Tissue and Cell 49 (2017) 495–502

Contents lists available at ScienceDirect

Tissue and Cell

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

A Comparative Study to Evaluate Myogenic Differentiation Potential of Human Chorion versus Umbilical Cord Blood-derived Mesenchymal Stem Cells

Nikoo Bana[a,1], Davood Sanooghi[b,1], Mansoureh Soleimani[c,d], Nasim Hayati Roodbari[a], Sepideh Alavi Moghaddam[a], Mohammad Taghi Joghataei[c,d,e], Forough Azam Sayahpour[f], Faezeh Faghihi[c,*]

[a] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[b] Department of Genetics, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
[c] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
[d] Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[e] Department of Neuroscience, School of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
[f] Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

ARTICLE INFO

Article history:
Received 21 January 2017
Received in revised form 1 May 2017
Accepted 10 May 2017
Available online 11 May 2017

Keywords:
Mesenchymal Stem Cells
5-Azacytidine
Umbilical Cord Blood
Chorion
Myogenic differentiation

ABSTRACT

Objective: Musculodegenerative diseases threaten the life of many patients in the world. Since drug administration is not efficient in regeneration of damaged tissues, stem cell therapy is considered as a good strategy to restore the lost cells. Since the efficiency of myogenic differentiation potential of human Chorion- derived Mesenchymal Stem Cells (C-MSCs) has not been addressed so far; we set out to evaluate myogenic differentiation property of these cells in comparison with Umbilical Cord Blood-derived Mesenchymal Stem Cells (UCB-MSCs) in the presence of 5-azacytidine.
Materials & methods: To do that, neonate placenta Umbilical Cord Blood were transferred to the lab. After characterization of the isolated cells using flowcytometry and multilineage differentiation capacity, the obtained Mesenchymal Stem Cells were cultured in DMEM/F12 supplemented with 2% FBS and 10 μM of 5-azacytidine to induce myogenic differentiation. Real-time PCR and immunocytochemistry were used to assess the myogenic properties of the cells.
Results: Our data showed that C-MSCs and UCB-MSCs were spindle shape in morphology. They were positive for CD90, CD73 and CD44 antigens, and negative for hematopoietic markers. They also differentiated into osteoblast and adipoblast lineages. Real-time PCR results showed that the cells could express MyoD, desmin and α-MHC at the end of the first week ($P < 0.05$). No significant upregulation was detected in the expression of GATA-4 in both groups. Immunocytochemical staining revealed the expression of Desmin, cTnT and α-MHC.
Conclusions: Results showed that these cells are potent to differentiate into myoblast- like cells. An upregulation in the expression of some myogenic markers (desmin, α- MHC) was observed in C-MSCs in comparison with UCB-MSCs.

1. Introduction

Musculodegenerative diseases threaten the life of many patients in the world. Since drug administration is not efficient in regeneration of damaged tissues, stem cell therapy is considered as a good strategy to restore the lost cells (Sohn and Gussoni, 2004).

Mesenchymal Stem Cells, by their self- renewal ability, multilineage differentiation capacity and immunomodulatory properties are currently the most desirable cell candidate to be used in cell therapy and regenerative medicine (Chan et al., 2004; Cossu and Mavilio, 2000; Cui et al., 2007). Mesenchymal Stem Cells can be isolated from various tissue types; including bone marrow (Antonisis et al., 2008), lung (Lama et al., 2007), fat (Gronthos et al., 2001),

* Corresponding author.
E-mail addresses: Faghihi.f@iums.ac.ir, faezefaghihi@yahoo.com (F. Faghihi).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tice.2017.05.003
0040-8166/

liver (Wang et al., 2016), umbilical cord (Claire Mennan et al., 2013), amniotic fluid (Kim et al., 2013), placenta (Vellasamy et al., 2012), and Umbilical Cord Blood (Sibov et al., 2012). There are some reports which indicate the differentiation potential of these cells into skeletal muscles (Galli et al., 2014), cardiomyocytes (Pittenger and Martin, 2004) and smooth muscles (Xiao et al., 2009). Among various source of cells, Human Umbilical Cord Blood and chorion tissues are two major ethically approved reservoirs of Mesenchymal Stem Cells. Ease of recruitment, immunomodulatory properties and multilinege differentiation capacity are criteria which made these cells as appropriate type of cells in tissue engineering and regenerative medicine (Kwon et al., 2016; Pittenger et al., 1999).

As a hypomethylating agent, 5-Azacytidine is widely recruited to influence the differentiation capability of stem cells nonspecifically (Bel et al., 2003). It has been shown that 5- azacytidine, as a DNA methylating agent, could induce the differentiation of cells into multiple cellular phenotypes such as myocytes (Tomita et al., 1999; Taylor and Jones, 1979; Namjoo et al., 2013) and β- cells (Pennarossa et al., 2013). Since the efficiency of myogenic differentiation potential of human Chorion- derived Mesenchymal Stem Cells (C-MSCs) has not been addressed so far, in this study we set out to evaluate myogenic differentiation property of these cells in comparison with Umbilical Cord Blood- derived Mesenchymal Stem Cells (UCB-MSCs) in the presence of 5- azacytidine.

2. Materials and methods

2.1. Isolation and culture of human Chorion- derived Mesenchymal Stem Cells (C-MSCs)

Human C-MSCs were isolated according to our lab protocol (Faghihi et al., 2016). Briefly, the neonate placenta was moved to the lab after obtaining maternal informed consent. Using of the cells for research was approved by the Ethical Committee of Iran University of Medical Sciences. In the lab, chorion tissue was washed in phosphate-buffered saline (PBS; Caisson, USA) to remove blood cells. After cutting into small pieces, the tissue was digested with 0.3% type II collagenase solution (Gibco, Germany) at 37 °C for 30 min. Then the enzyme was deactivated using PBS and the cell suspension was centrifuged at 1200 rpm for 5 min. The number of 5000 cell/cm^2 was plated in a tissue flask (SPL, Korea) that contained the expansion medium composed of DMEM/F12 (Caisson, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Germany), 10000 U/ml penicillin and 10000 μg/ml streptomycin (Caisson, USA). Cells were kept in a humidified chamber at 37 °C and 5% CO^2. The non-adherent cells were removed three days later. At 70–80% confluence, the cells were trypsinized and moved into three new culture flasks. Cells at passage 3 (P#3) were used in the following experiments.

2.2. Isolation of human Umbilical Cord Blood- derived Mesenchymal Stem Cells (UCB-MSCs)

Human Umbilical Cord blood mononuclear cells were isolated based on our lab protocol (Faghihi et al., 2008; Yousefi et al., 2017). To do that, human UCB was collected from the umbilical vein of the neonates after elective caesarean section. The use of cells for research was approved by the Ethics Committee at Iran University of Medical Sciences. To isolate mononuclear cells, blood samples were collected by anticoagulant- treated syringe. The samples were transferred into sterile tubes (Falcon, England) and processed within 4 h. Hydroxyethylstarch (10%, Sigma, USA) was used to remove red blood cells. The rest of the cells were diluted in washing buffer containing PBS, supplemented with 0.2% EDTA (Sigma,USA), 10000 U/ml penicillin, 10000 μg/ml streptomycin and 15% FBS. The mononuclear cells (MNCs) were obtained after centrifugation of the cell suspension over Ficoll-Hypaque (Inno- train, Germany) at 1500 rpm for 15 min. Number of 100,000 MNCs were seeded in each cm^2 of 75-cm^2 culture flasks containing DMEM/F12 supplemented with 10% fetal bovine serum, 10,000 U/ml penicillin, and 10,000 μg/ml streptomycin. The cultures were maintained at 37 °C in 5%CO_2 for 5 days. Then the Medium was changed every 3 days. At 70–80% confluence, the cells were trypsinized and then moved into three new culture flasks. Cells at passage 3 (P#3) were used in the following experiments.

Table 1
List of antibodies used in this study.

Antibody Name	Brand	Catalog No.
PE Mouse Anti-Human CD73	BD Biosciences	550257
Mouse Anti-Human CD45 FITC/CD34 PE	BD Biosciences	341071
PE Mouse Anti-Human CD90	BD Biosciences	561970
FITC Mouse Anti-Human CD44	BD Biosciences	347943
PE Mouse IgG1, κ Isotype Control	BD Biosciences	555749
FITC Mouse IgG1, κ Isotype Control	eBioscience	11-4714-71
Mouse Anti-Desmin antibody	Abcam	ab8470
Mouse Anti-Cardiac Troponin T antibody	Abcam	ab8295
Rabbit Anti-heavy chain Myosin antibody	Abcam	ab124205
Rabbit Anti-GATA4 antibody	Abcam	ab134057
Mouse Anti-MyoD antibody	Santa Cruz Biotechnology	sc-32758
Donkey Anti-Mouse IgG H&L (PE)	Abcam	ab7003
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	Abcam	ab150077

2.3. Characterization of the isolated cells

2.3.1. Flow cytometry

To investigate mesenchymal stemness properties of the isolated cells, Cytofluorimetric analysis was carried out for the cells at passage 3. According to our lab protocol (Faghihi et al., 2016), the approximate number of 10^5 cells was pre incubated with 10% goat serum (Sigma, USA) at 4 °C. One hour later, the serum was removed and the cells were incubated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies against human CD34, CD44, CD45, CD73, and CD90 (all from BD bioscience, USA) at 4 °C for 40 min. Isotype-matched antibodies were used as control. The level of fluorescence greater than 95% was defined as positive expression. Becton-Dickinson flow cytometer was used to detect the expression of the antigens, and data analysis was performed by Flomax software. The detailed information of the antibodies has been listed in the Table 1.

2.4. Differentiation into mesodermal lineages

To assess mesenchymal stemness properties of the isolated cells, we checked their ability to differentiate into osteoblast and adipoblast cells, according to our lab protocols (Faghihi et al., 2015). To do that, number of 50,000 cells were plated in a 6-well plates (SPL, Korea) containing expansion medium composed of DMEM/F12, 10% FBS and 10,000 U/ml penicillin and 10,000 μg/ml streptomycin for three days. At day three the medium was removed