Application Note 002

Differentiation of Porcine Induced Pluripotent Stem Cells into Electrophysiologically Active Motor Neurons.

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Abstract

Dragon Bio's porcine-induced pluripotent stem cells (piPSCs) possess key properties such as self-renewal and the capacity to differentiate into clinically relevant cell types, including motor neurons. Using a 24-day motor neuron differentiation protocol developed by Sania Therapeutics, Dragon Bio piPSCs were successfully differentiated into motor neurons. The resulting piPSC-derived motor neurons expressed canonical lineage markers, including HB9, PHOX2B, and ChAT. Functional analysis using microelectrode array (MEA) assays revealed robust electrophysiological activity, indicative of neuronal maturity and network functionality. These findings establish piPSCs as a valuable in vitro model for investigating motor neuron development and degeneration. Dragon piPSCs can be applied to disease modelling, high-throughput drug screening, and the development of cell-based therapeutic strategies for motor neuron disorders.

Introduction

Induced pluripotent stem cells (iPSCs) possess the remarkable capacity to differentiate into virtually any cell type, including motor neurons, making them invaluable tools for studying disease mechanisms and developing potential therapies. Human induced pluripotent stem cells (iPSCs) have been extensively used to model amyotrophic lateral sclerosis (ALS) and other motor neuron diseases (MNDs), providing insights into disease-specific phenotypes such as motor neuron degeneration, functional impairment, and oxidative stress [1-2]. Recent advances have demonstrated the successful differentiation of motor neurons from porcine iPSCs, underscoring their potential as an alternative large-animal model for investigating motor neuron differentiation and the pathophysiology of MNDs such as ALS [3-5]. Collectively, porcine iPSCs are a relevant and translational model system for advancing our understanding of motor neuron disease and accelerating the development of effective therapies.

Methods

piPSC Cell Culture

Porcine induced pluripotent stem cells (Dragon Bio, #DB_001) were clump-passaged using ReLeSR (Stem Cell Technologies), onto Geltrex coated 6-well plates (Thermofisher) in Lacey's Pluriplus Growth Medium (Dragon Bio, #DB_101).

Motor Neuron Differentiation

piPSCs were differentiated into motor neurons using a 24-day differentiation protocol (Sania Therapeutics). Briefly, piPSCs were dissociated into single cells and Embryoid Body formation was initiated. This was followed by differentiation into neural progenitor cells and further maturation into motor neurons.

Immunocytochemistry

piPSCs were immunostained using the following antibodies. For pluripotency analysis: Rabbit OCT4A (2890S, Cell Signalling Technology, 1:100), Mouse NANOG (#4893S, Cell Signalling Technology, 1:100) and nuclear DAPI stain (D1306, Invitrogen, 1:10,000). For motor neuron differentiation: Goat anti-CHAT antibody (Millipore, AB144p,1:100), Rabbit anti-β3-tubulin (Abcam, ab18207, 1:1,000. Rabbit anti-HB9 antibody (ThermoFisher, PA5-

67195,1:200), Chicken anti-MAP2 (Abcam, ab5392, 1:2,000), Mouse anti-PHOX2B (SantaCruz, Sc-376697, 1:100). Images were taken on a fluorescence microscope at 20x.

Microelectrode Array Electrophysiology Assays

Day 10 piPSC-derived neural progenitor cells were seeded onto 24-well MEA plates (Axion Biosystems). Neuronal activity was assessed by recording extracellular voltage traces and firing patterns across 16 electrodes per well over a 5-minute duration.

Results

Differentiation of Dragon Bio piPSCs into Motor Neurons

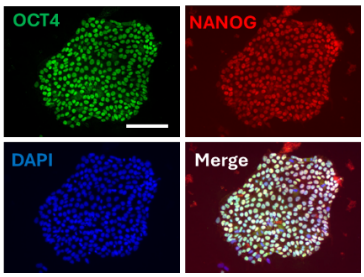

Figure 1: Dragon Bio Porcine iPSCs. Immunofluorescence staining of porcine iPSCs showing expression of pluripotency markers OCT4 (green) and NANOG (red), with nuclear counterstaining using DAPI (blue). Scale bar: 100µm.

Dragon Bio has generated porcine induced pluripotent stem cells (piPSCs) through non-integrating reprogramming of porcine fibroblasts. The resulting piPSCs exhibit typical colony morphology and express the pluripotency markers OCT4 and NANOG (Figure 1).

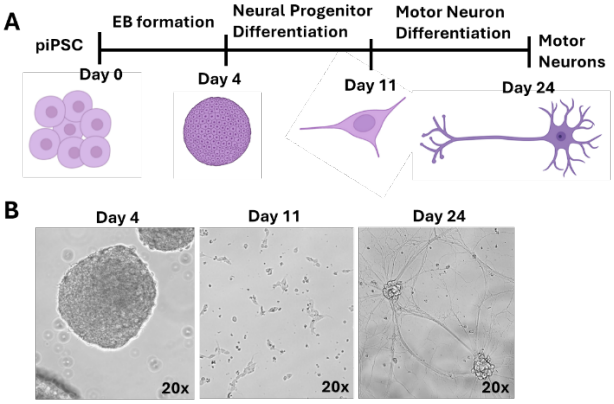

Figure 2. Morphological Characterization of piPSC-Derived Motor Neuron Differentiation. A) Schematic of Sania Therapeutics' Motor Neuron Differentiation Protocol. B) Brightfield microscopy images during each stage of motor neuron differentiation. All images were acquired at 20× magnification.

piPSCs were differentiated into motor neurons using a 24-day protocol developed by Sania Therapeutics (Figure 2A). By Day 4, piPSCs formed embryoid bodies during the neural induction phase. At Day 11, neural progenitor cells were observed. By Day 24, the cells had differentiated into mature motor neurons, exhibiting characteristic morphology of long axons and dendrites (Figure 2B).

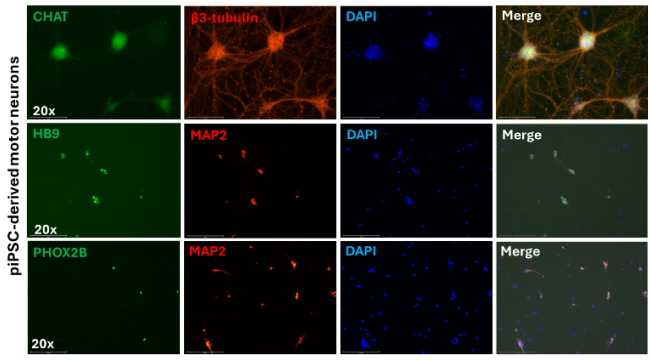

Figure 3. piPSC-Derived Motor Neurons Express Motor Neuron Markers. Top panel: Immunofluorescence staining of piPSC-derived motor neurons showing expression of ChAT (green), β3-tubulin (red), and DAPI (blue). Middle panel: Staining for HB9 (green), MAP2 (red), and DAPI (blue) in piPSC-derived motor neurons. Bottom panel: Expression of PHOX2B (green), MAP2 (red), and DAPI (blue) in piPSC-derived motor neurons. All images were acquired at 20× magnification.

To validate the identity and maturation status of motor neurons differentiated from porcine iPSCs, immunofluorescence staining was performed for key neuronal and motor neuron-specific markers. piPSC-derived motor neurons exhibited robust expression of choline acetyltransferase (ChAT), a hallmark of cholinergic motor neurons, and β3-Tubulin, confirming neuronal structure (Figure 3, Top panel). Additional staining revealed expression of HB9, a transcription factor critical for motor neuron specification, alongside MAP2, a dendritic marker (Figure 3, Middle panel). Furthermore, piPSC-derived motor neurons expressed PHOX2B, another motor neuron lineage marker (Figure 3, Bottom panel). These results confirm successful differentiation of piPSCs into mature motor neurons.

piPSC-Derived Motor Neurons Exhibit Electrophysiological Activity

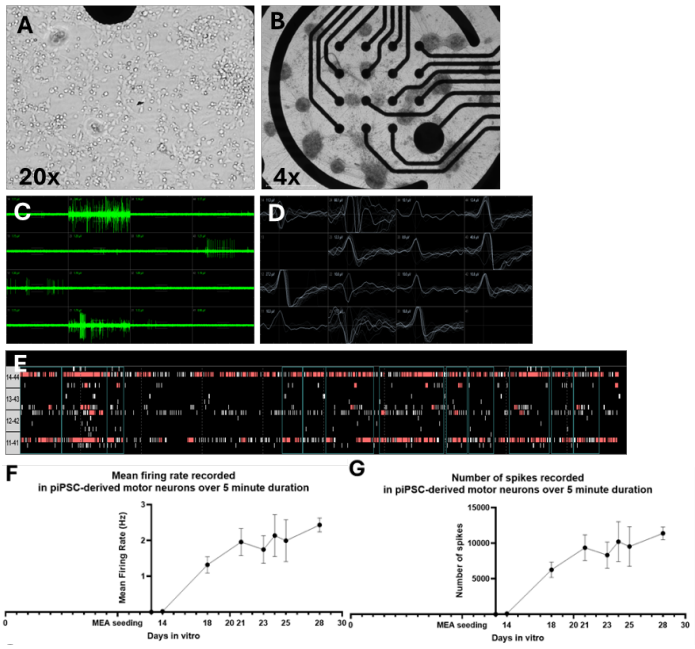

Figure 4: piPSC derived motor neurons display neuronal activity. Electrophysiological activity of piPSC-derived motor neurons. (A) Day 10 neural progenitors seeded onto 24-well MEA plates; recordings taken from Day 14 onward. (B) Day 28 neurons showing mature morphology. (C) Representative spike waveforms. (D) Extracellular voltage traces from active electrodes. (E) Raster plot illustrating synchronized firing across 16 electrodes. (F) Mean firing rate across the MEA plate. (G) Total spike count recorded over 5 minutes.

To assess the functional maturation of piPSC-derived motor neurons, multi-electrode array (MEA) analysis was performed. Day 10 piPSC-derived neural progenitor cells were seeded onto 24-well MEA plates, and electrophysiological recordings were conducted from Day 14 to Day 28 (Figure 4A). By Day 28, piPSC-derived motor neurons were visibly attached to the MEA surface and displayed mature neuronal morphology (Figure 4B). Continuous waveform plots captured on Day 28 revealed spontaneous spike activity (Figure 4C), with extracellular voltage traces recorded from multiple active electrodes (Figure 4D). A representative raster plot illustrated synchronised firing patterns across all 16 electrodes in a single well, indicative of network-level neuronal activity (Figure 4E).

Quantitative assessment across the MEA plate population showed that by Day 28, motor neurons exhibited a consistent mean firing rate of 2 Hz (Figure 4F) and generated over 1,000 spikes (Figure 4G). These findings confirm that piPSC-derived motor neurons are electrophysiologically active and capable of generating spontaneous action potentials *in vitro*, reflecting successful functional differentiation.

Discussion

The development of induced pluripotent stem cells (iPSCs) has transformed modelling of neurodegenerative diseases. Porcine iPSCs provide a physiologically relevant, translational model due to their similarity to humans and other large mammals. Using a 24-day Sania Therapeutics protocol, Dragon Bio piPSCs were differentiated into motor neurons expressing canonical markers HB9, PHOX2B, and ChAT, confirming lineage specification and maturation. By Day 28, MEA analysis demonstrated robust electrophysiological activity with characteristic firing patterns of functional neuronal networks.

These results establish Dragon Bio piPSCs as a scalable and ethically viable in vitro platform for modelling motor neuron development, disease, and therapeutic screening. Beyond human applications, piPSC-derived motor neurons also offer a valuable translational tool for veterinary medicine, enabling preclinical studies and regenerative therapy development for motor neuron disorders in companion and livestock species.

References

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