ELSEVIER

Brain & Development 35 (2013) 349–355

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Enhanced expression of myogenic differentiation factors and skeletal muscle proteins in human amnion-derived cells via the forced expression of *MYOD1*

Yoshika Akizawa [a,b], Hitoshi Kanno [a,c], Yayoi Kawamichi [b], Yoshio Matsuda [b], Hiroaki Ohta [d], Hisaichi Fujii [c], Hideo Matsui [b], Kayoko Saito [a,*]

[a] *Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan*
[b] *Department of Obstetrics and Gynecology, Tokyo Women's Medical University, Tokyo, Japan*
[c] *Department of Transfusion Medicine and Cell Processing, Tokyo Women's Medical University, Tokyo, Japan*
[d] *Sanno Medical Center, International University of Health and Welfare, Japan*

Received 15 December 2011; received in revised form 12 May 2012; accepted 21 May 2012

Abstract

Objectives: Mesenchymal stem cells are expected to be an ideal cell source for cellular and gene therapy. We previously showed that cells derived from the human placenta can be induced to differentiate into myotubes *in vitro* and to express dystrophin in mdx/scid mice *in vivo*. In this study, we examined whether amnion-derived cells can be efficiently transduced and differentiated using lentiviral vectors carrying human *MYOD1*. *Methods:* The amnion-derived cells were isolated from human preterm placentas. They were transduced with the *MYOD1* vector, and mRNA levels for *MYOD1*, *MYF5, MYOG, MYH2* and *DMD* were determined by quantitative-reverse transcriptase-polymerase chain reaction, and also examined immunocytochemically. *Results:* Approximately 70% of amnion-derived cells were efficiently transduced by the lentiviral vectors. *MYOD1* activates *MYF5* and *MYOG*, *MYH2* and *DMD* after a 7-day culture. The concerted upregulations of these myogenic regulatory factors enhanced *MYH2* and *DMD* expressions. *PAX7* was below the detectable level. Both myosin heavy chain and dystrophin were demonstrated by immunocytochemistry. *Conclusions: MYOD1* activates *MYF5* and *MYOG*, the transcription factor genes essential for myogenic differentiation, and the concerted upregulation of these myogenic regulatory factors enhanced *MYH2* and *DMD* expressions. The amniotic membrane is an immune-privileged tissue, making *MYOD1*-transduced amnion-derived cells an ideal cell source for cellular and gene therapy for muscle disorders. This is the first report showing that amnion-derived cells can be modified by exogenous genes using lentiviral vectors. Furthermore, *MYOD1*-transduced amnion-derived cells are capable of the dystrophin expression necessary for myogenic differentiation.

Keywords: Duchenne muscular dystrophy; Mesenchymal stem cell; Cellular therapy; Gene therapy; Dystrophin; Placenta

1. Introduction

Duchenne muscular dystrophy (OMIM# 310200) is an X-linked recessive inherited disorder that affects 1 in 3500 males. The onset of Duchenne muscular dystrophyis usually before the age of 3 years, and patients die of respiratory failure around the age of 20 [1]. Duchenne muscular dystrophyis caused by structural mutations in

* Corresponding author. Address: Institute of Medical Genetics, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjyuku-ku, Tokyo 162-0054, Japan. Tel.: +81 3 3353 8111; fax: +81 3 5269 7689.
E-mail address: saito@img.twmu.ac.jp (K. Saito).

0387-7604/$ - see front matter © 2012 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.braindev.2012.05.012

the dystrophin gene (*DMD*), which encodes dystrophin, a large membrane-associated protein that plays an important role in linking intracellular cytoskeletal actin filaments to the sarcolemmal membrane [2]. Approximately 60% of *DMD* mutations are large deletions or insertions, whereas 40% are nonsense, missense, or small insertion-deletion mutations.

No curative therapeutic approaches for Duchenne muscular dystrophy currently exist. However, cell-based treatments in addition to gene therapy [3], exon skipping therapy [4], and read-through therapy with aminoglycosides [5] remain promising options.

Mesenchymal stem cells (MSC) are expected to be an ideal cell source for cellular and gene therapy because they can easily be obtained from bone marrow, adipose tissues, and the placenta, they are abundant and non-tumorigenic, and they have the useful characteristics of homing and chemokine secretion. MSC are already utilized for the treatment of graft versus host disease [6] and inflammatory bowel disease [7]. Several laboratories have shown that MSC can be obtained from amnion-derived cells and induced to differentiate into myocytes [8].

Although the myogenic differentiation of MSC can be induced by treating them with demethylating agents such as 5-azacythidine (5AZA), there is no marked enhancement of either *MYOD1,* the human myogenic differentiation factor 1 gene, or *MYH2* expression, nor does5AZA treatment substantially increase the myogenic differentiation of MSC [9]. In addition, there have been several attempts to enhance the myogenesis by introducing *MYOD1* into cells [10]. It was recently shown that human adipose-derived cells displayed enhanced myogenic differentiation after being forced to express *MYOD1* [11], and another group showed that forced expression of *MYOD1* led to the trans-differentiation of human fibroblasts into myotubes [12].

In this study, we introduced human *MYOD1* into amnion-derived cells using a lentiviral vector and examined the precise gene expression levels of *MYF5*, *MYOG*, *MYH2* and *DMD*. We demonstrated significant upregulations of the genes for essential transcription factors involved in myogenesis. The potential applications of*MYOD1*-transduced amnion-derived cells are also discussed.

2. Materials and methods

2.1. Isolation of human amnion-derived cells

Ethics approval for the tissue collection was granted by the Institutional Review Board of Tokyo Women's Medical University, Japan. Written informed consent was obtained prior to sample collection. Amnion tissue samples were obtained from normal full-term pregnancies at the time of caesarean section before the onset of labor. None of these pregnancies were complicated by premature membrane rupture or chorioamnionitis. The placentas were processed within 24 h of collection; i.e., they were thoroughly washed with phosphate-buffered saline (a solution containing sodium chloride, sodium phosphate, potassium chloride and potassium phosphate), and, after separation from the placentas, the amnions were minced into 5 mm sections using knives on a clean bench. The amnion tissue was placed in collagen I coated dishes (Iwaki, Japan), and after 20 min, Mesenchymal Stem Cell Basal Medium (MSCBM, Lonza, USA) was carefully poured onto the attached cells, which were then maintained at 37 °C in 5% CO_2. After 48 h, the non-adherent cells were removed, and the medium was changed twice a week. After about one week, a few colonies were found in the dishes. At 70–80% confluence, the amnion-derived cells were harvested with 0.5% Trypsin–EDTA (Life Sciences, USA) and plated onto new dishes. Cells were processed from 24 placentas, and primary cultures from 8 placentas were used for this study.

2.2. Flowcytometric analysis

The amnion-derived cells were used for fluorescent activated cell sorting (FACS) analysis employing the EPICS ALTRA XL-MCL analyzer (Beckman Coulter, USA), and the data were analyzed with EXPO™32 ADC software (Beckman Coulter). Antibodies against human CD14, CD29, CD34, CD44, CD45, CD73, CD105, CD166, HLA-ABC, and HLA-DR were obtained from Beckman Coulter and BD Biosciences Pharmingen (USA), AbD Serotec (UK) and Cytognos (Spain).

2.3. Production of lentiviral vectors and MYOD1 transduction of human amnion-derived cells

A full-length human *MYOD1* cDNA clone (Genome Network Project Clone, WW01A62C23) was provided by the RIKEN Bioresource Center (Ibaraki, Japan) through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan [13–16]. A lentiviral vector carrying the *MYOD1* cDNA, pLenti6/human*MYOD1,* was constructed using the pLenti6/UbC/V5-DEST Gateway Vector kit and the ViraPower Lentiviral Expression System (Life Technologies, USA). A GFP expression vector, pRRL.PPT.SF.IRES-GFP, was kindly provided by Taiju Utsugisawa.

Three micrograms of the purified pLenti6/UbC/V5-DEST – human*MYOD1* cDNA and pRRL.PPT.SF. IRES-GFP were used for the transfection of 4×10^6 293FT cells together with Lipofectamine 2000 (provided with the kit) reagent and ViraPower packaging Mix (provided with the kit). After 48 h, the supernatant