Efficient Replication of West Nile Virus in Human Induced Pluripotent Stem Cells

Marta Trevisan (1), Alessandro Sinigaglia (1), Monia Pacenti (2), Giorgio Palù (1,2), Luisa Barzon (1,2)
1. Department of Molecular Medicine, University of Padova, Padova, Italy
2. Microbiology and Virology Unit, Padova University Hospital, Padova, Italy

Background

West Nile virus (WNV) is a mosquito-borne zoonotic virus that can incidentally infect humans. Most humans infected with WNV remain asymptomatic and only approximately 20–40% develop symptoms, which range from a mild flu-like syndrome to severe neuroinvasive disease in less than 1% of cases.

Studies using animal models, mainly on mice, have provided insights into WNV pathogenesis. However, the mouse model has important biological limitations (besides the ethical issue related to the experimental use of animals) and thus alternative infection models are highly desirable. Human induced pluripotent stem cells (iPSCs), i.e., undifferentiated pluripotent cells reprogrammed from adult somatic cells by a set of transcription factors, have been recently used to generate *in vitro* models of viral infection of human cells. By exploiting the iPSC technology, patient-specific iPSCs can be generated and differentiated into a variety of cells and tissues, which might be used as models to investigate patient's susceptibility to viral infectious diseases.

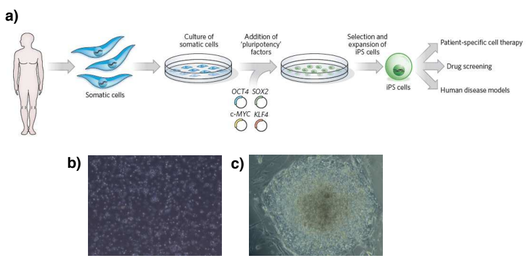


Fig. 1. a) Strategy for the generation of patient-specific iPSCs for disease modeling. **b)** PBMCs obtained from a patient with WNV infection and **c)** a clone of iPSCs that were derived from these PBMCs.

Objective

To setup *in vitro* models of WNV infection based on cells derived from human iPSCs, aim of this study was to evaluate the permissivity of iPSCs to WNV infection and if viral infection could change their pluripotency features.

Methods

Human iPSC clones were derived from human BJ fibroblasts by episomal vector nucleofection and their stemness features were confirmed by alkaline phosphatase assay, pluripotency gene expression analysis, and embryo body test. BJ fibroblasts and Vero E6 cells were used as controls.

Clones of human iPSC were infected by the laboratory-adapted WNV Eg101 strain at MOI 0.1 and 0.01 pfu/cell. Viral replication kinetics was analyzed by quantifying the viral load using plaque assay and qRT-PCR on supernatants and cell lysates collected from WNV-infected and control mock infected iPSCs in a time-course experiment.

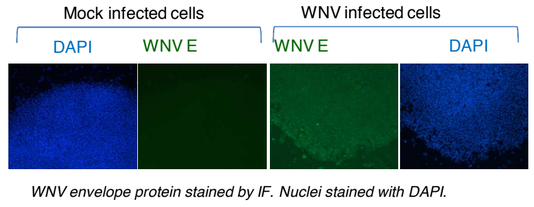
To test the permissiveness of human iPSC to clinical isolates of WNV, cells were also infected with the Ita09 WNV lineage 1 strain and with the Italy/Rovigo/2013 WNV lineage 2 strain.

Pluripotency features in infected cells were evaluated by analysis of pluripotency marker gene expression by immuofluorescence and qRT-PCR.

Cellular innate immune response to WNV infection was evaluated by qRT-PCR analysis of IFN type I expression.

Results

Viral titration in time course experiments demonstrated that WNV could infect and replicate very efficiently in iPSCs, as demonstrated by a progressive increase of the viral load in infected cells, up to 10^9 pfu/mL at 96 hours p.i., with viral loads similar to those achieved in Vero E6 cells (i.e., the cells that are used for WNV isolation and growth). At variance, WNV replication in parental human BJ fibroblast cells was less efficient, as shown in Fig. 2 and 3.



WNV envelope protein stained by IF. Nuclei stained with DAPI.

Fig. 2. Immunofluorescence analysis of WNV E protein expression in iPSCs infected with WNV lineage 1 Eg101 and in mock infected controls.

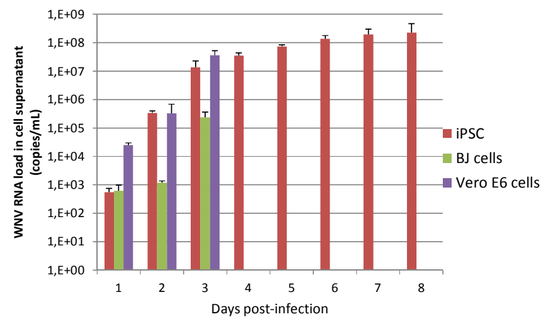


Fig. 3. Replication kinetics of a clinical isolate of WNV lineage 2 in human iPSCs, in parental fibroblast BJ cells, and in control Vero E6 cells. WNV RNA load was measured by qRT-PCR.

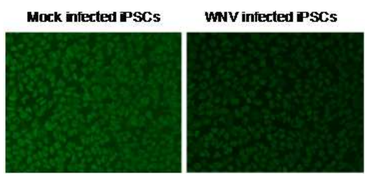


Fig. 4. IF analysis of Oct4 expression in human iPSC infected with WNV lineage 2 (MOI 0.1 at 72 h p.i.) or mock infected.

During the first 72-96 h p.i., microscopy examination showed that WNV infection induced partial differentiation of iPSCs, as indicated also by decreased expression of some pluripotency markers (e.g., Oct4 and Nanog) (Fig. 4).

Notwithstanding efficient viral replication, CPE and cell death occurred only at late time points p.i. (7-8 day p.i with MOI 0.1).

To test the mechanisms of iPSC permissiveness to WNV infection, expression of type I IFNα and IFNβ was measured by qRT-PCR in infected cells. This analysis demonstrated low expression of IFN type I in iPSCs and weak induction after WNV infection, while parental BJ cells expressed higher IFN levels, which markedly increased as a response to WNV infection (Fig. 5).

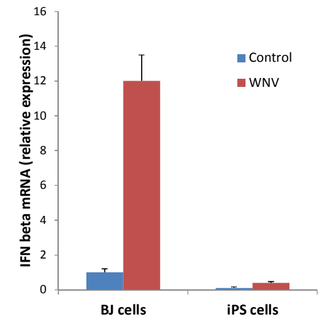


Fig. 5. qRT-PCR analysis of IFNβ expression in human iPSC and in parental BJ fibroblasts infected with WNV lineage 2 (MOI 0.1 at 72 h pi).

Conclusion

Human iPSCs are permissive to WNV replication probably as a consequence of defective IFN type I response and efficient control of host RNAi by a virus that has adapted to alternate life cycles between mosquitoes and vertebrates. Besides the relevance for WNV pathogenesis, these results are preliminary to further studies on the setup of *in vitro* patients-specific iPSC-derived models of WNV disease.

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DMM Dipartimento di Medicina Molecolare Università di Padova