Studying brain disorders with human PSC/iPSCderived cellular models

Yingjun Liu 01.09.2020

High-throughput 3D screening for differentiation of hPSC-derived cell therapy candidates

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Science Advances, 2020

CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons

Neuron, 2019

Ruilin Tian,^{1,2,3,7} Mariam A. Gachechiladze,^{4,7} Connor H. Ludwig,^{1,3,7} Matthew T. Laurie,¹ Jason Y. Hong,^{1,3} Diane Nathaniel,^{1,3} Anika V. Prabhu,⁵ Michael S. Fernandopulle,⁴ Rajan Patel,⁴ Mehrnoosh Abshari,⁶ Michael E. Ward,^{4,*} and Martin Kampmann^{1,3,8,*}

Genome-wide CRISPRi/a screens in human neurons link lysosomal failure to ferroptosis

Ruilin Tian^{1,2,*}, Anthony Abarientos¹, Jason Hong¹, Sayed Hadi Hashemi³, Rui Yan⁴, Mike A. Nalls^{5,6}, Andrew B. Singleton⁵, Ke Xu⁴, Faraz Faghri^{3,5,6}, Martin Kampmann^{1,7,*}

BioRxiv, 2020

High-throughput 3D screening for differentiation of hPSC-derived cell therapy candidates



- 1200 combinatorial culture conditions (with 4 repeats for each) were screened for high efficient generation of OPC, consuming less than 0.2% of the reagent volumes of a corresponding 96-well plate format.
- Could be generalized for the generation of other cell types

Riya Muckom, Douglas S. Clark and David V. Schaffer et al., Science Advances, 2020

Seeding and cell viability on the micropillar culture chip



The starting point: current OPC differentiation protocol



Timeline of exogenous signals for in vitro Production of OPCs from pluripotent stem cells

- LDN/SB (LDN193189/SB431542): Smad/BMP signaling inhibitors to induce neuroectodermal differentiation of hPSCs
- CHIR99021: wnt signals
- SAG: SHH agonist

Snapshots of screening of 1200 conditions...





Generalization of the approach (for dopaminergic neurons)



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BioRxiv, 2020

hiPSC cell line with inducible NGN2 expression



Chao Wang, Michael E. Ward and Li Gan et al., Stem cell reports, 2017





Basics of CRISPR mediated gene repression and activation



Martin Kampmann, Nature Reviews Neurology, 2020

hiPSC cell lines with inducible NGN2 expression and inducible CRRSPRi/a





Ruilin Tian, Mariam A. Gachechiladze, Michael E.Ward and Martin Kampmann et al., Neuron, 2019 Ruilin Tian and Martin Kampmann et al., BioRxiv, 2020

4 neuron

/ 14 neuron -/- TMP

4 neuron

Whole-genome CRISPRi/a screening of neuron-survival genes



Whole-genome CRISPRi/a screening of genes control ROS, lipid peroxidation and toxicity



Whole-genome CRISPRi/a screening of genes control ROS, lipid peroxidation and toxicity



Loss of prosaposin induces ROS and lipid peroxidation in neurons and causes neuronal ferroptosis in the absence of antioxidants



WT

PSAP KO







Loss of prosaposin induces ROS and lipid peroxidation in neurons and causes neuronal ferroptosis in the absence of antioxidants







Z-VAD-FMK: a pan-caspase inhibitor Iron chelator deferoxamine (DFO) and Ferrostatin-1: ferroptosis inhibitors

Loss of prosaposin induces ROS and lipid peroxidation in neurons and causes neuronal ferroptosis in the absence of antioxidants







Combining CRISPRi/a with single cell RNA-sequencing





Examples of CRISPRi/a-CROP-Seq









NQO1 overexpression vs. control

Thank you for your attention!