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This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix. Methods

Generation and culture of hiPSC

Adult human dermal fibroblasts were transduced with retroviruses encoding Oct3/4, Klf4, Sox2, c-Myc overnight. On day 3, the transduced fibroblasts were treated with 20mM VPA (Sigma) for 7 days. On day 4, the fibroblasts were plated onto a MEF feeder layer with HUES hESC medium. The colonies were picked at 2 weeks when visible. They were initially passaged by mechanical dissociation and subsequently adapted to Accutase (Innovative Cell Technologies). HiPSC lines were characterized for their ability to differentiate into cells of the 3 germ layers and for the expression of biomarkers for pluripotency.

Neural differentiation and isolation of NSC and neurons by FACS

Protocol was previously described. Briefly, PA6 cells were plated in a 10 cm dish and seeded with 100,000 cells iPSC next day. To enhance neural induction, some cultures were treated with 500ng/ml Noggin (R&D Systems) and 10µM SB431542 (Tocris) for the first 6 days of differentiation. On day 12, NSCs were sorted using cell surface signature CD24+/CD184+/CD44-/CD271-. NSCs were expanded in NSC growth medium, containing DMEM:F12+Glutamax, 0.5X B27, 0.5X N2, 1X P/S (Life Technologies) and 20ng/ml bFGF (R&D, Biopioneer). When the culture was 80% confluent, medium was changed to neuron differentiation medium: DMEM:F12+Glutamax, 0.5X B27, 0.5X N2, 1X P/S, 20ng/ml BDNF, 20ng/ml GDNF (both from Peprotech) and 0.5mM dibutyryl cyclic AMP (Sigma) for 3 weeks. Three-

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week differentiation cultures were dissociated with Accutase and sorted for CD24+/CD44-/CD184- neuronal population by FACS Aria (BD Biosciences).

Embryoid bodies (EB) generation

iPSC colonies were treated with dispase (BD Biosciences) for 30 minutes at 37°C then dissociated by pipeting. Clusters of the dissociated cells were placed in low-adhesion plates (Corning) with iPSC media without FGF. Media was changed every three days. After 7 days, the clusters of cells grew to EBs and were seeded on coverglass coated with Matrigel (BD Biosciences). Cultures were maintained in either neuron differentiation media or fibroblast media for another 7 days.

Quantitative PCR for retroviral reactivation

Primers targeted to the blastocidin resistance region were used for detection. FastStart PCR master mix was used (Roche). Reactions were applied by the Applied Biosystems Real-Time PCR System. Forward primer TCATGTGGGAGCGGCAATTCG, Reverse primer TGCACTACCAATCGCAATGGCT.

Immunofluorescent imaging of cells in culture

Cells were fixed with 4% paraformaldehyde. Primary unconjugated antibodies were stained with secondary antibodies (goat anti-rabbit and goat anti-mouse conjugated to Alexa 488, 647, 568; Molecular Probes). Antibodies and dilutions used include: AFP (Dako) 1:500, Desmin (BD) 1:1000, Map2b (Sigma) 1:1000, Sox1 (BD) 1:1000, Pax6
(Millipore) 1:1000, Ki-67 (BD) 1:1000. Cell nuclei were counterstained with DAPI. Cells were imaged on Nikon Axiophot. Scale bar 100µm.

IP/Western

Conditioned media was concentrated with Centricon spin columns (Millipore). The concentrated media was incubated with 4µg of the B436 anti-Aβ1-12 mouse monoclonal antibody ⁴ for 2-3 hours, then incubated with 30µl IgG beads overnight. The beads were extracted with Laemmlı gel sample dilution buffer and the IP was resolved on an 8% urea gel. The electrophoresed proteins were transferred to a nitrocellulose membrane and the blot was probed with 0.1µg/ml 82E1 (IBL) and amplified with biotin (Vector Lab)/streptavidin (Jackson ImmunoResearch). The blot was developed with Pierce ECL Western Blotting Substrate (Pierce).

eReferences


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**eFigure 1.** (A to D) Differentiation to three different germ layers: bright field image of embryoid bodies (EB) (A), differentiated EB stained with Map2b (B), Desmin (C) and AFP (D). (E) G-banding of iPSC reveals normal karyotype. (F) Evaluation of retroviral reactivation by qPCR. (G) Bright field image of neural rosette. (H-J) The NSC expressed Nestin and most of the NSC express Ki-67. (K-N) Immunofluorescent staining of sorted neurons showing DAPI (K), expression of β-III-tubulin (L), expression of Map2b (M), and merged image (N). Scale bar 100μm.
eFigure 2. Treatment of different cell types with GSM-4. Aβ 40, 42, 42/40 and total Aβ of (A) NDC1 and PS1-1 fibroblasts treated with GSM-4, (B) NDC1 and PS1-1 iPSC treated with GSM-4, (C) NSC derived from iPSC of NDC1 and PS1-1 mutant treated with GSM-4, (D) differentiated neuronal cultures from NDC1 and PS1-1 mutant treated with GSM-4. Statistical analysis performed using one-way ANOVA and Dunnett’s multiple comparison test. NS = not statistically significant, * p <0.05, ** p<0.01, *** p<0.005, **** p<0.0001, error bar +/- SEM, N=3.

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eFigure 3. (A) Linear regression analysis comparing NDC1 fibroblast, iPSC, NSC and neuron in response to GSM-4 treatment. (B) Linear regression analysis comparing PS1-1 fibroblast, iPSC, NSC and neuron in response to GSM-4 treatment.

eFigure 4. Treatment of APPdup NSC with GSM-4. Aβ 40, 42, 42/40 and total Aβ of APPdup NSC treated with GSM-4.