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Paper Poster Session III

Emerging viruses

Efficient replication of West Nile virus in human induced pluripotent stem cells

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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% 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.

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. After characterization, clones of human iPSCs were infected with the WNV Eg101 strain at MOI 0.1 and 0.01 pfu. Viral replication kinetics was analyzed by quantifying the viral load using plaque assay and qRT-PCR on supernatants collected from WNV-infected and control mock-infected iPSCs in a time-course experiment. Pluripotency features in infected cells were evaluated by analysis of pluripotency marker gene expression.

Results: Viral titration 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^{11} pfu/mL at 96 hours 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), but it did not cause typical CPE.

Conclusion: Human induced pluripotent stem cells were demonstrated to be permissive to WNV replication and could be used to culture WNV. WNV infection did not cause typical CPE in iPSCs, but induced a partial cellular differentiation. Besides the relevance for WNV pathogenesis, these results are preliminary to further studies on the setup of *in vitro* models of WNV infection based on iPSCs-derived human cells.