Journal of the Neurological Sciences 351 (2015) 160–167

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Analysis of differentially expressed lncRNAs in differentiation of bone marrow stem cells into neural cells

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ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 11 February 2015
Accepted 5 March 2015
Available online 12 March 2015

Keywords:
Differentiation
Bone marrow stem cells
Neural cells
lncRNA
mRNA
Microarray analysis

ABSTRACT

Many studies have reported micro RNAs involved in the differentiation of bone marrow mesenchymal stem cells (BMSCs) into neural cells; however, the roles of long non-coding RNAs (lncRNAs) in the differentiation of BMSCs into neural cells remain poorly understood. We used microarray assays to compare the lncRNA and messenger RNA (mRNA) expression profiles in BMSCs and neural-induced BMSCs. We found a total of 24 lncRNAs and 738 mRNAs that were upregulated and 32 lncRNAs and 682 mRNAs that were downregulated in samples induced for 3 h; 27 lncRNAs and 864 mRNAs that were upregulated and 37 lncRNAs and 968 mRNAs that were downregulated in 6 h samples; and 23 lncRNAs and 1159 mRNAs that were upregulated or downregulated in both the 3 h and 6 h samples. For 23 differentially lncRNAs and 83 differentially mRNAs, 256 matched lncRNA-mRNA pairs were found. GO (Gene ontology) analysis showed that these lncRNAs were associated with biological processes, cellular components, and molecular functions. Twenty-five pathways were identified by pathway analysis. Then, RT-qPCR validation of the differentially expressed H19, Esco2, Pcdhb18, and RGD1560277 genes confirmed the microarray data. Our study revealed the expression patterns of lncRNAs in the differentiation of BMSCs into neural cells, and many lncRNAs were differentially expressed in induced BMSCs, suggesting that they may play key roles in processes of differentiation. Our findings may promote the use of BMSCs to treat neurodegenerative diseases and trauma.

1. Introduction

Stem cells have great potential in treatments of spinal cord and brain injuries, neurodegenerative disorders, and stroke [1–4]. The self neural stem cells or embryonic stem cells were hard to obtain; however, one type of stem cells, bone marrow mesenchymal stem cells (BMSCs) can be easily isolated, expanded, and induced to differentiate into neuron-like cells under certain conditions [5–7]. In addition, compared to strategies involving neural stem cell or embryonic stem cell transplantation, the BMSCs could be obtained from the adult patient himself, and therefore, could avoid ethical dilemmas and immunological problems, and BMSCs are also of very low risk to develop into malignancy [8,9].

Long non-coding RNAs (lncRNAs) are a subset of noncoding RNAs longer than 200 nucleotides; they are participating in gene expression, such as epigenetic, transcriptional, and post-transcriptional regulation [10]. Altered lncRNA expression has been observed in differentiating embryonic stem (ES) cells [11], muscle cells [12], and somatic tissue progenitor cells [13]. Many studies proved that some microRNAs can promote the differentiation of BMSCs into neural cells [14,15]. However, the functions of lncRNAs in the differentiation of BMSCs into neural cells are still unknown.

To identify a novel molecule mechanism in the differentiation of BMSCs into neural cells, we investigated differences in lncRNA and mRNA expression profiles between BMSCs and neural differentiated BMSCs via microarray and bioinformatics analyses.

Table 1
Primers used for RT-qPCR analysis.

Primer name	Primer sequence (5′-3′)	Length (bp)
R-H19-S	CAGAGGGATTTTACAGCAAGGA	138
R-H19-A	ACTGAGCGGTTAGGGCATACA	
R-Pcdhb18-S	TGTTTGGCAGGTTCTGAGTTTG	89
R-Pcdhb18-A	CCCAGATCCTTTGCTAGATTCAC	
R-RGD1560277-S	GCGCTCTGAACTCACTACCACT	175
R-RGD1560277-A	CTCCTCTGTATCCCTCGCTCTA	
R-Esco2-S	ATCTCCAACATCACCACCGAT	81
R-Esco2-A	CCCAAAACTCTGCTACTATCCGT	
R-β-actin-S	TGCTATGTTGCCCTAGACTTCG	240
R-β-actin-A	GTTGGCATAGAGGTCTTTACGG	

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http://dx.doi.org/10.1016/j.jns.2015.03.011
0022-510X/

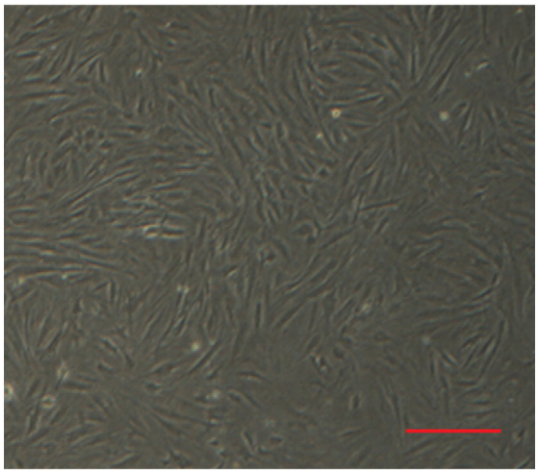

Fig. 1. BMSCs grew to be more spindle-shaped, fibroblastic morphology as the cell cultures approached confluency. Bar: 100 μm.

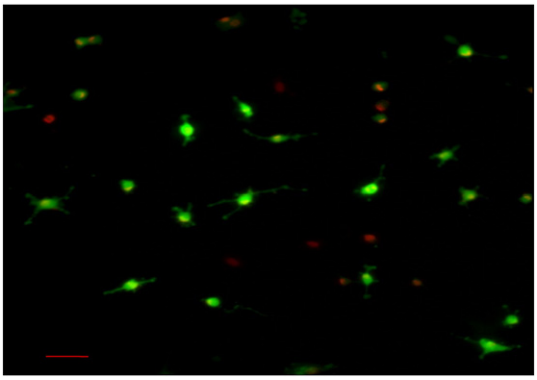

Fig. 3. NSE-positive image of the 6-h differentiated BMSCs by immunofluorescence staining. Bar: 50 μm. The red channel was stained by PI-stained nuclei.

2. Materials and methods

2.1. Ethics statement

All experiments in this study were approved by *the animal care and experimentation committee of Wenzhou Medical University*.

2.2. Cell culture and identification

We harvested the BMSCs from femurs of 3–4 week old Sprague–Dawley rats according to the method of Ni et al. [7]. The cells were suspended in α-minimal essential medium (α-MEM) (Gibco, USA), then, separated by Ficoll (Sigma, USA) density gradient centrifugation. Mononuclear cells, which were suspended in α-MEM, were plated in culture flasks (25-cm^2), and cultured at 37 °C, 5% CO_2 condition. Nonadherent cells were removed after about 24–48 h after culture. The BMSCs were spindle-shaped, fibroblastic morphology after the nonadherent cells were removed. Flow cytometry was used to identify BMSCs when they were at passage 4; antibodies include CD34 PE (Santa Cruz, USA), CD45 PE (Invitrogen Germany), CD73 FITC (BD Biosciences, USA), CD90 FITC (Invitrogen, Germany). The BMSCs were used for neuronal induction and further experiments at passage 5.

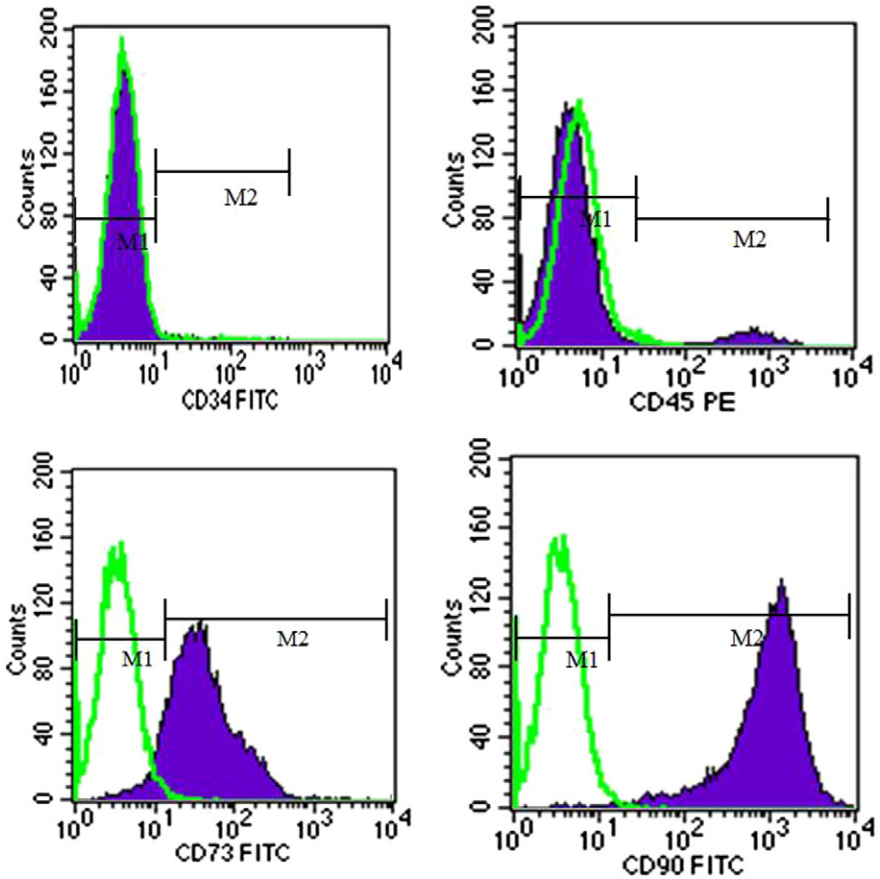

Fig. 2. Flow cytometry showed that BMSCs were strongly positive for CD73 and CD90 and negative for the hemopoietic markers CD34 and CD45.