Transcription Factor-Based Rapid Differentiation of Human iPSCs into Inhibitory, Excitatory and Sensory Neurons

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Introduction

There are barriers in obtaining cells and tissues from patients to study neurological diseases and disroders due to their limited availability for research as well as ethical concerns. Human brain structure is quite distinct from those of experimental animals further impeding human neural research. Thus there is a critical need for establishing an accessible human cell-based disease model. Human induced pluripotent stem cells (hiPSCs) have been used extensively as an unlimited source of neurons of human origin. When appropriate cues are provided at the right times, hiPSCs can differentiate into various types of neurons, making hiPSC-derived neurons an attractive resource. However, commonly used stepwise differentiation methods are often not ideal due to the long duration of culture which typically extends for several weeks. Our differentiation method provides efficient differentiation of human iPS or embryonic stem (ES) cells into neuronal subtypes such as GABAergic, excitatory, or sensory neurons, within 10 days. We utilize four serial deliveries of a cocktail of synthetic messenger RNAs encoding transcription factors. In this manner, their expression levels can be sustained highly enough to rapidly reprogram the epigenetic marks associated with pluripotency without leaving a genetic footprint. In the case of GABAergic neurons, the expression of characteristic marker proteins such as PVALB and GAD65/GAD67 was confirmed by immunoflourescence microscopy in 74% and 65% of TUBB3-positive cells, respectively, in the culture 10 days after differentiation induction. Furthermore, GABA secretion was detected by ELISA in the supernatant of the cultures of GABAergic neurons 17 days (282±27.0 ng/ml) and 24 days (791±75.5 ng/ml) after the initiation of differentiation. Likewise, 57% of TUBB3 positive cells (93%) exhibited VGLUT1 expression in day 10 cultures of excitatory neuron differentiation. On day 10 of sensory neuron differentiation 89% of PRPH-positive neurons were SCN9A positive. This approach has been successfully applied to several different hiPSC lines derived from healthy donors as well as from patients with various neurological disorders. Collectively, this technique is amenable to accelerate our understanding of human brain development as well as neurological disorders. This study was sponsored by our internal fund and the authors declare no financial conflict of interest.

Methods

Figure 1. Overview of the critical steps in the differentiation process and corresponding phase contrast images of (A) Quick-Neuron™- Excitatory neurons (B) Quick-Neuron™- GABAergic neurons and (C) Quick-Neuron™- Sensory neurons

Acknowledgements

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★ Please visit booth # 733, www.elixirgensci.com , or scan the QR code for more information.

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Results

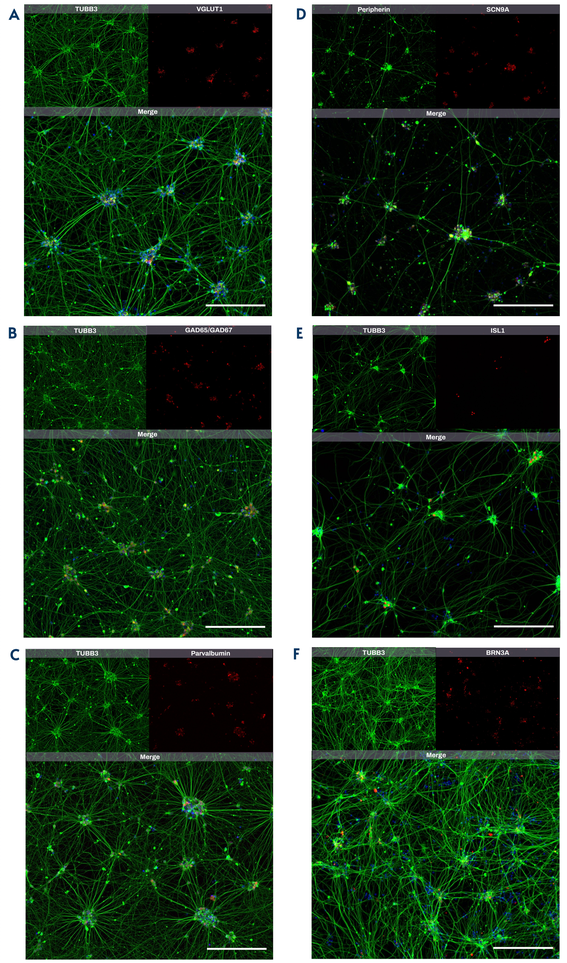

Figure 2. Immunofluorescent staining confirms expression of proteins associated with each population of neurons (A) 57% of TUBB3 positive cells express the excitatory neuron-associated marker VGLUT1 at Day 10 of excitatory neuron differentiation. (B) 65% of TUBB3 positive cells express the GABAergic Neuron-associated marker GAD65/GAD67 at Day 14 of GABAergic differentiation (C) 74% of TUBB3-positive cells express the GABAergic neuron-associated marker PVALB at Day 14 of GABAergic differentiation (D) 89% of PRPH-positive cells express the Sensory Neuron-associated marker SCN9A at Day 10 of sensory neuron differentiation (E) 40% of TUBB3-positive cells express the sensory neuron-associated marker ISL1 at Day 10 of sensory neuron differentiation and (F) 14.6% of TUBB3-positive cells express the Sensory Neuron-associated marker BRN3A at Day 10 of sensory neuron differentiation.

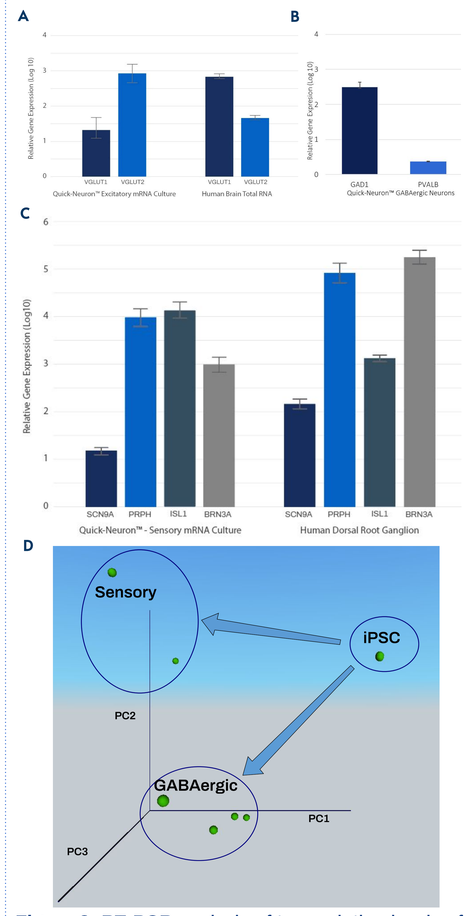

Figure 3. RT-PCR analysis of transcription levels of specific genes was performed on day 10 of differentiation. Graphs show comparisons between **(A)** Quick-Neuron™ Excitatory Neurons and human brain total RNA **(B)** Quick-Neuron™ GABAergic Neurons and iPSC RNA. **(C)** Quick-Neuron™ Sensory Neurons and human dorsal root ganglion RNA. The relative gene expression is normalized to phosphoglycerate kinase 1 (PGK1). N=3 for all neuron types. Error bars show standard deviation. **(D)** Principal component analysis (PCA). PCA indicates that Quick-Neuron™GABAergic Neurons display completely different gene expression than that of human iPSC and Quick-Neuron™ Sensory Neurons at day 10 of differentiation. PC1 (the x-axis) clearly separates the gene expression profile of hiPSCs from those of GABAergic neurons and sensory neurons. Furthermore, PC2 (the y-axis) shows the progression of neural maturation of sensory neurons.

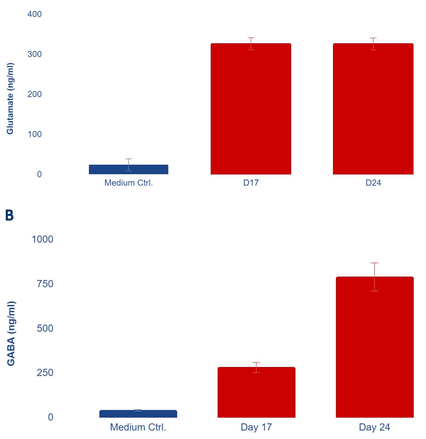

Figure 4. ELISA assay **(A)** Glutamate expression level was determined by ELISA from Quick-Neuron™ Excitatory neuronal culture, using supernatants collected at day 17 and day 24 post differentiation (n=2). Data is expressed as the mean of 2 biological replicates ±SD. **(B)** GABA expression level was determined by ELISA from Quick-Neuron™ GABAergic neuronal culture, using supernatants collected at day 17 and day 24 post differentiation (n=2). Data is expressed as the mean of 2 biological replicates ±SD. Supernatants of Quick-Neuron™ Excitatory neuronal culture at day 24 post differentiation were used as negative control.

Conclusions

Our Quick-Neuron™ mRNA Kits allow researchers to quickly, easily, and efficiently differentiate their iPS or ES cell line of choice into specific types of neurons. Elixirgen Scientific's hiPSC-derived differentiation protocols provide :

- **Robust differentiation.** Immunostaining and RT-PCR clearly indicate successful differentiation towards desired cell types displaying characteristic markers and with gene expression patterns distinct from iPSC on PCA analysis.
- **High reproducibility.** Results are similar across different experiments and sources of iPSC lines.
- **Time-saving differentiation process.** Starting from iPSCs, within 10 days, our protocols deliver differentiated neural cells.