Cytotherapy, 2018; 20: 919–929

International Society
ISCT
Cell & Gene Therapy

Epigenetic changes in umbilical cord mesenchymal stromal cells upon stimulation and culture expansion

SAMANTHA F.H. DE WITTE[1], FLEUR S. PETERS[1], ANA MERINO[1], SANDER S. KOREVAAR[1], JOYCE B.J. VAN MEURS[2], LISA O'FLYNN[3], STEVE J. ELLIMAN[3], PHILIP N. NEWSOME[4,5,6], KARIN BOER[1], CARLA C. BAAN[1] & MARTIN J. HOOGDUIJN[1]

[1]Nephrology and Transplantation, [2]Endocrinology, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands, [3]Orbsen Therapeutics Ltd., Galway, Ireland, [4]National Institute for Health Research Biomedical Research Centre at University Hospitals Birmingham National Health Service, Foundation Trust and the University of Birmingham, [5]Centre for Liver Research, Institute of Immunology and Immunotherapy, University of Birmingham, and [6]Liver Unit, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Abstract

Background. Mesenchymal stromal cells (MSCs) are studied for their immunotherapeutic potential. Prior to therapeutic use, MSCs are culture expanded to obtain the required cell numbers and, to improve their efficacy, MSCs may be primed *in vitro*. Culture expansion and priming induce phenotypical and functional changes in MSCs and thus standardisation and quality control measurements come in need. We investigated the impact of priming and culturing on MSC DNA methylation and examined the use of epigenetic profiling as a quality control tool. *Methods.* Human umbilical cord–derived MSCs (ucMSCs) were cultured for 3 days with interferon (IFN)γ, transforming growth factor (TGF)β or a multi-factor combination (MC; IFNγ, TGFβ and retinoic acid). In addition, ucMSCs were culture expanded for 14 days. Phenotypical changes and T-cell proliferation inhibition capacity were examined. Genome-wide DNA methylation was measured with Infinium MethylationEPIC Beadchip. *Results.* Upon priming, ucMSCs exhibited a different immunophenotype and ucMSC(IFNγ) and ucMSC(MC) had an increased capacity to inhibit T-cell proliferation. DNA methylation patterns were minimally affected by priming, with only one significantly differentially methylated site (DMS) in IFNγ- and MC-primed ucMSCs associated with autophagy activity. In contrast, 14 days after culture expansion, ucMSCs displayed minor phenotypical and functional changes but showed >4000 significantly DMSs, mostly concerning genes involved in membrane composition, cell adhesion and transmembrane signalling. *Discussion.* These data show that DNA methylation of MSCs is only marginally affected by priming, whereas culture expansion and subsequent increased cellular interactions have a large impact on methylation. On account of this study, we suggest that DNA methylation analysis is a useful quality control tool for culture expanded therapeutic MSCs.

Key Words: *culture expansion, DNA methylation, epigenetics, mesenchymal stromal cell, priming, quality control*

Introduction

Mesenchymal stromal cells (MSCs) have been extensively examined in clinical trials regarding their immunotherapeutic potential [1–4]. Prior to their application in the clinic, MSCs are commonly expanded to obtain clinically relevant numbers. However, during long-term *in vitro* culture expansion the phenotype and function of MSCs are affected [5–7]. Previous studies have shown that during long-term expansion their proliferative capacity decreases [7,8]. In addition, long-term culture expansion affects the immunomodulatory properties of MSCs, for instance, their capacity to inhibit T-cell proliferation [8]. Recently, there has been growing interest in the optimization of the immunomodulatory properties of MSCs *in vitro*. MSCs can be primed with stimuli to enhance their immunomodulatory properties with the aim to improve their therapeutic efficacy [9–17]. Prior to their clinical application, MSCs are routinely tested for multiple parameters to assess their safety and functionality, such as karyotype, morphology (spindle-shape) and viability, as well as their cell surface protein expression and differentiation capacity according to the recommendations of the International Society for Cellular Therapy [1–4,18–21]. These tests give a global

Correspondence: **Martin J. Hoogduijn,** PhD, Department of Internal Medicine, Erasmus Medical Center, Postbus 2040, 3000 CA Rotterdam, the Netherlands.
E-mail: m.hoogduijn@erasmusmc.nl

(Received 24 January 2018; accepted 8 May 2018)

ISSN 1465-3249 Copyright © 2018 International Society for Cellular Therapy. Published by Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.jcyt.2018.05.005

indication of the state of MSCs. However, MSCs have a great ability to adapt and culture expansion and priming may, therefore, modify MSCs on a different level. Therefore, we endeavored to perform a more in-depth analysis of the effects of culture expansion and stimulation on MSCs.

Epigenetic modifications of the genome can be both hereditary as well as environmentally influenced. These epigenetic modifications affect gene expression without altering the genomic sequence and are important regulators of cellular function [22–25]. Methylation of cytosines at cytosine-phosphate-guanine (CpG) sites in the DNA is one of the main mechanisms of epigenetic modifications. Methylation at a CpG site may block the start of transcription, and, in particular, methylation of CpG islands at transcriptional start sites (TSSs) is associated with long-term gene silencing [22]. *In vitro* procedures may affect DNA methylation, potentially resulting in changes in their gene expression and subsequently their phenotype and function.

Previously, it was demonstrated that there is an association between osteogenic differentiation of MSCs and their DNA methylation pattern [26–29]. In addition, other studies demonstrated that during long-term culture expansion, where MSC were cultured more than 10 passages, MSCs became senescent and their DNA methylation patterns changed [30,31]. However, no study to date has addressed the effect of priming MSCs *in vitro* with various stimuli to optimize their immunomodulatory properties on the DNA methylation. Furthermore, it remains unclear whether during culture expansion DNA methylation patterns of MSCs are affected. Elucidation of the effects of MSC expansion and priming on DNA methylation may result in additional quality control tools that help in development and application of MSC therapy in the clinical setting. This will ensure the use of better standardized MSC therapeutic products. Therefore, in this study, we investigated the changes in methylation in the epigenome of MSCs during priming by various stimuli and also after 2 weeks of culture expansion.

Materials and methods

Isolation and culture of MSCs

Human umbilical cord tissue was collected by Tissue Solutions Ltd. from Caesarean section deliveries from virally screened healthy donors. Whole cord tissue of the neonatal side was used for MSC isolation. All cord tissues provided by Tissue Solutions were obtained according to the legal and ethical requirements of the country of collection, with the approval of an ethics committee (or similar) and with anonymous consent from the donor. Isolation of the $CD362^{+}$ subset of human umbilical cord–derived MSCs (ucMSCs) was performed according to previous studies by de Witte *et al.* [8,10]. After isolation, each cell fraction was counted, seeded for expansion and cryopreserved at passage two for shipment to Erasmus Medical Center. Here ucMSCs were cultured in minimum essential medium Eagle alpha modification (MEM-α; Sigma-Aldrich) containing 2 mmol/L L-glutamine (Lonza), 1% penicillin/streptomycin solution (P/S; 100IU/mL penicillin, 100IU/mL streptomycin; Lonza) and supplemented with 15% batch tested fetal bovine serum (FBS; Lonza) and 1ng/mL basic fibroblast growth factor (bFGF) (Sigma) and kept at 37°C, 5% CO_2 and 20% O_2. Once a week the medium was refreshed and ucMSCs were passaged using 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA; Life Technologies) at ~80–90% confluence. All ucMSCs used in the experiments were between passage three and six.

Characterization of ucMSCs was performed using flow cytometric analysis of the cell surface markers: CD31 (Pacific blue; BD Biosciences), CD45 (APC-H7: Allophycocyanin-Horizon7; BD Biosciences), CD13 (Phycoerythrin-cyanine7; BD Biosciences), CD73 (Phycoerythrin; BD Pharmingen), CD90 (Allophycocyanin; R&D Systems) and CD105 (Fluorescein isothiocyanate conjugate; R&D Systems). After labeling the cells were washed and measured on the FACSCanto II flow cytometer (BD Biosciences; Supplementary Figure 1).

Experimental design

The experimental design consists of two parts: 'Priming of MSC' and 'Culture expansion of MSC' (Figure 1).

Priming of ucMSCs

ucMSCs of four different umbilical cord donors were stimulated with factors that were known to modify MSC function or phenotype, as demonstrated in previous studies [10,32]. At day 0, MSCs (confluent culture) were stimulated for 3 days with interferon gamma (IFNγ; 50 ng/mL; Life Technologies), transforming growth factor beta 1 (TGFβ; 10 ng/mL; R&D systems) or a multi-factor combination (MC) of IFNγ, TGFβ and retinoic acid (RA; 100 μmol/L; Sigma). At day 3, cells were trypsinised and either used for experiment or snap frozen in pellets containing 300,000 cells and stored at −80°C.

Culture expansion of MSCs

At day 0, MSCs of four different umbilical cord donors were seeded (250,000cells/T175 flask). Medium was partly refreshed (50%) every 5/6 days. At day 14, cells were used for experiment or snap frozen as pellets for future use.

Download English Version:

https://daneshyari.com/en/article/8956269

Download Persian Version:

https://daneshyari.com/article/8956269

Daneshyari.com