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Culture expansion induces non-tumorigenic aneuploidy in adipose tissue-derived mesenchymal stromal cells

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Abstract

Background aims. Adipose tissue-derived mesenchymal stromal cells (ASCs) are of interest as a cell therapeutic agent for immunologic and degenerative diseases. During *in vitro* expansion, ASCs may be at risk for genetic alterations, and genetic screening is a prerequisite. We examined the presence of aneuploidy in ASCs and its origin and development during culture and evaluated the implications of aneuploidy for therapeutic use of ASCs. *Methods.* Adipose tissue of healthy individuals was used for isolation and expansion of ASCs. Chromosome copy numbers were studied using fluorescence *in situ* hybridization analysis. Aneuploidy was studied in freshly isolated ASCs, in ASCs cultured for 0–16 passages and in senescent cultures. To evaluate the plasticity of ploidy, ASCs were cloned, and the variation of ploidy in the clones was examined. Tumorigenicity was studied by subcutaneous injection of aneuploid ASCs in immunodeficient NOD/SCID mice. *Results.* No aneuploidy was detected in freshly isolated ASCs. In low passages (passages 0–4), aneuploidy was detected in 3.4% of ASCs. Prolonged culture expansion of ASCs (passages 5–16) resulted in a significant increase of aneuploidy to 7.1%. With senescence, aneuploidy increased further to 19.8%. Aneuploidy was observed in clones of diploid ASCs, demonstrating the *de novo* development of aneuploidy. No transformation of ASCs was observed, and in contrast to cancer cell lines, aneuploid ASCs were incapable of tumor formation in immunodeficient mice. *Conclusions.* ASC cultures contain a stable percentage of aneuploid cells. Aneuploidy was not a predecessor of transformation or tumor formation. This finding indicates that aneuploidy is culture-induced but unlikely to compromise clinical application of ASCs.

Key Words: *adipose tissue, aneuploidy, clinical application, mesenchymal stromal cell, senescence, transformation*

Introduction

Mesenchymal stromal cells (MSCs) have immunomodulatory and regenerative capacities (1–4). They are of interest as a cell therapeutic agent for various medical conditions, including Crohn disease, graft-versus-host disease, and solid-organ transplantation (1,5,6). At the present time, >300 clinical trials are registered to study the applicability and effects of MSC administration (April 2013, http://clinicaltrials.gov). In the absence of a specific marker, MSCs are defined by the criteria set by the International Society for Cellular Therapy as plastic adherent cells with a $CD73^+$, $CD90^+$, $CD105^+$, $CD14^-$, $CD34^-$, $CD45^-$ and human leukocyte antigen (HLA)-DR^- immunophenotype and the capacity to differentiate into osteoblasts, adipocytes and chondrocytes (7). The primary source of MSCs is bone marrow; however, MSCs reside in virtually all tissues (8), of which adipose tissue is the most accessible and most suitable for the isolation of cells for research and therapeutic purposes. Adipose tissue-derived mesenchymal stromal cells (ASCs) are very similar to bone marrow-derived MSCs (9,10), although un-cultured ASCs express CD34 (11,12).

For most clinical applications, ASCs are expanded in culture to obtain sufficient numbers of cells. During expansion, ASCs may be at risk for transformation. To ensure patient safety, ASCs should be subjected to strict release criteria,

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similar to MSCs (13). However, until now, genetic screening of clinical-grade MSCs has been limited to karyotyping of a small number of cells (1,2,13,14).

Pre-clinical research evaluating the genetic stability of MSCs has resulted in contradicting outcomes. Studies reporting spontaneous transformation of MSCs in culture (15,16) were retracted when cell cultures appeared to be contaminated with tumor cell lines (17,18). Nevertheless, there is evidence that aneuploidy occurs in cultured MSCs derived from both bone marrow and adipose tissue (19–22). These chromosomal aberrations also have been detected in MSCs used in clinical trials (23). Because aneuploidy has been suggested to be associated with cancer (24), aneuploidy in MSC cultures may be a worrisome finding, and its detection in MSC cultures warrants thorough investigation.

To date, it is unknown whether aneuploidy in ASCs is already present *in vivo* or whether it is induced during the culture process. How aneuploidy evolves during further extensive culture expansion is also unknown. Additionally, it is unknown whether aneuploidy is associated with transformation or tumor formation and whether it compromises the safety of clinical application of ASCs. The aim of this study is to examine the occurrence and functional implications of aneuploidy in ASCs.

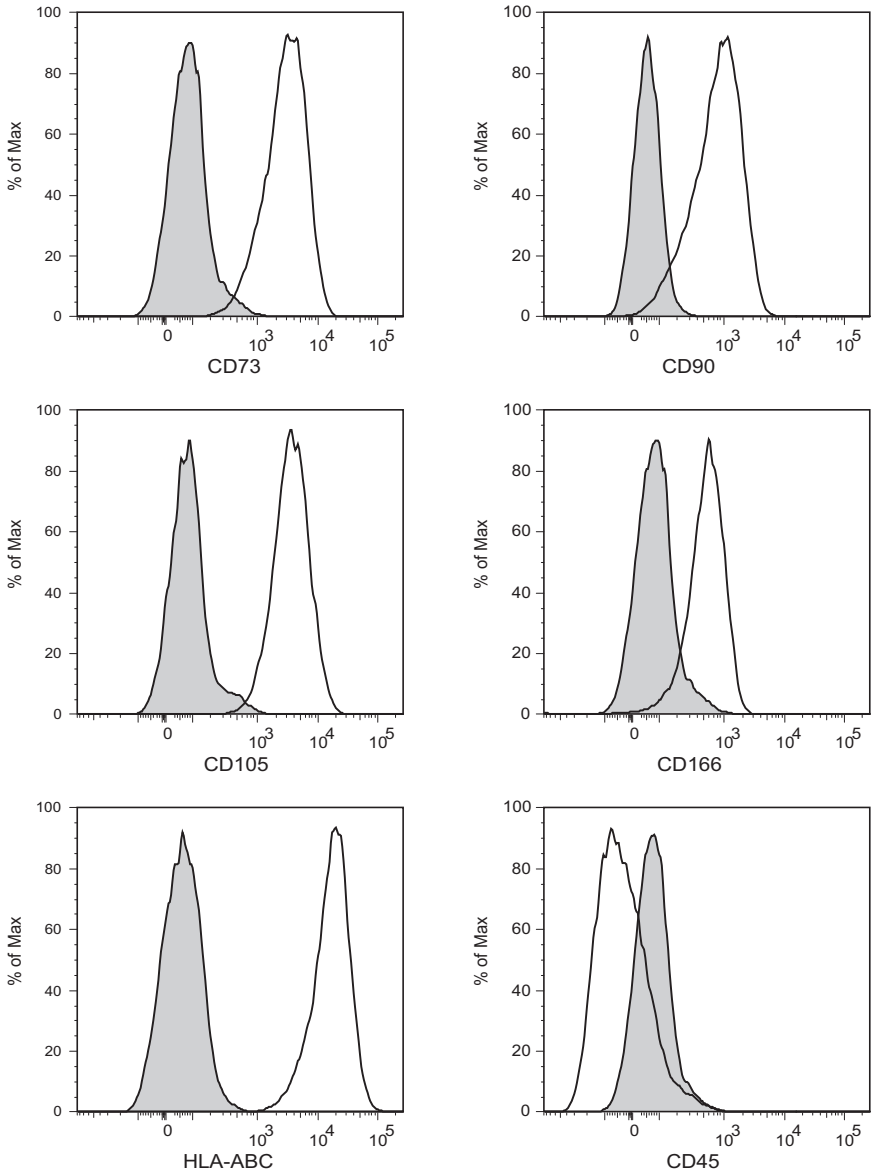

Figure 1. Immunophenotype of ASCs. Gray histograms represent unstained cells, and open histograms represent stained cells. Representative culture is shown.