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Letter to the Editor

Monkeypox virus productively infects human induced pluripotent stem cell-derived astrocytes and neural progenitor cells

Dear Editor,

Shortly after the outbreak of monkeypox virus (MPXV) in multiple countries in Europe and North America, Orviz and colleagues described confirmed MPX cases in Madrid, Spain, in a recent study published in this journal.[1] Aside from typical symptoms such as fever, lymph node enlargement, asthenia, and skin lesions, patients developed neurological signs including headaches and myalgia.[1] In addition, some groups reported MPX individuals with less typical neurological manifestations, such as seizures and encephalitis, who were more severely affected.[2] Studies in infected animals, such as rodents, suggested that the virus can also penetrate the brain.[3,4] However, it is unknown whether MPVX may infect cells in the central nervous system (CNS). We take advantage of our established human induced pluripotent stem cell (hiPSC) platform to address this knowledge gap.

Here, we investigated the susceptibility of hiPSC-derived neural progenitor cells (NPCs), neurons, and astrocytes to MPXV. An A.2 strain virus was isolated from an MPX patient in Thailand, propagated in Vero cells, and titered in HeLa cells. The derivation of human neural cells from hiPSCs has been described elsewhere.[5] Briefly, NPCs were derived from commercially available hiPSCs (ATCC, ACS-1019) using the dual SMAD inhibition protocol via embryoid bodies and rosette selection and maintained in N2B27 media supplemented with basic fibroblast growth factor (bFGF). Neurons were generated from NPCs by the removal of bFGF from the NPC culture media. Astrocytes were derived from NPCs in astrocyte differentiation and maturation media (STEMCELL Technologies). All derived neural cells expressed specific markers as expected. (**Supplementary Fig. S1**).

We initially assessed if MPXV infected hiPSC-derived neural cells by visual observation of the cultures over time and by immunofluorescence assays (IFA). hiPSC-derived NPCs, neurons, and astrocytes were infected with MPXV at multiplicities of infection (MOIs) of 0.0001, 0.001, and 0.1 in their corresponding media (N2B27 with bFGF, N2B27 and AM (Sciencell), respectively). Cell morphology was observed and imaged every 24 h (**Supplementary Fig. S2**). We noticed changes in the morphology of astrocytes 48 h post-infection (hpi), when they began to cluster (**Supplementary Fig. S2C**). At 96 hpi, cells were fixed for IFA as described in **Supplementary Fig. S1**. We found that MPXV-infected NPCs were less densely packed as compared to mock-infected NPCs (**Fig. 1A, top and Supplementary Fig. S1A**), likely due to an increase in cell detachment as a result of infection. Surprisingly, we found no cytopathic effect (CPE) in infected neurons at any MOI (**Fig. 1A, middle and Supplementary Fig. S1B**). In contrast, CPE is most pronounced in infected astrocytes, as indicated by the abundant formation of syncytia, larger cells with multiple nuclei (**Fig. 1A bottom and B).** As MOI rises, the occurrence of such CPE also increases (**Fig. 1A, bottom and Supplementary Fig. S2C**). Unfortunately, due to the unavailability of antibodies against MPXV proteins, we were unable to demonstrate co-labeling of cell type-specific markers and viral proteins. Instead, we assessed whether productive infection with MPXV occurs in these hiPSC-derived neural cells. Note that we have added 45-day-old hiPSC-derived neurons in this replication study in case the age of neurons might play a role in permissiveness to MPXV infection. NPCs, neurons (day 14 and day 45) and astrocytes, as well as Vero cells as positive controls, were infected with MPXV at an MOI of 1.0 for 1 h before being washed with PBS and replenished with the appropriate media. Supernatants were collected at 0, 24, 48, 72 and 96 hpi, the cells were imaged (**Supplementary Fig. S3**), and viral DNA was extracted from free virus for qPCR. Primers and conditions have been described elsewhere.[6] Cycle threshold (Ct) values of MPXV qPCR in NPCs and astrocytes as well as in Vero cells significantly decreased over time compared with the remaining input virus at 0 hpi (**Fig. 1C and Supplementary Table S1**), suggesting that MPXV could productively infect NPCs and astrocytes similar to Vero cells. On the other hand, such a change is not pronounced in 14-day-old and 45-day-old neurons (**Fig. 1C and Supplementary Table S1)**, suggesting that they are not permissive to MPXV. This supports our IFA findings. Intracellular viruses were not examined in this study, but the increased detection of viral genomic DNA in cell culture supernatants is a strong indicator of viral replication. It is also important to note that although the differences in Ct values between NPCs and astrocytes were relatively small, NPCs were seeded at a density four times higher than astrocytes, suggesting that among the three types of neural cells in this study, astrocytes may be the most permissive to or productive for MPXV infection.

Astrocytes play an important role in CNS homeostasis, such as maintenance of synapses, regulation of neurotransmitters, release of neurotrophic factors, and uptake of glutamate. Upon CNS injury or infection, they can become either neuroprotective for CNS repair or neurotoxic by promoting neuroinflammation.[7] These dynamic states have been reported to be associated with various neurodegenerative diseases such as Alzheimer's disease[8] and Parkinson's disease,[9] as well as infections.[10] Given the importance of astrocytes, it is logical to suppose that neurons in patients' brains would respond differently to MPXV infection than our findings, which demonstrated no change in the shape or distribution of hiPSC-derived neurons. They may be damaged not by the infection directly but by the changed properties of the infected astrocytes. More research employing more advanced models, such

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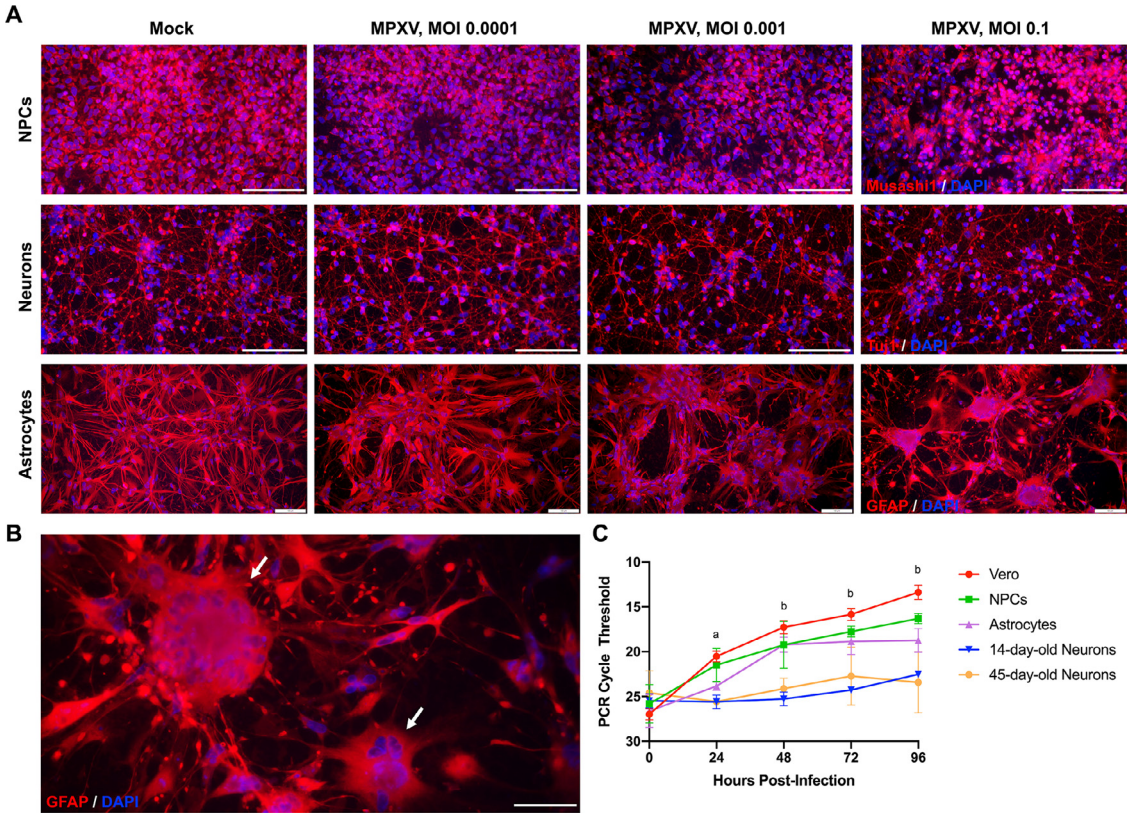

Fig. 1. MPXV infection in hiPSC-derived NPCs, neurons and astrocytes
(A) Representative images of NPCs (top), neurons (middle) and astrocytes (bottom) in MPXV and mock infections. Cells were seeded onto matrigel-coated 24-well plates at 4×10^5, 4.5×10^5, and 10^5 cells/well, respectively. Cells were then incubated with the virus at MOIs of 0.0001, 0.001 or 0.1. At 96 hpi, cells were fixed with 80% ice-cold acetone and immunofluorescently stained for cell-type specific markers (Musashi1, TuJ1 and GFAP, respectively) according to the protocol described in Supplementary Fig. S1. Bar, 100 μm. (B) Higher magnification of syncytia (white arrows) observed in MPXV-infected astrocyte culture. Bar, 50 μm. (C) PCR cycle threshold value of MPXV qPCR in NPCs, neurons (day 14 and day 45), and astrocytes as well as Vero cells at each timepoint. Cells were prepared as described above, infected with MPXV at an MOI of 1.0 for 1 h, washed, and fed with fresh culture media. Supernatants were collected at 0, 24, 48, 72, and 96 hpi. Viral DNA extraction was performed using the Viral Genome Extraction Kit II (Geneaid), and qPCR using primers specific for MPXV[6] was conducted. Statistical analyses were performed by two-way ANOVA. Multiple comparisons were performed within cell type, comparing cycle threshold at 24, 48, 72, and 96 hpi against 0 hpi. a, Vero and NPC values significantly different from 0 hpi, $p < 0.0001$; astrocyte values, $p = 0.0004$. b,Vero, NPC and astrocyte values significantly different from 0 hpi, $p < 0.0001$.

as co-culture of neurons and astrocytes or brain organoids more closely resembling the human brain, might provide more insight into the effects of MPXV infection on neurons in a more relevant setting.

In summary, we found that hiPSC-derived NPCs and astrocytes were susceptible to MPVX infection, causing dramatic changes in cell distribution and morphology, especially in astrocytes. Increased levels of viral DNA were detected in culture supernatants, suggesting productive replication. To our knowledge, this is the first study reporting differences in MPXV permissiveness between hiPSC-derived NPCs and astrocytes versus hiPSC-derived neurons, shedding light on the association between MPXV and reported neurological complications on a cellular level and guiding us towards a deeper understanding of MPXV pathogenesis in the brain.

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Declaration of Competing Interest

None.

CRediT authorship contribution statement

Thanathom Chailangkarn: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Samaporn Teeravechyan:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Khemphitcha Attasombat:** Methodology, Investigation, Data curation. **Theeradej Thaweerattanasinp:** Methodology, Investigation. **Kitpong Sunchatawirul:** Methodology, Investigation. **Pawita Suwanwattana:** Methodology, Investigation. **Krit Pongpirul:** Methodology, Investigation. **Anan Jongkaewwattana:** Conceptualization, Methodology, Investigation, Writing – review & editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.10.016.

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