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Chromosomal aberrations and deoxyribonucleic acid single-strand breaks in adipose-derived stem cells during long-term expansion *in vitro*

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Abstract

Background aims. Adipose-derived stem cells (ASCs) are a promising mesenchymal cell source for tissue engineering approaches. To obtain an adequate cell amount, *in vitro* expansion of the cells may be required in some cases. To monitor potential contraindications for therapeutic applications in humans, DNA strand breaks and chromosomal aberrations in ASCs during *in vitro* expansion were examined. *Methods.* After isolation of ASC from human lipoaspirates of seven patients, *in vitro* expansion over 10 passages was performed. Cells from passages 1, 2, 3, 5 and 10 were used for the alkaline single-cell microgel electrophoresis (comet) assay to detect DNA single-strand breaks and alkali labile as well as incomplete excision repair sites. Chromosomal changes were examined by means of the chromosomal aberration test. *Results.* During *in vitro* expansion, ASC showed no DNA single-strand breaks in the comet assay. With the chromosomal aberration test, however, a significant increase in chromosomal aberrations were detected. *Conclusions.* The study showed that although no DNA fragmentation could be determined, the safety of ASC cannot be ensured with respect to chromosome stability during *in vitro* expansion. Thus, reliable analyses for detecting ASC populations, which accumulate chromosomal aberrations or even undergo malignant transformation during extensive *in vitro* expansion, must be implemented as part of the safety evaluation of these cells for stem cell–based therapy.

Key Words: *adipose tissue–derived stem cells, ASC, chromosomal aberration, comet assay, DNA strand breaks, genetic stability*

Introduction

Mesenchymal stromal cells (MSCs) of the bone marrow (BMMSCs) and adipose tissue (ASCs) can be used as multi-potent cell sources for stem cell research (1–15) and clinical applications (16–22).

In the 1970s, Friedenstein *et al.* (23–27) identified mesenchymal progenitors in the bone marrow by isolating spindle-shaped and plastic-adherent cells with a high replicative capacity *in vitro* and an osteogenic differentiation potential. Years later, the terms "stromal stem cells" and "mesenchymal stromal cells" were established by Owen (28) and Caplan (29), respectively. Pittenger *et al.* (2) demonstrated the differentiation potential into the adipogenic, chondrogenic and osteogenic lineage of BMMSCs after their *in vitro* expansion. Subsequently, Owen (28) proposed that the marrow stromal system might be part of a wider stromal system in the body. In addition, Caplan (29) postulated that MSCs are present not only in the bone marrow but also in periosteum and muscle-connective tissue and considered the possibility of a "future self-cell therapy." In the following years, MSCs were isolated from almost all adult tissues, such as bone marrow (26,27,29–32), adipose tissue (4,5,27,31,32), periosteum (27,29,31,32), synovium (27,31–33), dental pulp (31,32,34), muscle (30–32) and solid organs (eg, brain, kidney, spleen, liver and pancreas (27,30,31).

In the mid-1960s, Rodbell *et al.* (35) used collagenase digestion to separate mature adipocytes from the stromal vascular fraction (SVF) cells. Van and Roncari (36) and Pettersson *et al.* (37,38) described adipocyte precursor cells in the stromal vascular

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fraction of rat and human adipose tissue with the ability to develop into adipocytes. Zuk *et al.* (4) supported the existence of ASCs in 2001 and showed clonal multipotent ASCs 1 year later (5). Over the past decade, ASCs have been increasingly focused on for stem cell research (3–15) and clinical applications (17–22,39–41). Adipose tissue is abundantly available and ASCs can be easily harvested with less discomfort, lower donor-site morbidity (42,43) and higher yield (6,42,44–46) compared with BMMSCs, which can be isolated at a percentage of 0.001–0.01% (2). As opposed to embryonic stem cells, the application of MSCs is not ethically restricted (44,47) and thus ASCs are considered to be the preferred human adult stem cell source for cell-based approaches in the future (48).

In 2006, the International Society for Cellular Therapy defined minimal criteria for BMMSCs (49) and, although ASCs meet two of the three criteria including plastic adherence and multi-lineage differentiation potential (3–15,20,50–55), they do not entirely share immunophenotype characteristics with BMMSCs (46).

The native progenitor/stem population of adipose progenitors have been identified as both pericytes (56) and adventitial progenitors (48,57). The identified cell source of BMMSCs, however, which was defined as a subendothelial progenitor, is rare (58,59). Because the freshly isolated SVF consists of multiple cell types (20), especially an initially high amount of endothelial and mesenchymal progenitors (48,56,57), the SVF may be sufficient to be used immediately for cellular therapies (39,59). However, although a large amount of ASCs can be isolated primarily, *in vitro* expansion of the cells may be required for some applications, for example, in the case of large defects (40).

This may raise concerns about the safety of BMMSCs and ASCs, which is demonstrated by various studies reporting the increased risk of cytogenetic instability, spontaneous transformation and malignant tumor formation *in vivo*. Several reports describe spontaneous *in vitro* transformation of mouse (60–63), rat (64) or rabbit (65) MSCs from bone marrow or adipose tissue. Accumulation of chromosomal aberrations, elevation in telomerase activity and malignant tumor formation *in vivo* after systemic administration of the transformed cells could be observed after long-term passaging (60,62,63). However, there appears to be a remarkable variability for spontaneous tumorigenesis in the mouse models because Jeong *et al.* (66) reported on malignant tumor formation after implantation of even short-term cultured BMMSCs. Ren *et al.* (67) described spontaneous transformation of adult MSCs from cynomolgus macaques *in vitro* in their publication, which was criticized subsequently because of the lack of DNA fingerprinting (68). In 2012, Ren *et al.* (69) added short tandem repeat (STR) analyses and could confirm their previous findings.

Data concerning human BMMSCs or ASCs, which can currently be found in the literature, are contrary to the animal data. There is no indication for spontaneous immortalization, transformation and tumor formation (60,70,71), rapid proliferation (60,70) or chromosomal abnormalities (60,70,72) of human MSCs. Besides these reports, there are two publications on spontaneous transformation of human BMMSCs (73) and ASCs (74) during long-term expansion. However, both articles were retracted because the authors could not reproduce their results and suspected a cross-contamination artifact during culture (75,76).

At present there is no clear evidence for the spontaneous transformation of human MSCs, whereas animal data may leave skepticism as to the safety of MSC applications. To contribute additional information to the potential changes in MSCs during *in vitro* expansion, our study investigated DNA strand breaks and chromosomal aberrations in human ASCs during continous passaging up to passage 10.

Methods

Isolation and cell culture of human ASCs

Human ASCs were isolated from subcutaneous adipose tissue of healthy donors (n = 7) undergoing liposuction surgery for aesthetic reasons. The study was approved by the Ethics Board of the Medical Department of the Julius Maximilian University of Wuerzburg (grant No. 72/06), and informed consent was obtained from all individuals included in the study.

The isolation procedure was performed as previously described (77–79). Adipose tissue in sterile bottles was rinsed twice with phosphate-buffered saline (PBS; Roche Diagnostics, Mannheim, Germany) plus 1% penicillin/streptomycin (P/S; Biochrom AG, Berlin, Germany). Afterward, digestion with sterile-filtered collagenase P solution containing 10 mg of collagenase P (Roche Diagnostics) in PBS per 100 mL of lipoaspirate material for 3 h at 37°C, under continuous shaking, followed. The pelleted SVF was separated from the adipose cell fraction by centrifugation for 5 min at 500*g* at room temperature. After the supernatant containing mature adipocytes was discarded, erythrocytes in the remaining cell pellet were eliminated by incubation with erythrocyte lysis buffer (154 mmol/L ammonium chloride [NH_4Cl], Sigma-Aldrich, Steinheim, Germany; 10 mmol/L