Experimental Neurology 307 (2018) 155–163

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research paper

The human motor neuron axonal transcriptome is enriched for transcripts related to mitochondrial function and microtubule-based axonal transport

Renata Maciel[a,b], Dana M. Bis[b], Adriana P. Rebelo[b], Cima Saghira[b], Stephan Züchner[a,b], Mario A. Saporta[a,b,*]

[a] Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33136, USA
[b] Department of Human Genetics, Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL 33136, USA

ARTICLE INFO

Keywords:
Human motor neurons
Axonal transcriptome
Induced pluripotent stem cells
RNA sequencing

ABSTRACT

Local axonal translation of specific mRNA species plays an important role in axon maintenance, plasticity during development and recovery from injury. Recently, disrupted axonal mRNA transport and translation have been linked to neurodegenerative disorders. To identify mRNA species that are actively transported to axons and play an important role in axonal physiology, we mapped the axonal transcriptome of human induced pluripotent stem cell (iPSC)-derived motor neurons using permeable inserts to obtain large amounts of enriched axonal material for RNA isolation and sequencing. Motor neurons from healthy subjects were used to determine differences in gene expression profiles between neuronal somatodendritic and axonal compartments. Our results demonstrate that several transcripts were enriched in either the axon or neuronal bodies. Gene ontology analysis demonstrated enrichment in the axonal compartment for transcripts associated with mitochondrial electron transport, microtubule-based axonal transport and ER-associated protein catabolism. These results suggest that local translation of mRNAs is required to meet the high-energy demand of axons and to support microtubule-based axonal transport. Interestingly, several transcripts related to human genetic disorders associated with axonal degeneration (inherited axonopathies) were identified among the mRNA species enriched in motor axons.

1. Introduction

Local translation of proteins from messenger RNAs that are selectively transported from the neuronal cell body to axons and the synaptic terminal (Wang et al., 2010) allows for local modulation of protein synthesis in response to synaptic activation (Sutton and Schuman, 2006). The local translation of proteins in axons seems to also have a role in regulating axon outgrowth and regeneration (Zheng et al., 2001; Taylor et al., 2009), as well as in synapse formation and remodelling (Sutton and Schuman, 2006).

Disrupted axonal RNA metabolism has recently emerged as a new pathomechanism of distinct neuromuscular disorders, including Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis (Fallini et al., 2016; Rotem et al., 2017). Moreover, given the significant disruption in axonal transport observed in several types of axonal Charcot-Marie-Tooth disease (CMT), including those associated with mutations in *MFN2* (Baloh et al., 2007; Misko et al., 2010; Misko et al., 2012), *NEFL* (Saporta et al., 2015; Gentil et al., 2012) and *HSPB1* (D'Ydewalle et al., 2011), it is likely that the transport of mRNAs to the distal axons is also impaired in these inherited axonopathies. As a first step towards the goal of identifying mRNA species that may be dysregulated in human axonopathies, we mapped the axonal transcriptome of several healthy induced pluripotent stem cell (iPSC)-derived motor neuron lines. Interestingly, multiple transcripts known to be associated with inherited axonopathies, including CMT and Hereditary Spastic Paraplegia (HSP), were found to be enriched in the axons of human motor neurons, suggesting that their local expression is essential for axonal maintenance. The transcriptome map assembled by this study will be a useful tool to investigate axonal mRNA dysregulation in human neurodegenerative diseases that specifically affect spinal motor neuron axons.

2. Results

2.1. Production of an enriched population of mature motor neurons

Human iPSCs were differentiated into spinal motor neurons using a modified dual SMAD inhibition protocol (Supplementary Fig. 1A). This protocol produces around 21–25% mature motor neurons (ISL1/HB9 positive cells) (Supplementary Fig. 1B), in a heterogeneous cell

* Corresponding author at: Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33136, USA.
E-mail address: mas638@med.miami.edu (M.A. Saporta).

https://doi.org/10.1016/j.expneurol.2018.06.008
Received 22 November 2017; Received in revised form 5 June 2018; Accepted 15 June 2018
Available online 20 June 2018
0014-4886/

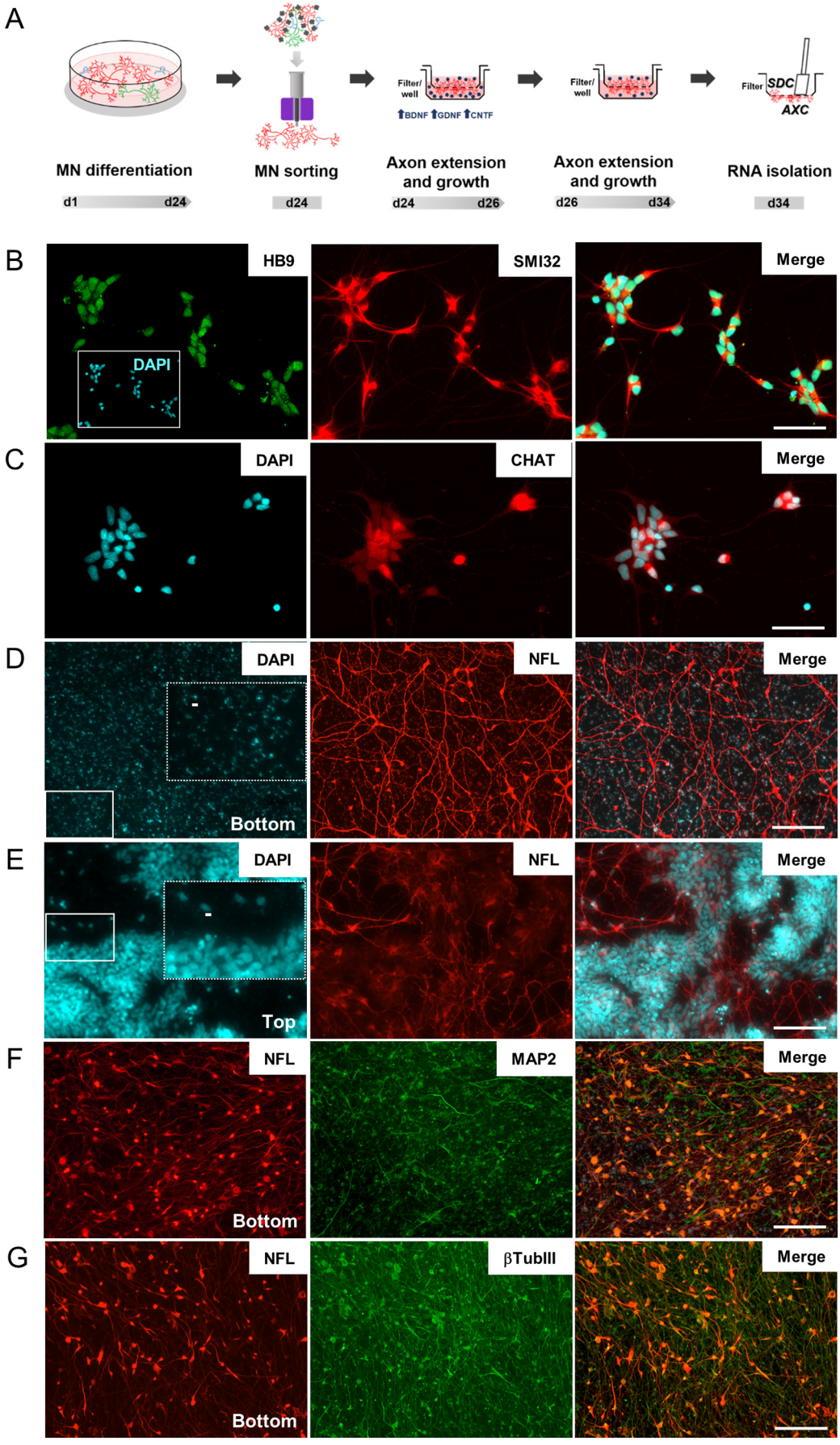

Fig. 1. Isolation of axons from iPSC-derived motor neurons. (A) Timeline showing motor neuron differentiation protocol and separation of cell compartments for RNA isolation. Representative images of sorted motor neurons stained for (B) HB9 and SMI32 and (C) CHAT. (D & E) Permeable inserts bearing a 1.0-μm porous membrane were used to obtain large amounts of (D) enriched axonal material from iPSC-derived motor neurons. No neuronal nuclei were identified on DAPI staining at filter-bottom compartment, as opposed to the (E) numerous nuclei observed in the filter top. Dashed rectangles in D and E are zoomed images from fields indicated by solid rectangles showing (D) blue auto fluorescence of filter pores in bottom compartment and (E) nuclei staining in top compartments. Scale bar 5 μm. (F & G) Representative images of bottom compartments showing that neuronal projections (β-TubIII) are mainly composed of axons (NFL) and few dendrites (MAP2). HB9: Homeobox HB9; SMI32: neurofilament heavy chain; CHAT: choline acetyltransferase; NFL: neurofilament light chain; MAP2: microtubule associated protein 2;βTubIII: Tubulin, beta 3 class III. Scale bar 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)