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Enhanced expression of *FNDC5* in human embryonic stem cell-derived neural cells along with relevant embryonic neural tissues

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ABSTRACT

Availability of human embryonic stem cells (hESCs) has enhanced the capability of basic and clinical research in the context of human neural differentiation. Derivation of neural progenitor (NP) cells from hESCs facilitates the process of human embryonic development through the generation of neuronal subtypes. We have recently indicated that fibronectin type III domain containing 5 protein (*FNDC5*) expression is required for appropriate neural differentiation of mouse embryonic stem cells (mESCs). Bioinformatics analyses have shown the presence of three isoforms for human *FNDC5* mRNA. To differentiate which isoform of *FNDC5* is involved in the process of human neural differentiation, we have used hESCs as an in vitro model for neural differentiation by retinoic acid (RA) induction. The hESC line, Royan H5, was differentiated into a neural lineage in defined adherent culture treated by RA and basic fibroblast growth factor (bFGF). We collected all cell types that included hESCs, rosette structures, and neural cells in an attempt to assess the expression of *FNDC5* isoforms. There was a contiguous increase in all three *FNDC5* isoforms during the neural differentiation process. Furthermore, the highest level of expression of the isoforms was significantly observed in neural cells compared to hESCs and the rosette structures known as neural precursor cells (NPCs). High expression levels of *FNDC5* in human fetal brain and spinal cord tissues have suggested the involvement of this gene in neural tube development. Additional research is necessary to determine the major function of *FDNC5* in this process.

1. Introduction

Mouse fibronectin type III domain-containing 5 protein (*FNDC5*) cDNA, originally cloned by Ferrer-Martinez et al. and Teufel et al. in 2002, encodes a protein with 209 amino acid residues.

Amino acid analysis has shown the presence of two hydrophobic regions and a fibronectin type III domain in the structure of this protein. There are three peptides, serine–lysine–isoleucine (SKI) similar to peroxisomal targeting signal type1 (PTS1) at the C-terminus of *FNDC5*. Previously, it has been suggested that SKI directs this protein into the matrix of peroxisomes (Ferrer-Martinez et al., 2002; Ostadsharif et al., 2009, 2010) thereby suggesting that *FNDC5* is a peroxisomal matrix protein (Tanhaie et al., 2009). However, recently it has been reported that when the N-terminal signal peptide of *FNDC5* is removed, a glycosylated mature protein, known as Irisin, is proteolytically cleaved and released into the extracellular space. Irisin is synthesized under the regulation of PGC1-α and is secreted mainly from muscle into the blood which activates a thermogenic function in adipose tissues (Boström et al., 2012). Primary Northern blot analyses have revealed that *FNDC5* is mainly expressed in the heart, skeletal muscle, and brain tissues (Ferrer-Martinez et al., 2002). Our previous data have shown that the expression of *FNDC5* transcripts markedly increased after retinoic acid (RA) induction

Abbreviations: bFGF, Basic fibroblast growth factor; BSA, Bovine serum albumin; DAPI, 4,6-Diamidino-2-phenylindole; *FNDC5*, Fibronectin type III domain containing 5 protein; *GAPDH*, *Glyceraldehyde 3-phosphate dehydrogenase*; hESCs, Human embryonic stem cells; mESCs, Mouse embryonic stem cells; NCBI, National Center for Biotechnology Information; NP, Neural progenitor; NPCs, Neural precursor cells; PBS, Phosphate buffered saline; PTS1, Peroxisomal targeting signal type1; PVDF, Polyvinylidenedifluoride; RA, Retinoic acid; SEM, Standard error of mean; SKI, Serine–lysine–isoleucine; HRP, Horseradish peroxidase; RT-PCR, Reverse transcription-polymerase chain reaction

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during the neural differentiation of mouse embryonic stem cells (mESCs) in neural precursor cells (NPCs) and neurospheres (Ostadsharif et al., 2011). Knockdown expression of *FNDC5* during the process of NPC formation caused a significant reduced expression of both neural progenitors (NPs) and mature neuronal markers which resulted in the reduction of both neuronal and astrocyte maturation (Hashemi et al., 2013). In humans, the role of *FNDC5* in neural differentiation has yet to be determined. Therefore this study examined the expression profile of *FNDC5* mRNA and its three predicted isoforms during the neural differentiation process in human embryonic stem cells (hESCs).

2. Materials and methods

2.1. Human embryonic stem cells (hESCs) culture and neural differentiation

The hESC line, Royan H5, was used for in vitro production of neural differentiation (Baharvand et al., 2006). Cells were passaged and maintained under feeder-free conditions, then subjected to neural differentiation as previously described (Baharvand et al., 2007). The neural differentiation procedure, as outlined in Fig. 1A, is divided into five substages (steps 1–5). Briefly, hESCs were allowed to proliferate for six days in hESc medium that contained DMEM/F12 medium, 20% knock-

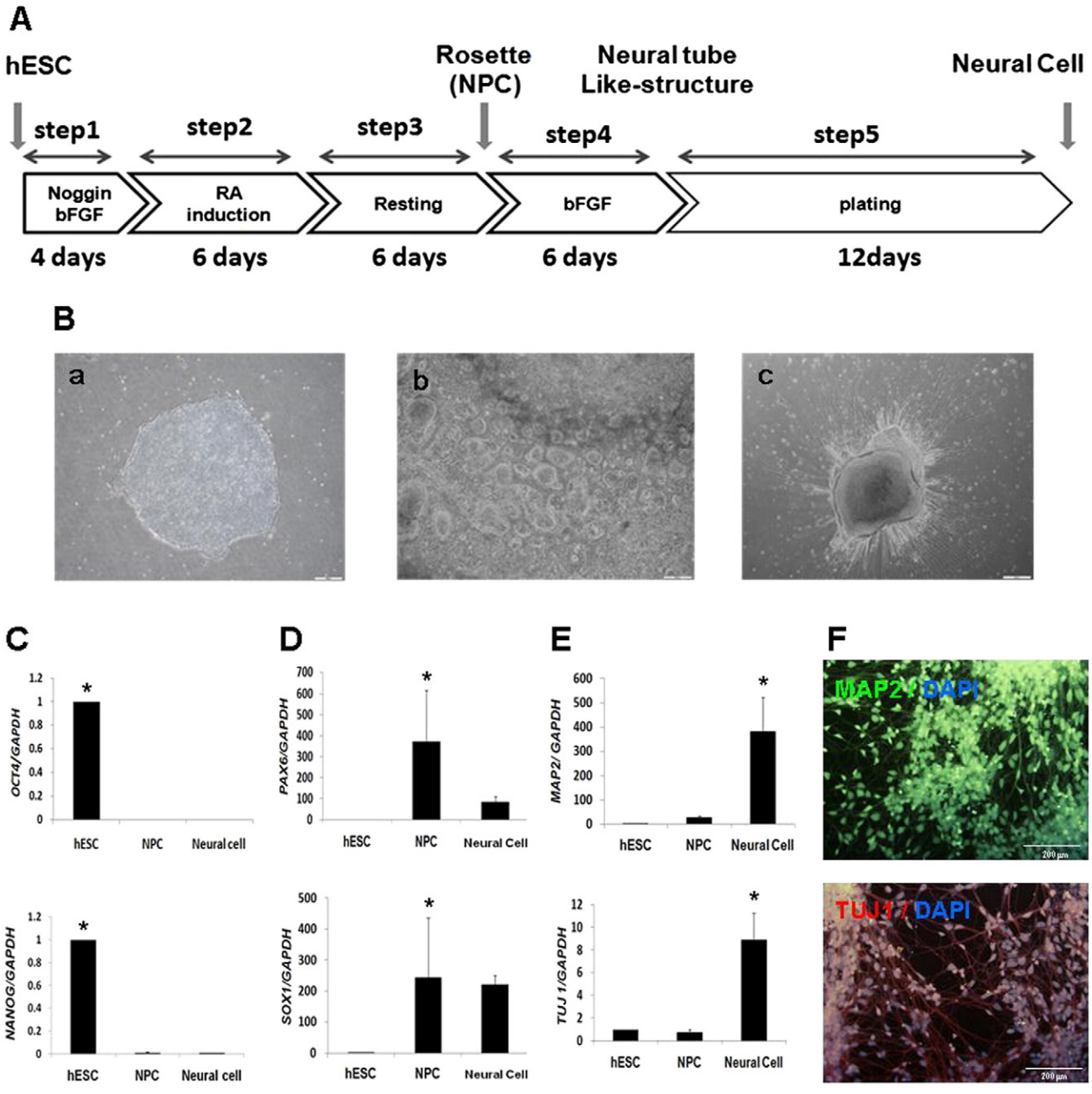

Fig. 1. Characterization of human embryonic stem cell (hESC)-derived cells during neural differentiation. Schematic illustration of the protocol of neural differentiation from hESCs (A). Phase contrast of cells selected to evaluate *FNDC5* expression during neural differentiation (B). A colony of hESCs grown on extracellular matrix at day 6 (a); typical rosette-neural progenitors appeared after retinoic acid (RA) induction in the outer margin of the colony (b); maturing neural cells produced in neurobasal medium at day 12 after plating of neural tubes (c). Evaluation of expressions of stem cell specific markers, *OCT4* and *NANOG* (C); Rosette-neural progenitor markers, *PAX6* and *SOX1* (D); and mature neural cell markers, *TUJ1* and *MAP2* (E). Relative expression of target genes normalized with *GAPDH*. Immunofluorescence staining of neural cells with antibodies against *MAP2* and *TUJ1* as mature neuron markers (F). Represented value bars are the mean of triplicate independent experiments ± SEM.*$p < 0.05$. Bar: 200 µm.