Cytotherapy, 2016; 18: 336–343

International Society for Cellular Therapy
ISCT®

Chromosomal stability of mesenchymal stromal cells during *in vitro* culture

BRIAN G. STULTZ, KATHLEEN MCGINNIS, ELAINE E. THOMPSON, JESSICA L. LO SURDO, STEVEN R. BAUER & DEBORAH A. HURSH

Division of Cell and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA

Abstract

Background aims. Mesenchymal stromal cells (MSCs) are being investigated for use in cell therapy. The extensive *in vitro* expansion necessary to obtain sufficient cells for clinical use increases the risk that genetically abnormal cells will arise and be propagated during cell culture. Genetic abnormalities may lead to transformation and poor performance in clinical use, and are a critical safety concern for cell therapies using MSCs. *Methods.* We used spectral karyotyping (SKY) to investigate the genetic stability of human MSCs from ten donors during passaging. *Results.* Our data indicate that chromosomal abnormalities exist in MSCs at early passages and can be clonally propagated. The karyotypic abnormalities observed during our study diminished during passage. *Conclusions.* Karyotyping of MSCs reveals characteristics which may be valuable in deciding the suitability of cells for further use. Karyotypic analysis is useful for monitoring the genetic stability of MSCs during expansion.

Key Words: *genomic stability, mesenchymal stromal cells, MSC, SKY, spectral karyotyping*

Introduction

Human mesenchymal stromal cells (hMSCs) are derived from several tissues including bone marrow. They are plastic-adherent in tissue culture; can differentiate into osteoblasts, chondrocytes and adipocytes; and express cell surface markers CD73, CD90, CD105, but not CD11b, CD14, CD19, CD34, CD45 and CD79α [1]. MSCs are being investigated in clinical trials for a variety of indications.

MSCs are often significantly expanded to obtain sufficient cells for therapy, which increases the probability that genetic changes may arise. Genetic stability and potential transformation leading to tumor growth are safety concerns for stem cell therapies [2]. Chromosomal alterations are associated with increased tumorigenicity and the inability to reach desired differentiation states [3,4]. Additionally, there is donor-to-donor variability in the genetic stability of cells used for therapy [5]. To explore effects of extended passage and donor source on genetic stability, we performed karyotypic analysis of hMSCs as part of a larger analysis of attributes of these cells that might predict safety and performance in clinical use [6–12].

High rates of aneuploidy, escape from replicative senescence and transformation in rodent MSCs prompted concern for the use of hMSCs [13,14]. However, studies on hMSCs report low levels of chromosomal aberrations among donor samples [15,16] and during culture expansion [5,17,18]. Low levels of non-clonal chromosomal aberrations in MSCs used in clinical trials have been observed, but there are no reports of malignant transformation [19]. The evidence of malignant transformation during *in vitro* expansion is mixed [5,20] because initial reports for hMSCs were a result of contaminated cell lines [21,22]. However, a recent article reported that verified hMSCs exhibited spontaneous tumorigenic transformation associated with genomic alterations during culture [23], and thus MSCs tumorigenic potential remains unresolved. Additionally, the significance of chromosomal alterations or the effect of genetic instability on therapeutic performance is poorly understood.

Multiple methods are available to assess chromosomal stability of cells including Giemsa (G) banding, fluorescence in situ hybridization (FISH), spectral karyotyping (SKY) and comparative genomic hybridization (CGH). SKY is a rapid FISH-based method

Correspondence: **Deborah A. Hursh**, PhD, Division of Cell and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, Building 72, Room 3216, Silver Spring, MD 20993, USA. E-mail: deborah.hursh@fda.hhs.gov

(Received 11 June 2015; accepted 18 November 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.11.017

in which chromosome-specific fluorescent labels are used to visualize all chromosomes in a single hybridization [24,25]. Using SKY, we identified genomic aberrations in bone marrow–derived hMSCs from multiple donors and at multiple passages to analyze the chromosomal stability of each cell line during culture. We found that chromosomal aberrations both exist and arise in culture-expanded MSCs and could be clonally propagated. However, in all cases, the aberrations diminished with extended culture, suggesting that they did not offer a replicative advantage, providing further evidence of the general genetic stability of hMSCs.

Methods

Cell culture

Bone marrow–derived hMSCs from 10 donors were purchased from All Cells or Lonza as passage 1 vials and designated with identifiers indicating donor. Both AllCells and Lonza use standard protocols for isolation of MSCs from bone marrow and culture cells in Mesencult (Stem Cell Technologies) or MSCGM (Lonza), respectively. MSCs were expanded in culture medium composed of α-minimum essential medium (Life Technologies), 1% L-glutamine (Life Technologies), 1% penicillin and streptomycin (Life Technologies) and 16.5% fetal bovine serum (JM Bioscience) to passages 3, 5 and 7 (P3, P5 and P7), as previously described [7]. Passage number equals times cells were trypsinized until cryopreservation. Expansion lots PCBM1641, PCBM1632, 167696, 110877, 8F3560, PCBM1662 and 127756 were tested for expression of cell surface markers by flow cytometry [7], and growth kinetics using an IncuCyte Live Cell Imager [8]. Additionally, colony-forming units, quantitative adipogenic differentiation, proteomic profiling, transcriptome analysis, T-cell immunomodulation and changes in chromatin modifications have been described for these lots [6–12].

Metaphase spreads

Cryopreserved cells were cultured to 70–80% confluence, then 10 μL/mL of 10% demecolcine (Sigma Aldrich) was added. After 4–5 hours, cells were trypsinized, centrifuged and resuspended in a few drops of media, and a hypotonic solution (0.2% potassium chloride, 0.2% sodium citrate, and 0.01% fetal bovine serum) was added dropwise. Cells were incubated at 37°C for 20 min, centrifuged and resuspended in hypotonic solution. Fixative (3:1 solution of methanol/acetic acid) was added dropwise. Cells were incubated 15 min at room temperature, centrifuged and resuspended in fixative twice. Resuspended cells were dropped onto clean slides over a 60°C water bath and air-dried. Slides were microscopically examined for metaphase spreading and stored 5 to 15 days at room temperature before SKY.

Karyotyping

Chromosome hybridization for spectral karyotyping was performed using the human SkyPaint and CAD detection kits (Applied Spectral Imaging) according to the company protocol. Metaphase chromosomes were analyzed on a Nikon Eclipse E800 microscope equipped with the HISKY system and software (Applied Spectral Imaging). We observed variable random loss of chromosomes, likely because of the technical preparation of slides. Unless multiple karyotypes exhibited the same chromosomal loss, these data were excluded from the analysis. Karyotyping followed the International System for Chromosome Nomenclature 2009 [26].

Giemsa stained mitotic chromosome preparation (G-banding) was performed by the WiCell cytogenetics laboratory. Twenty metaphase spreads were analyzed for each sample.

Statistical analysis

The percent abnormal karyotypes were calculated for all samples and technical replicates averaged. This resulted in heteroscedastic data requiring nonparametric analysis. Friedman's test, a repeated-measures analysis of variance on ranks, was performed. To test sampling bias within donors, contingency tables were constructed from counts of normal and abnormal spreads and two-tailed P values calculated using Fisher's exact test for 2×2 tables or the Freeman-Halton test for larger tables. Data was analyzed using SAS system for Windows, Version 9.3 (Copyright 2012, SAS Institute).

Results

Primary characterization of MSCs

We used SKY to analyze chromosomal stability of MSCs derived from different donors expanded through seven passages. The International System for Human Cytology Nomenclature 2009 report [26] recommends scoring a minimum of 20 mitotic spreads and defines a clonal population as two of the same abnormal karyotype. To increase the robustness of our data, we attempted to collect a minimum of 40 mitotic figures per sample; however, this was not always feasible because of the diminishing proliferative potential of some MSC lines. MSCs were derived from young or middle-aged donors (Figure 1A).

The primary karyotype of PBMC1632 displayed a Robertsonian Translocation of chromosomes t(13;14) (Figure 1B). We also identified two MSC lines with karyotypically abnormal subpopulations at passage 3