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Molecular analyses and Sindbis virus pathogenesis in human neuroblastoma cell line

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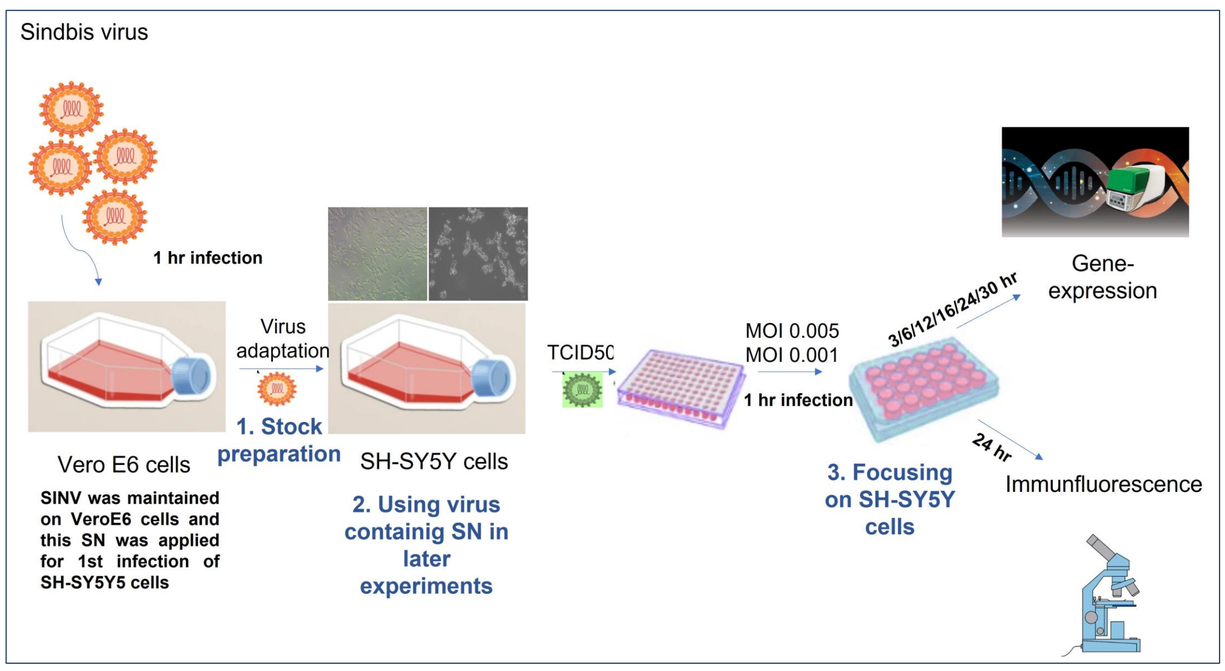
INTRODUCTION & AIM

The Sindbis virus (SINV) is an arbovirus with a positive-sense ssRNA genome. Sindbis virus (SINV) circulates in an enzootic cycle between its vectors mosquitoes (primarily *Aedes*/*Culex* spp.) and birds, however, can infect various animals. In humans, SINV triggers musculoskeletal syndromes characterized by fever, rash, acute and chronic polyarthritis *etc*., but relatively few studies are available about the virus's impact on the nervous and immune systems. It is imperative to comprehend the response of brain cells, including neurons and glial cells, to SINV infection, given its propensity to target the central nervous system. The SH-SY5Y cell line, which originates from neuroblastoma, is a widely employed subject in neurobiological and virological studies. These cells facilitate the examination of the SINV's effects on the level of viral replication, gene expression, and cell damage/apoptosis caused by the virus. ***Therefore, we aimed to study the mechanism of viral infection in vitro and examine early immune markers during SINV infection.***

METHOD



In view of the insufficient knowledge of the virus, all experimental designs had to be optimized. Following the preparation of virus stock on SH-SY5Y cells, the median tissue culture infectious dose (TCID50) was utilized to determine the multiplicity of infection (MOI).

1. MOI 0.005 and MOI 0.001 were applied during the majority of experiments in which only early signs of infection were visible. Inspection of the cells was conducted on a continuous basis using an inverted light microscope, with the objective of distinguishing the virus-induced cytopathogenic effect.
2. With regard to the viral aspect, a Taqman probe-based RT-qPCR was developed for the detection of SINV nucleic acid (Fig. 3.). Furthermore, immunofluorescence-based dsRNA antibody staining was utilized to reinforce virus replication (Fig. 2).
3. Monitoring of signs of apoptosis (Caspase-3/Bid-positivity using immunofluorescence) was conducted through microscopic observation (Fig. 4.).
4. In order to monitor the expression of a number of immune-related genes (*pattern recognition receptors* (*PRRs*; *TLR3/7*, *RIG1/MDA5*), a *regulator gene* (*β-catenin*), and *cytokines* (*IL-1b*, *IL-6*, *IL-10*, *TNFα*, *IFNβ*) at various times (3, 6, 12, 16, 24, and 30 hours), qPCR was also performed (Fig. 5).

RESULTS & DISCUSSION

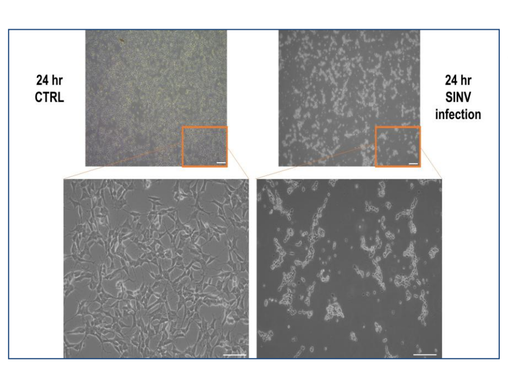


Figure 1. Representative light microscopic images of SH-SY5Y cells followed by 24 hr SINV infection (MOI 1). Left: CTRL cells, scale bars: 100 and 50 µm. Right: SINV infected cells, scale bars: 100 and 50 µm.

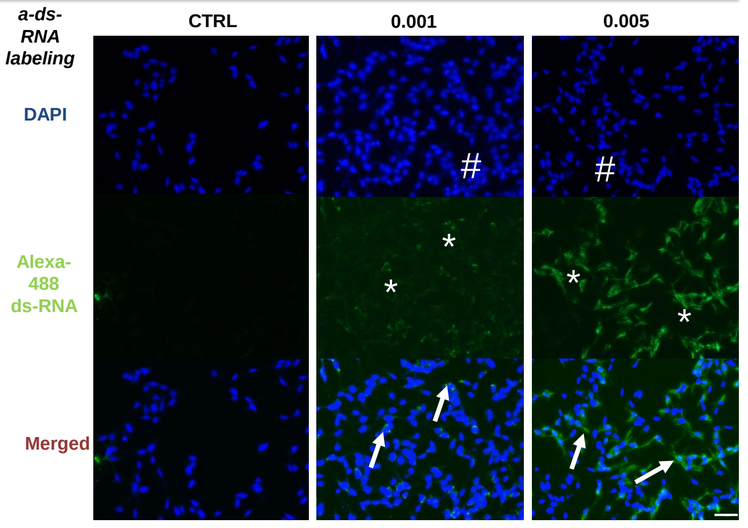


Figure 2. Fluorescence detection of ds-RNA positive cells (the sign of virus replication) in CTRL SH-SY5Y cells, and MOI 0.001 or MOI 0.005 SINV infected SH-SY5Y cells after 24 hr. *Number signs:* cell nuclei (blue), *asterisks*: ds-RNA positive cells (green), *arrows*: merged ds-RNA positive cells. Scale bars: 50 µm.

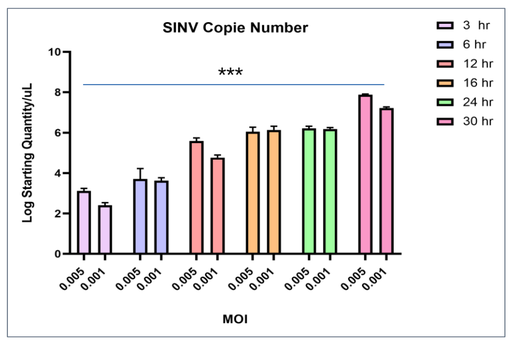


Figure 3. Detection of *SINV* nucleic acid in time-dependent manner. Taqman probe-based specific RT-qPCR was design. MOI 0.005 and 0.001 viral quantities were used for SH-SY5Y cells infection for 3 hr to 30 hr. Log_{10} viral quantities are presented (n=3±SEM, **p*<0.05, ** *p*<0.01, *** *p*<0.001)

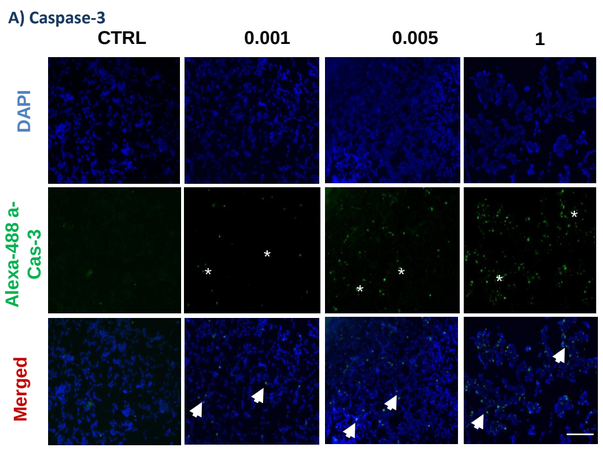


Figure 4. Fluorescence detection of apoptotic markers and localization in CTRL SH-SY5Y cells, and MOI 0.001 or MOI 0.005 or MOI 1 SINV infected SH-SY5Y cells after 24 hr. A) Caspase-3 and B) Bid-positive cells were labeled upon SINV infection. *Number signs*: cell nuclei (blue), *asterisks*: Caspase-3 (upper) or Bid positive cells (lower) (green), *arrows*: merged Caspase-3 positive cells (upper) or merged Bid-positive cells (lower). Scale bars: 100 (A) or 50 µm (B).

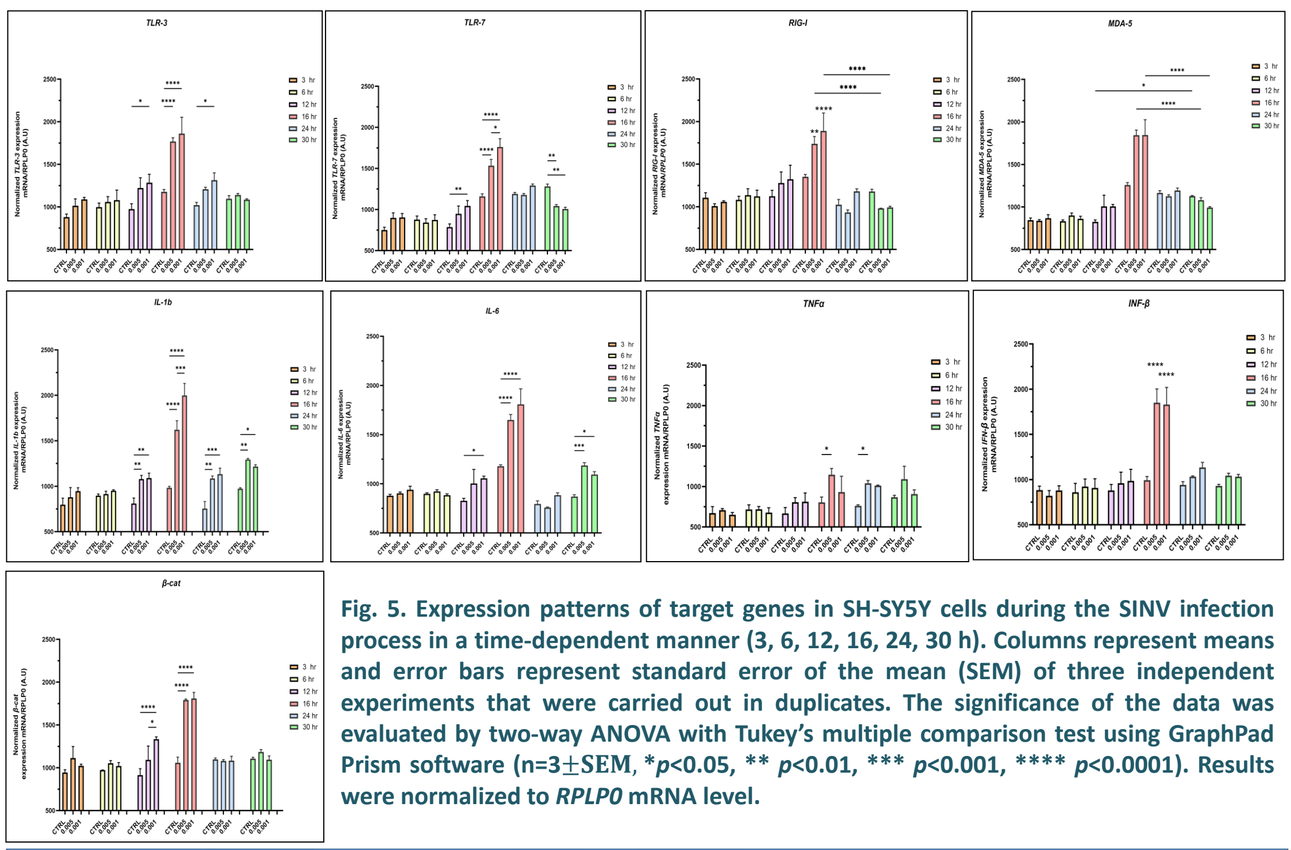


Fig. 5. Expression patterns of target genes in SH-SY5Y cells during the SINV infection process in a time-dependent manner (3, 6, 12, 16, 24, 30 h). Columns represent means and error bars represent standard error of the mean (SEM) of three independent experiments that were carried out in duplicates. The significance of the data was evaluated by two-way ANOVA with Tukey's multiple comparison test using GraphPad Prism software (n=3±SEM, **p*<0.05, ** *p*<0.01, *** *p*<0.001, **** *p*<0.0001). Results were normalized to *RPLP0* mRNA level.

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CONCLUSION

This study on SH-SY5Y cells infected with SINV revealed important findings in SINV-immunology:

1.The TCID50 assay showed significant morphological differences and virus replication in SINV-infected cells, activating the apoptotic pathway and suggesting possible in vivo tissue damage and encephalitis.

2.PRRs, especially *TLR 3/7*, exhibited early mRNA activation, increasing by 12/16 hr, confirming SINV presence and initiating interferon-mediated responses.

3.Inflammatory cytokines *(IL-1β*, *IL-6*, *TNF-α*) elevated significantly at 12/16 hr but decreased by 30 hr (except *TNF-α*), contributing to both infection control and potential tissue damage.

4.Molecules like *IL-10* regulate inflammation, while raised *β-catenin* indicates activated immune processes.