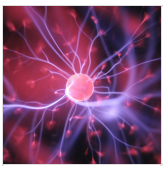

Sep 16, 2020

Protocols for Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

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ABSTRACT

Proneural transcription factors (TFs) drive highly efficient differentiation of pluripotent stem cells to lineage-specific neurons. However, current strategies mainly rely on genome-integrating viruses. Here, we used synthetic mRNAs coding two proneural TFs (Atoh1 and Ngn2) to differentiate induced pluripotent stem cells (iPSCs) into midbrain dopaminergic (mDA) neurons. mRNAs coding Atoh1 and Ngn2 with defined phosphosite modifications led to higher and more stable protein expression, and induced more efficient neuron conversion, as compared to mRNAs coding wild-type proteins. Using these two modified mRNAs with morphogens, we established a 5-day protocol that can rapidly generate mDA neurons with >90% purity from normal and Parkinson's disease iPSCs. After in vitro maturation, these mRNA-induced mDA (miDA) neurons recapitulate key biochemical and electrophysiological features of primary mDA neurons and can provide high-content neuron cultures for drug discovery. Proteomic analysis of Atoh1-binding proteins identified the nonmuscle myosin II (NM-II) complex as a new binding partner of nuclear Atoh1. The NM-II complex, commonly known as an ATP-dependent molecular motor, binds more strongly to phosphosite-modified Atoh1 than the wild type. Blebbistatin, an NM-II complex antagonist, and bradykinin, an NM-II complex agonist, inhibited and promoted, respectively, the transcriptional activity of Atoh1 and the efficiency of miDA neuron generation. These findings established the first mRNA-driven strategy for efficient iPSC differentiation to mDA neurons. We further identified the NM-II complex as a positive modulator of Atoh1-driven neuron differentiation. The methodology described here will facilitate the development of mRNA-driven differentiation strategies for generating iPSC-derived progenies widely applicable to disease modeling and cell replacement therapy.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6344911/

THIS COLLECTION ACCOMPANIES THE FOLLOWING PUBLICATION

Xue Y, Zhan X, Sun S, et al. Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. Stem Cells Transl Med. 2019;8(2):112–123. doi:10.1002/sctm.18-0036

ATTACHMENTS

Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons.pdf

COLLECTION CITATION

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https://protocols.io/view/protocols-for-synthetic-mrnas-drive-highly-efficie-9e5h3g6

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KEYWORDS

ipsc, Atoh1, Ngn2, phosphosite modification, midbrain dopaminergic, differentiation, nonmuscle myosin II, NM-II

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GUIDELINES

All results reported should represent at least three independent replications. Perform statistical analysis using Prism software. Post hoc tests should include the Student's *t* test and the Tukey multiple comparison tests as appropriate. For neurophysiological recordings, the recorded data should first be visualized with Clampfit 9.2 and exported to MATLAB for further analysis and plotting. Visualize recording traces with Igor Pro 6.0. All data to be represented as mean ± SEM.

BEFORE START

Obtain approval to work with human stem cells from an appropriate Institutional Review Board.

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for safety and environmental hazards.

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