Using Human Induced Pluripotent Stem Cells To Investigate Neurodevelopmental Effects Of Human Cytomegalovirus

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1. INTRODUCTION: Neuronal Stem/Progenitor (NS/P) cells derived from human induced pluripotent stem cells (iPSC) provide an unprecedented opportunity to study human brain development and model neurodegenerative and neuro- developmental diseases.

We have initiated neurodevelopmental studies using human cytomegalovirus (HCMV) as a tool to perturb normal neural differentiation. HCMV infects neural stem cells and neuroprogenitor cells located in ventricular and sub-vetricular zone. It is a major cause of prenatal encephalitis and mental retardation. HCMV has been investigated using neurospheres prepared using forebrain tissues from fetal abortuses. These models provide important information, but have obvious limitations. Hence we have investigated CMV effects on human iPSC-derived NS/P cells.

2. METHODS: We generated iPSCs from adult human fibroblasts. iPSCs were differentiated into neurospheres that were expanded as monolayer cultures of NS/Ps. The neurospheres were further differentiated into functional neurons. NS/P cells and neurons were infected with human cytomegalovirus (HCMV) at multiplicity of infection (MOI=3).

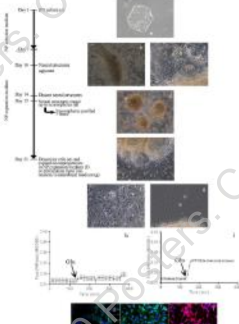

FIGURE 1: NEURONAL DIFFERENTIATION FROM IPSCS GENERATED FROM FIBROBLASTS. Left: Schematic diagram of differentiation of iPS cells into neurospheres. Right: (a) A typical morphology of the iPS colony cultured for 7 days on matrigel with mTeSR1 medium. (b-c) Neural tube-like structures. (d) Neurospheres formed 1 day after culturing in suspension dissected neural tube-like structures. (e) Neural rosettes forming after replating neurospheres on matrigel-coated plates. (f) Neuroprogenitors. (g) Neurons. (h, i) Measurements of calcium influx in neuroprogenitors (h) and neurons (i) induced by administration of 10μm glutamate [Glu]. The significant increase ofglutamate-mediated Ca^{2+} influx indicates these iPS-derived neurons are functional. (j-l) Staining of iPS-derived neurons with β-tubulin III (Tuj1), Tyroxine hydroxylase (TH), and NR4A2, respectively.

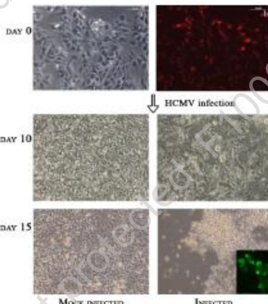

FIGURE 2: CYTOPATHIC EFFECT (CPE) IN PROGENITORS CULTURE INDUCED BY HCMV AT DAY 8 AFTER INFECTION. (a) microphotograph of neuroprogenitors before infection. (b) The percentage of progenitors in the culture approaches 90% as showed by staining for nestin. (c, e) Mock infected progenitors. (d, f) CPE in monolayer culture of progenitors. Staining for HCMV early immediate gene expression [green] in inset (f).

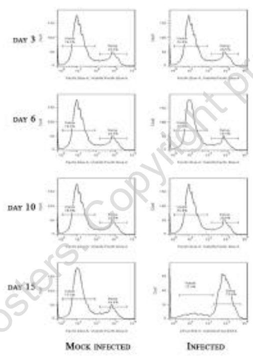

FIGURE 3: FACS ANALYSIS OF PROGENITOR VIABILITY AFTER HCMV INFECTION, GATED ON THE NESTIN-POSITIVE AND B- TUBULIN-III-POSITIVE CELLS.

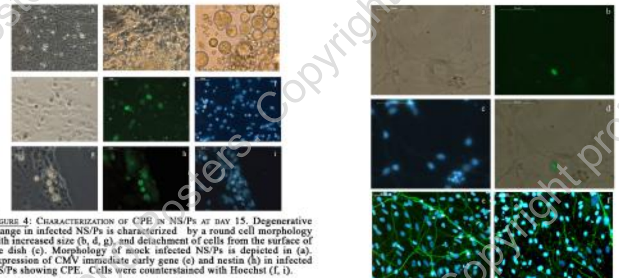

FIGURE 4: CHARACTERIZATION OF CPE IN NS/PS AT DAY 15. Degenerative change in infected NS/Ps is characterized by a round cell morphology with increased size (b, d, g), and detachment of cells from the surface of the dish (c). Morphology of mock infected NS/Ps is depicted in (a). Expression of CMV immediate early gene (e) and nestin (h) in infected NS/Ps showing CPE. Cells were counterstained with Hoechst (f, i).

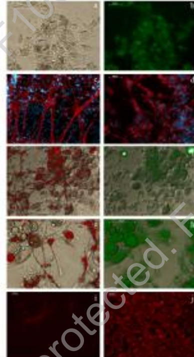

Figure 5: HCMV infection of neural rosettes prevents neuronal differentiation. To investigate the effect of HCMV on neural stem cells, neural rosettes were infected with HCMV at an MOI of 3. At day 10 p.i. most of the cells in the infected cultures displayed the classic signs of CPE (a). Immunostaining for pp65, a major component of the viral tegument, showed the presence of the protein in the nucleus in the very large majority of the infected cells (b). Immunostaining for Tuj1 showed a significant difference in neuronal density between mock-infected (c) and infected (d) cultures. Weak to moderate staining for pp65 was detected only in few neurons differentiated from infected neural rosettes, and only few of them developed CPE. The merge of the staining for TuJ1 (red) and pp65 (green) emphasizes the expression of β-tubulin III and the viral antigen in neurons differentiating from infected neural rosettes (e-h). A conspicuous fraction of the infected cells displayed a strong staining for Tuj1 (j), whilst in mock infected cultures, only cells with neuronal morphology were stained with Tuj1 (i).

FIGURE 6: MATURE NEURONS EXPOSED TO HCMV. Neuron-enriched culture were infected with HCMV at MOI of 3. Expression of HCMV proteins was detected in some neurons at day 3 post-infection. (a) Bright field. (b) Staining for early and late HCMV antigens. (c) Nuclei counterstained with Hoechst. (d) Overlay of HCMV antigen and bright field. At day 15 post-infection, most neurons showed degeneration as highlighted by immunostaining for Tuj1 (f), while mock infected neurons maintained their integrity (e).

3. RESULTS: Cytopathic effects of HCMV were observed on the 10th day post infection in neuroprogenitor cells. HCMV infection of neural rosettes did affect neural differentiation. In infected neuroprogenitor cells, cytopathic effects were observed at day 10 post-infection. Earlier, the adherence of these cells to the underlying matrix was reduced. Neurons were much more refractory to infection. Reduced cell density and neuron degeneration was only observed at day 15 post-infection.

4. CONCLUSIONS: Human iPS cells can efficiently generate neurospheres, which can be expanded as monolayer cultures of NS/P cells or differentiated into neurons. IPSC-derived NS/P cells and neurons offer powerful cellular models to investigate the effects of neurotropic viral agents on human neurodevelopment.

5. REFERENCES

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