1), serine and arginine rich splicing factor 11 (SRSF11), and spectrin repeat containing nuclear envelope protein 1 (SYNE1) and zinc finger protein 251 (ZNF251), to determine the expression level of these genes, as well as to determine whether they are significantly upregulated or downregulated in ZIKV infection. This could serve to act as a prescreening method to identify which of the genes are the best candidates to perform "in detail" follow-up experiments.

2. Objectives

1) To determine the expression levels of gene encoding for ZIKV E protein-interacting proteins including ATP1B3, CEP192, COPS5, RSL24D1, SRSF11, SYNE1and ZNF251, during ZIKV infection.
2) To identify whether the aforementioned interacting proteins exhibit upregulation or downregulation in gene expression after ZIKV infection.

3. Materials and Methods

3.1 Cells and viruses

Adenocarcinomic human alveolar basal epithelial cells (A549; ATCC CCL-185) were cultured in Dulbecco's modified eagle's medium (DMEM) (Gibco, Invitrogen, Grand Island, NY), which was supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Invitrogen), and incubated at 37°C with 5% CO2.

The ZIKV strain SV0010/15, a kind gift from the Armed Forces Research Institute of Medical Sciences (AFRIMS) and the Department of Disease Control, Ministry of Public Health, Thailand (ZIKV-T, Thai isolate) was originally obtained from a Thai patient sample and was passaged once in *Toxorhynchites splendens* mosquitoes before being passed twice in C6/36 (*Aedes albopictus*) cells. Subsequently, the virus was passed twice more in C6/36 cells, four times more in rhesus monkey kidney epithelial cells (LLC-MK2), and twice more in C6/36 cells. Confirmation of the virus identity was carried out by DNA sequencing; the complete sequence of this strain can be found in GenBank with accession number KX051562 (SV0010/15).

3.2 ZIKV infection of A549 cells

Approximately 80% confluence was reached within 24 hours after seeding A549 cells into a 6-well plate under standard conditions. Following this, the cells were infected with ZIKV in a serum-free medium with a multiplicity of infection (MOI) of 0.5 for 2 hours or left uninfected (mock-infected). The infection was then halted by replacing the virus medium with DMEM containing 10% FBS, after which the cells were incubated at 37°C with 5% CO2 for 24 hours until they were collected for further experiments.

3.3 RNA extraction, semi-quantitative and quantitative RT-PCR analysis

A549 mock-infected or infected cells with ZIKV were harvested at 6-, 12-, 24-, 36-, and 48 hpi, and TRIzol reagent (Life Technologies, Carlsbad, CA) was used to extract total RNA from the cell pellets. The RNA concentrations of the purified PCR product were measured at 260 nm using a NanoDrop 2000 spectrophotometer, and 1 µg of extracted RNA was treated with DNaseI (RNase-free) (Ambion). The cDNA was then synthesized by reverse transcriptase using random hexamers (Thermo Fisher Scientific Inc., Waltham, MA). The reaction mixture comprised 1X reaction buffer, 1 mM dNTPs, 1 unit of RiboLock RNase Inhibitor (Thermo FisherScientific Inc.), and 10 units of RevertAid Reverse Transcriptase (Thermo Fisher Scientific Inc.) The reaction was carried out in a Veriti Cycler (Applied Biosystems, Foster City, CA) with the following cycling conditions: 25°C for 5 minutes, 42°C for 90 minutes, and 70°C for 10 minutes.

3.4 Sequencing of ZIKV

ZIKV strain SV0010/15 RNA was extracted from the supernatant and used as a template to generate cDNA with random hexamers from the above protocol. Conventional PCR was performed to amplify products using primers ZikaFront_1800-1820 5'-CTG AGA TGG ATG GTG CAA AGG-3' and Rev_ZikaFront_3779-3779 5'-TCA TTT CCG CGA AGG TGG CAC-3' with Phusion DNA polymerase. The thermocycling conditions were as follows: 98°C for 30 seconds for initial denaturation, then 30 cycles of denaturation at 98°C for 10 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 60 seconds. Subsequently, a final extension was done for 5 minutes at 72°C. Gel electrophoresis was performed to visualize the PCR product, where 1% agarose was used and stained with ethidium bromide (EtBr). The targeted fragments on the gel were excised and purified using a FavorPrepTM GEL/PCR purification kit, after which the primers were sent to Macrogen Inc. (Seoul, Korea) for verification.

3.5 Detection of mRNA expression levels of genes encoding ZIKV E protein-interacting proteins by real-time PCR

The cDNA was subjected to real-time PCR conducted in Mastercycler Realplex (Eppendorf, Hauppauge, NY) using Eppendorf 96-well plates. The amplification reaction was prepared by adding 2 µL of either diluted virus cDNA or standard control, 1X KAPA SYBR FAST Master Mix (2X) ABI Prism, and 0.2 µl each of forward and reverse primers. Table 1 shows the specific primer sequences. Subsequently, real-time PCR was set for initial denaturation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 20 seconds, and extension at 72°C for 20 seconds. The cycle was followed by a melting curve analysis with temperature steps at 95°C for 15 seconds, 60°C for 15 seconds, and a gradual increase to 95°C by 20 minutes. The relative change in the gene expression levels was determined by the 2-ΔΔCt method, with β-Actin serving as an internal control.

Table 1. Primer sequences for quantitative real-time polymerase chain reaction

Gene name	NCBI Reference sequence	Primer sequence (5' – 3')
ATP1B3	NM_001679.3	Forward ATGACGAAGAACGAGAAGAAG
		Reverse AGCCAGGAACCCATAAAAAAC
CEP192	NM_032142.3	Forward ACCTCAATTTCCACTCCATC
		Reverse TGCTTCTCACTGTTGCTTAT
COPS5	NM_006837.2	Forward GGGTGAATACGTTGAGTTCT
		Reverse CTTCTGATTTTCGGTCATGC
RSL24D1	NM_016304.2	Forward GATGCGATGAAGAGAGTTGA
		Reverse CATCCTCTTGTAACTGCTGT
SRSF11	NM_004768.3	Forward ACAGTCCCTAATTTCTGCTG
		Reverse GCCTGTGTCTAGAAGATGAG
SYNE1	NM_182961.3	Forward CCCAATCTTCCCTGAAATCA
		Reverse AAGGCAGTTTATCCATCTGG
ZNF251	NM_138367.1	Forward GAGAAAGAACCCAAGAGTGT
		Reverse GTGACTTCTCTGGTGTCTAC
β-Actin	NM_001101.5	Forward GAAGATGACCCAGATCATGT
		Reverse ATCTCTTGCTCGAAGTCCAG

4. Results and Discussion

Quantitative real-time PCR analysis showed that COPS5, RSL24D1, SRSF11, and SYNE1 mRNA were significantly increased by ZIKV infection at the late time points, specifically at 36 and 48 hours post-infection (hpi), compared to the mock-infected control (Fig. 1C, 1D, 1E, & 1F). Meanwhile, the expression levels of ATP1B3 and CEP192 (Fig. 1A & 1B) genes increased significantly during the first 36 hours post-

infection (hpi) and then decreased significantly at 48 hpi. As for the ZNF251 gene, it showed the same pattern as the rest of the genes during 36 hpi with increased expression. However, there was no significant difference in the 48 hpi.

Certain genes, specifically COPS5, RSL24D1, SRSF11, and SYNE1, were observed to be significantly upregulated upon ZIKV infection. Very little is known about the involvement of these genes in ZIKV infection (or indeed any flavivirus infection). However, three of these four genes are associated with different types of cancers. COP9 signalosome subunit 5 (COPS5), also called JAB1, is an important component of the COP9 signalosome (CSN). COPS5 is a conserved multi-protein complex essential for regulating the cell cycle, DNA damage response, and apoptosis in eukaryotes. The primary role of COPS5 is to remove ubiquitin-like modifier NEDD8 from cullin-RING ligases (CRLs) that help break down specific proteins. The overexpression of this gene has been linked to various human cancer entities, including hepatocellular carcinoma (HCC), colon adenocarcinoma, and breast cancer (Jumpertz et al., 2017). ZIKV infection results in apoptosis, which may explain the involvement of this gene in ZIKV infection. Another gene linked to HCC is the spectrin repeat containing nuclear envelope protein 1 (SYNE1), which encodes for the nuclear envelope protein nesprin-1, essential in connecting the nucleus to the cytoskeleton. Mutation of this gene has primarily been associated with cerebellar ataxia. In addition, recent studies showed that changes in its expression levels, single nucleotide polymorphisms, and mutations are related to the occurrence and development of various forms of cancer, such as lung cancer, gastric cancer, and oral cancer (Li et al., 2020). In a recent study by Harbin, Lin, Ueland, & Kolesar (2023), SYNE1 mutation also correlated with an increase in immune cell infiltration in ovarian cancer. The involvement of this gene in ZIKV infection may be a consequence of the extensive cytoskeletal rearrangements that occur in ZIKV infection. Meanwhile, serine and arginine-rich splicing factor 11 (SRSF11) is a serine/arginine-rich protein family member involved in alternative splicing (AS). Pan et al. (2022) found that the overexpression of this gene was associated with a poor prognosis in colorectal cancer (CRC) and had a pro-metastatic effect by inhibiting the alternative splicing of HSPA12A pre-RNA. The involvement of this gene in ZIKV infection is somewhat ambiguous as the ZIKV genome does not undergo alternative splicing, but flaviviruses are known to co-opt many RNA processing factors.

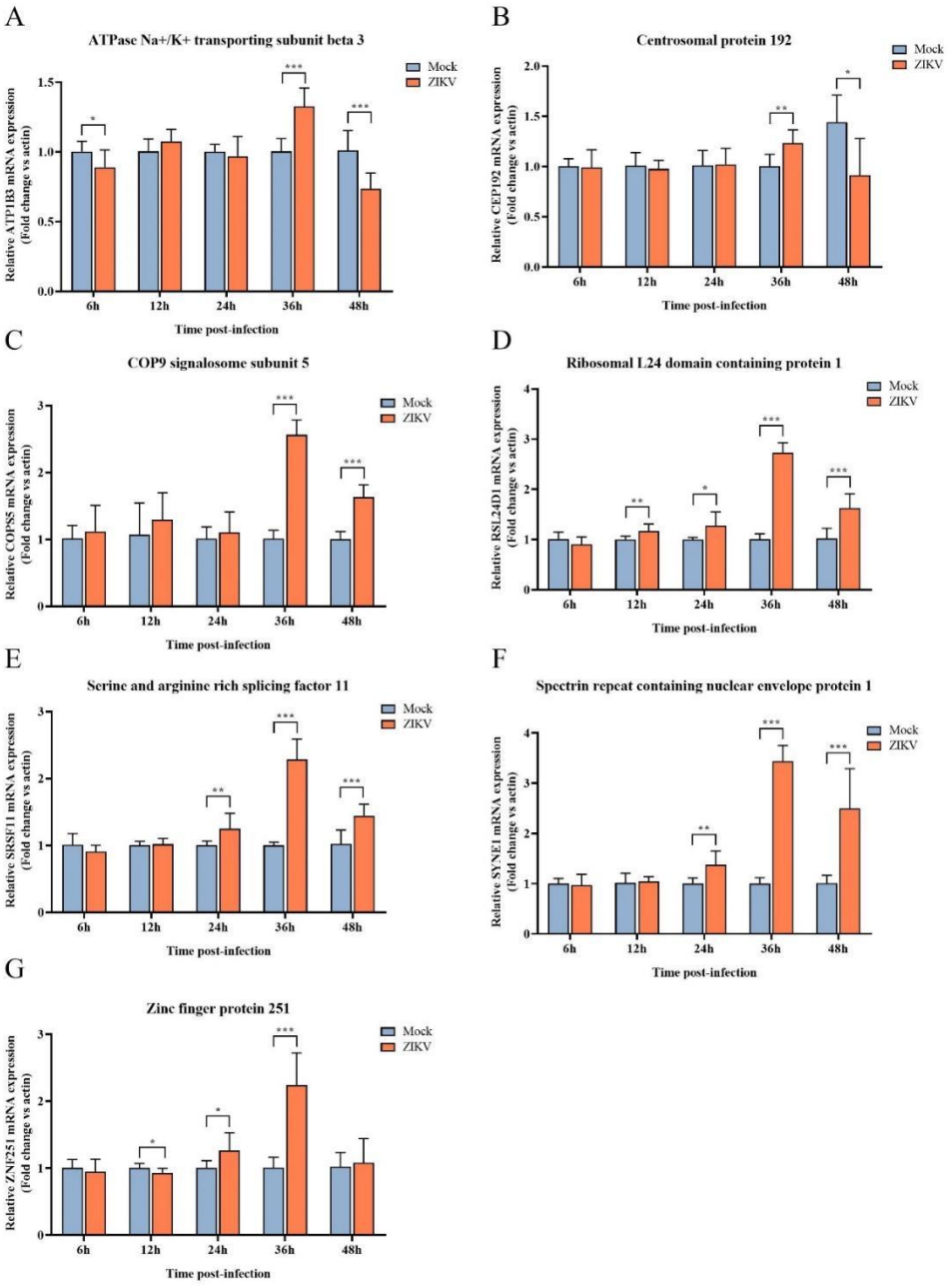

Figure 1. Gene expression of ZIKV E protein-interacting proteins during ZIKV infection

Figure 1 shows the expression levels of genes encoding for ZIKV E protein-interacting proteins during ZIKV infection. A549 cells were mock infected or infected with ZIKV-T at MOI 0.5 and then analyzed for expression levels of different genes by quantitative real-time PCR. The relative change in the expression levels of genes was determined by the 2-ΔΔCt method, with β-Actin used as an internal control. (A) ATP1B3, (B) CEP192, (C) COPS5, (D) RSL24D1, (E) SRSF11, (F) SYNE1, and (J) ZNF251 (* p value; *p<0.05, **p<0.01, ***p<0.001).

Conversely, other genes have been implicated in various functions. Ribosomal L24 domain containing 1 (RSL24D1), a gene similar to yeast Rlp24, is expressed at high levels in both human and mouse

pluripotent stem cells. Depletion of RSL24D1 alters embryonic stem cell (ESC) self-renewal and lineage commitment choices, which can affect ESC homeostasis (Durand et al., 2023). Zinc finger protein 251 (ZNF251) is a C2H2 ZFP family member with a Krüppel-associated box domain predicted to be a transcriptional repressor. Kim et al. (2023) examined glucose intolerance in mice due to ZNF251 deficiency, which impacted the expression of genes linked with adipocyte differentiation according to their conducted gene ontology (GO) analysis. The involvement of these genes in ZIKV infection remains unclear, though ZIKV is believed to be modulated by glucose levels.

ATP1B3 and CEP192 genes were found to be downregulated at 48 hours post-infection. It is noteworthy to correlate the similar pattern with the expression levels of ATPase Na+/K+ transporting subunit beta 3 (ATP1B3) and the result of ATPase Na+/K+ transporting subunit beta 1 (ATP1B1) in the study of Poonthavee et al. (2023), where both genes were upregulated in 36 hpi and significantly downregulated at 48 hpi. These two genes encode different subunits of the same protein, sodium/potassium-transporting ATPase subunit beta (Na+/K+-ATPase β subunit). Na+/K+ -ATPase plays an important role in maintaining the electrochemical gradients of Na and K ions across the plasma membrane, which are essential for osmoregulation and nerve excitability. ATP1B3 was the only gene analyzed in this study found to be implicated in other viral infections, where its overexpression significantly prevented Enterovirus 71 (EV71) replication in rhabdomyosarcoma (RD) cells through Type-I interferon production (Lu et al., 2016). The identification of two subunits of this protein suggests that this protein is a critical mediator of ZIKV infection. Centrosomal protein 192 (CEP192) located in the centrosome, centriole, and cytosol, functions to allow phosphatase binding activity for the mitotic spindle assembly. CEP192 is predicted to have an oncogenic role in HCC since it is highly expressed in the fetal liver but not in normal adult liver tissue (Liu et al., 2022). Again, the reason for the involvement of this protein in ZIKV infection remains unclear.

In terms of limitations, it should be noted that this study was undertaken in A549 (human lung alveolar cells), which is not a normal target cell for ZIKV infection. As such, further investigation of the role of these genes in ZIKV infection may be suitable by being undertaken in a more representative target cell line.

Overall, it is significant that all of these proteins identified through interaction with ZIKV E protein are transcriptionally regulated. These results suggest that these proteins are either a host cell response to combat or limit ZIKV or are possibly pro-viral replication factors. However, the results of this study suggest that these proteins are worth investigating more fully using other techniques.

5. Conclusion

ZIKV remains a significant public health concern due to its potential to cause congenital malformations and neurological complications. While the World Health Organization (WHO) terminated its emergency status in 2016, ongoing monitoring and research are crucial. ZIKV, along with other mosquito-transmitted pathogens, poses a threat to global health, highlighting the importance of understanding its transmission dynamics and clinical manifestations. Recent studies have shed light on the molecular interactions between ZIKV and host cells, particularly on the ZIKV E protein and its interaction with other protein-encoding genes. The investigation of genes encoding these interacting proteins during ZIKV infection revealed the significant upregulation of certain genes associated with various cancers, implicating potential pathways for further exploration.

This study likewise identified patterns of gene expression changes over time post-infection, providing insights into the dynamics of ZIKV-host interactions. Notably, genes encoding subunits of the sodium/potassium-transporting ATPase exhibited differential expression, suggesting their involvement in ZIKV infection and potential implications for therapeutic interventions. Overall, continued research into ZIKV pathogenesis, transmission, and host interactions is crucial for developing efficient preventive and therapeutic strategies. Understanding the molecular mechanisms underlying ZIKV infection can better mitigate its impact on global health and prevent future outbreaks.

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7. References

Burger-Calderon, R., Carrillo, F. B., Gresh, L., Ojeda, S., Sanchez, N., Plazaola, M., ... & Harris, E. (2020). Age-dependent manifestations and case definitions of pediatric Zika: a prospective cohort study. *The Lancet. Infectious diseases*, *20*(3), 371–380. https://doi.org/10.1016/S1473-3099(19)30547-X

Durand, S., Bruelle, M., Bourdelais, F., Bennychen, B., Blin-Gonthier, J., Isaac, C., ... & Gabut, M. (2023). RSL24D1 sustains steady-state ribosome biogenesis and pluripotency translational programs in embryonic stem cells. *Nature Communications*, *14*(1), Article 356. https://doi.org/10.1038/s41467-023-36037-7

Grard, G., Caron, M., Mombo, I. M., Nkoghe, D., Mboui Ondo, S., Jiolle, D., ... & Leroy, E. M. (2014). Zika virus in Gabon (Central Africa)–2007: a new threat from Aedes albopictus?. *PLoS Neglected Tropical Diseases*, *8*(2), Article e2681. https://doi.org/10.1371/journal.pntd.0002681

Harbin, L. M., Lin, N., Ueland, F. R., & Kolesar, J. M. (2023). SYNE1 Mutation Is Associated with Increased Tumor Mutation Burden and Immune Cell Infiltration in Ovarian Cancer. *International Journal of Molecular Sciences*, 24(18), 14212. https://doi.org/10.3390/ijms241814212

Hasan, S. S., Sevvana, M., Kuhn, R. J., & Rossmann, M. G. (2018). Structural biology of Zika virus and other flaviviruses. *Nature structural & molecular biology*, *25*(1), 13-20. https://doi.org/10.1038/s41594-017-0010-8

Jumpertz, S., Hennes, T., Asare, Y., Schütz, A. K., & Bernhagen, J. (2017). CSN5/JAB1 suppresses the WNT inhibitor DKK1 in colorectal cancer cells. *Cellular Signalling*, *34*, 38-46. https://doi.org/10.1016/j.cellsig.2017.02.013

Khongwichit, S., Sornjai, W., Jitobaom, K., Greenwood, M., Greenwood, M. P., Hitakarun, A., ... & Smith, D. R. (2021). A functional interaction between GRP78 and Zika virus E protein. *Scientific Reports*, *11*(1), Article 393. https://doi.org/10.1038/s41598-020-79803-z

Kim, E., Ham, S. A., Hwang, J. S., Won, J. P., Lee, H. G., Hur, J., & Seo, H. G. (2023). Zinc finger protein 251 deficiency impairs glucose metabolism by inducing adipocyte hypertrophy. *Molecular and Cellular Endocrinology*, *562*, Article 111838. https://doi.org/10.1016/j.mce.2022.111838

Li, M. I., Wong, P. S. J., Ng, L. C., & Tan, C. H. (2012). Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (linnaeus) to Zika virus, 6(8), Article e1792. https://doi.org/10.1371/journal.pntd.0001792

Li, P., Xiao, J., Zhou, B., Wei, J., Luo, J., & Chen, W. (2020). SYNE1 mutation may enhance the response to immune checkpoint blockade therapy in clear cell renal cell carcinoma patients. *Aging (Albany NY)*, *12*(19), Article 19316. https://doi.org/10.18632/aging.103781

Liu, Y., Liang, W., Chang, Y., He, Z., Huang, Q., He, M., & Peng, K. (2022). CEP192 is a novel prognostic marker and correlates with the immune microenvironment in hepatocellular carcinoma. *Frontiers in Immunology*, *13*, Article 950884. https://doi.org/10.3389/fimmu.2022.950884

Lu, Y., Hou, H., Wang, F., Qiao, L., Wang, X., Yu, J., ... & Sun, Z. (2016). ATP1B3: a virus-induced host factor against EV71 replication by up-regulating the production of type-I interferons. *Virology*, *496*, 28-34. https://doi.org/10.1016/j.virol.2016.05.013

Masmejan, S., Musso, D., Vouga, M., Pomar, L., Dashraath, P., Stojanov, M., ... & Baud, D. (2020). Zika virus. *Pathogens*, *9*(11), Article 898. https://doi.org/10.3390/pathogens9110898

Pan, Y. J., Huo, F. C., Kang, M. J., Liu, B. W., Wu, M. D., & Pei, D. S. (2022). Alternative splicing of HSPA12A pre-RNA by SRSF11 contributes to the metastasis potential of colorectal cancer. *Clinical and Translational Medicine*, *12*(11), Article e1113. https://doi.org/10.1002/ctm2.1113

Pomar, L., Musso, D., Malinger, G., Vouga, M., Panchaud, A., & Baud, D. (2019). Zika virus during pregnancy: From maternal exposure to congenital Zika virus syndrome. *Prenatal diagnosis*, *39*(6), 420-430. https://doi.org/10.1002/pd.5446

Poonthavee, S., Sornprasert, S., Ramphan, S., Sornjai, W., & Smith, D. R. (2023). Analyzing the expression levels of Zika E protein interacting protein-encoding genes upon Zika infection. *Proceeding in the*

30th FAOBMB & 8th BMB Conference 2023, pp. 385 - 389. Bangkok. Retrieved from https://www.bmbconference.org/faobmb2023/wpcontent/uploads/2023/12/FAOBMB_2023_Abstract-Book.pdf

Wikan, N., & Smith, D. R. (2016). Zika virus: history of a newly emerging arbovirus. *The Lancet Infectious Diseases*, *16*(7), e119-e126. https://doi.org/10.1016/S1473-3099(16)30010-X

Yakob, L. (2022). Zika virus after the public health emergency of international concern period, Brazil. *Emerging infectious diseases*, *28*(4), 837-840. https://doi.org/10.3201/eid2804.211949