Abstract number: 367

A Human iPSC-derived Neurosphere Model for Studying HIV-1 CNS Infection and Persistence

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Introduction

HIV-1 remains incurable, and HIV-associated neurocognitive disorders (HAND) continue to affect nearly half of patients despite combination antiretroviral therapy (cART), a consequence of persistent neuroinflammation and the release of neurotoxic viral proteins. Conventional 2-D cultures fail to capture the cellular complexity of the human central nervous system (CNS), limiting their utility for modeling viral pathogenesis and therapeutic response. This ongoing burden underscores the need for advanced, human-relevant 3-D models that more accurately mimic CNS biology and HIV-1 infection dynamics. To address this gap, we developed an induced pluripotent stem cell (iPSC)-derived 3-D neurosphere platform for modeling HIV-1 infection. iPSC-derived neural progenitor cells (NPCs) were aggregated into ultra-low attachment plates to form single, uniform spheres and were matured for at least two weeks. Differentiated neurospheres exhibited consistent morphology, and cellular diversity was confirmed by immunostaining for SOX2 (neural progenitors), TH (dopaminergic neurons), GFAP (astrocytes), and IBA-1 (microglia-like cells). Neurospheres were challenged with three different HIV-1 strains (89.6, JR-CSF, CHO40), and infection was assessed by western blot detection of Pr55, p24, and Nef proteins and RT-qPCR quantification of TAR and env transcripts. Robust viral replication was observed across strains, with high-level RNA (~10^9 TAR; ~10^7 env) and accumulation of viral proteins compared to uninfected controls. Treatment with a clinically relevant cART cocktail suppressed viral protein expression and significantly reduced TAR/env transcripts, demonstrating pharmacologic responsiveness. Importantly, Alu-gag PCR confirmed integration of HIV-1 proviral DNA into the host genome, and long-term cART-treated cultures maintained latent virus that was reactivated upon induction. These findings underscore the model's relevance for studying CNS reservoirs and persistence and its potential for dissecting reservoir dynamics within cell types implicated in HAND. Additionally, the platform was leveraged to model CNS repair processes, where treatment with stem cell–derived extracellular vesicles (EVs) enabled assessment of neuroprotective and regenerative responses within the same human-relevant 3-D context. Collectively, this platform offers a scalable, human-relevant system for studying HIV-1 neuropathogenesis and evaluating antiviral strategies. Furthermore, it has the potential to be adapted to study other neurotropic pathogens, neuroinflammatory conditions, and CNS disorders, advancing disease modeling beyond conventional 2-D cultures and animal models.

Methods

Neurosphere formation: The iPSC line ATCC-BXS0117 (ATCC® ACS-1031™) was differentiated via embryoid body (EB) formation and neural induction to produce the NPCs (ATCC® ACS-5003™) that were used for this study. NPCs were expanded in STEMdiff Neural Progenitor Medium (STEMCELL technologies) in cell matrix-coated flasks. To generate neurospheres, NPCs were seeded in ultra-low attachment 96-well plates. After approximately 48 hours, the media was removed and replaced with DMEM:F12 (ATCC® 30-2006™) + Dopaminergic Neuron Differentiation Kit (ATCC® ACS-3004™) for 4 days. To further induce differentiation the medium was spiked with the following cytokines: IL-34 (100 ng/mL), M-CSF (25 ng/mL), and TGFβ-1 (50 ng/mL) for an additional 7 days. Media was replaced every 2-3 days. The overall workflow for neurosphere generation is shown in **Figure 1**.

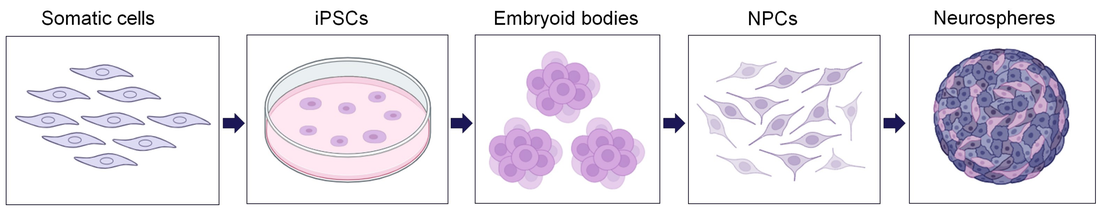


Figure 1: Generation of iPSC-derived neurospheres. Schematic illustrating progression from somatic cells to iPSCs, EBs, NPCs, and self-assembled 3-D neurospheres. Figure created with BioRender.com.

HIV-1 infection of neurospheres: Differentiated neurospheres were exposed to HIV-1 strains 89.6, JR-CSF, or CHO40 (MOI:10) using Infectin (day 0; ~200 µL total volume). After 48 hours, cultures were washed and maintained in fresh differentiation media with or without a cART cocktail (lamivudine, tenofovir disoproxil fumarate, emtricitabine, and indinavir; 2.5 µM each). For select conditions, stem cell–derived EVs were added at an approximate 1:250 cell:EV ratio. For HIV-1 89.6 infections, cART and EV treatments were replenished every 2–3 days. Neurospheres and supernatants were collected after 7–14 days for downstream analyses. Cytokines in supernatants were enriched using Nanotrap particles (NT082/NT080; Ceres Nanosciences) following established protocols. Long-term cultures were maintained for up to 6 months with bi-weekly media replenishments under continuous cART treatment.

Extracellular vesicle isolation and characterization: Human bone marrow mesenchymal stem cells (MSCs; ATCC® PCS-500-012™) and human iPSCs (ATCC® ACS-1019™) were cultured in media with EV-depleted FBS or serum-free/xeno free medium, respectively. EVs were isolated using a stepwise process of centrifugation, tangential flow filtration (TFF), and ultrafiltration (**Figure 2**) to obtain the final product. EV samples were aliquoted and frozen at −20°C for downstream analysis and functional assays. The size distribution and concentration of EVs were assessed via nanoparticle tracking analysis (NTA) using the NanoSight NS300. EV identity was confirmed using western blot for canonical EV surface markers and morphology was assessed by transmission electron microscopy.

Figure 2: EV production. Cells are scaled up to final volume (up to 5 L). Bulk conditioned medium is spun at 200x g to remove cellular debris, followed by diafiltration and concentration via TFF. The TFF product is further concentrated via ultrafiltration to obtain high yield EVs (e.g., >1x10^{10} particles/mL). EVs undergo standard ATCC® characterization assays (concentration, identity, sterility) prior to functional analysis. Figure created with BioRender.com.

Results

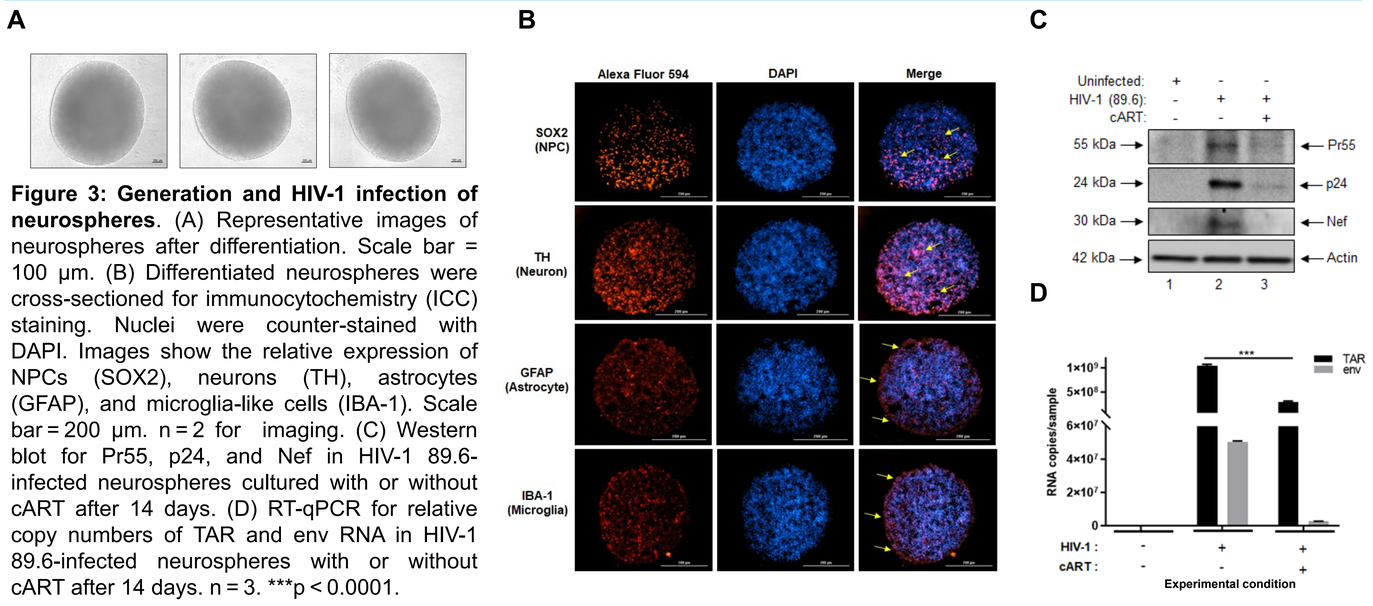


Figure 3: Generation and HIV-1 infection of neurospheres. (A) Representative images of neurospheres after differentiation. Scale bar = 100 µm. (B) Differentiated neurospheres were cross-sectioned for immunocytochemistry (ICC) staining. Nuclei were counter-stained with DAPI. Images show the relative expression of NPCs (SOX2), neurons (TH), astrocytes (GFAP), and microglia-like cells (IBA-1). Scale bar = 200 µm. n = 2 for imaging. (C) Western blot for Pr55, p24, and Nef in HIV-1 89.6-infected neurospheres cultured with or without cART after 14 days. (D) RT-qPCR for relative copy numbers of TAR and env RNA in HIV-1 89.6-infected neurospheres with or without cART after 14 days. n = 3. ***p < 0.0001.

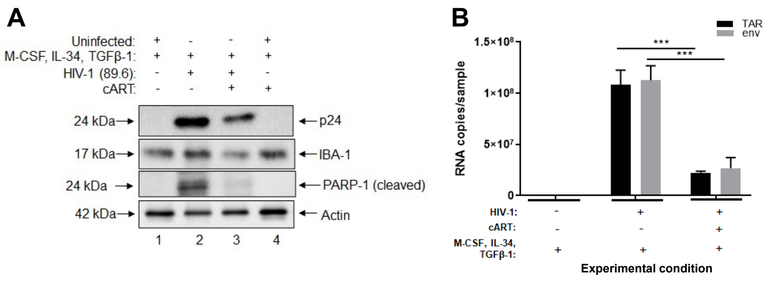


Figure 4: Cytokine induction of neurospheres enhances HIV-1 infection. Differentiated neurospheres were treated with IL-34 (100 ng/mL), M-CSF (25 ng/mL), TGFβ-1 (50 ng/mL) every other day for 7 additional days and were then exposed to dual tropic HIV-1 89.6 for 14 days with or without cART. (A) Western blot for viral protein (p24), microglia (IBA-1), and apoptosis (PARP-1). (B) RT-qPCR was performed to quantify the relative copy numbers of TAR and env RNA. n = 3. *** p < 0.0001.

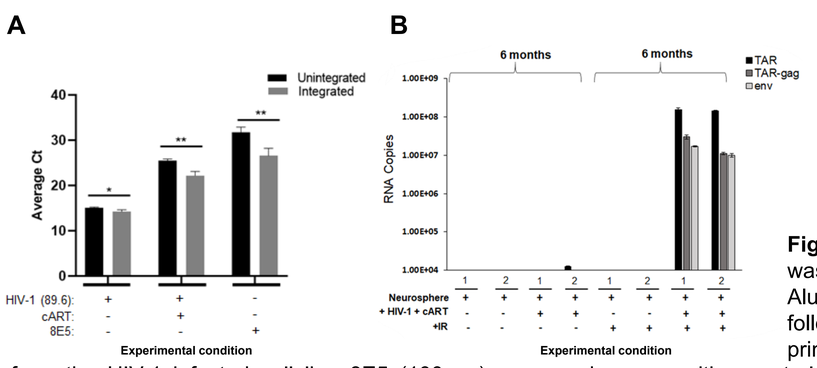
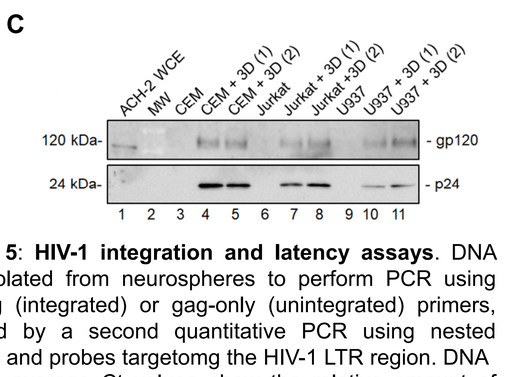


Figure 5: **HIV-1 integration and latency assays**. DNA was isolated from neurospheres to perform PCR using Alu-gag (integrated) or gag-only (unintegrated) primers, followed by a second quantitative PCR using nested primers and probes targetomg the HIV-1 LTR region. DNA from the HIV-1 infected cell line 8E5 (100 ng) was used as a positive control. (A) average Ct values show the relative amount of unintegrated and integrated transcripts. A two-tailed Student's t-test was used to assess significance. n = 3. * p < 0.05, ** p < 0.01. (B) After approximately 6 months in culture, with or without cART, HIV-1 infected neurospheres were exposed to 0.5 Gy ionizing radiation, and viral RNA was detected in 50 µL of supernatant after 3 days by RT-qPCR. n =3 measurements per sample. (C) Supernatants from irradiated cells were added to uninfected cells (CEM, Jurkat, or U937) with Infectin, incubated for 4 hours at 37°C, washed, and media was replaced. After 48 hours virus was isolated from supernatants using Nanotrap particles followed by western blot for gp120 and p24.



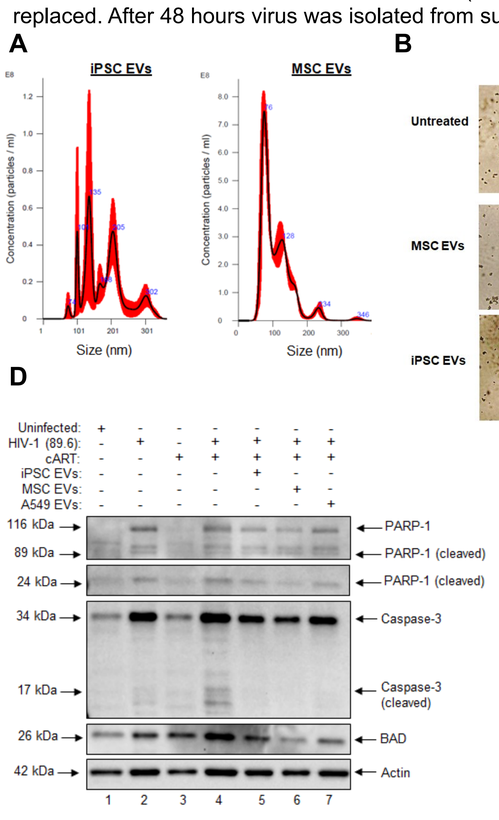


Figure 6: Reparative properties of stem cell EVs in HIV-1 infected neurospheres. EVs were isolated from MSCs and iPSCs using our previously published protocols. (A) EV concentration and size were measured by NTA. Histograms show that the majority of vesicles are within the expected size range of 500 to 200 nm. (B) EVs were fluorescently labeled with BODIPY 493/503. After removal of excess dye, EVs were added to differentiated neurospheres at an approximate ratio of 1:250 (recipient cell to EV ratio). After 24 hours the media was replaced. Representative brightfield and fluorescent images show the relative uptake of EVs after 8 days. Scale bar = 200 µm. HIV-1 infected neurospheres were treated with EVs at an approximate ratio of 1:250 (recipient cell to EV ratio). (C) Western blot was performed on neurosphere lysates to evaluate the expression of apoptotic proteins PARP-1, Caspase-3, and BAD. (D) Western blot was performed on neurosphere supernatants to evaluate the relative expression of inflammatory cytokines TNFα, IL-8, and IL-1β.

Conclusions

- A human iPSC-derived 3-D neurosphere model supports robust HIV-1 infection, recapitulating CNS cell diversity and enabling physiologically relevant studies of viral replication.
- cART suppresses viral activity, while long-term cultures maintain inducible latent reservoirs, supporting modeling of HIV-1 persistence in the CNS.
- This platform enables simultaneous evaluation of antiviral responses and EV-mediated neuroprotection, providing a system for studying neuropathogenesis and therapeutic interventions.
- The model is adaptable to long-term studies and can be extended to other neurotropic pathogens and CNS disease contexts beyond HIV-1.

Learn more about our iPSCs

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