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Comparative analysis of multilineage properties of mesenchymal stromal cells derived from fetal sources shows an advantage of mesenchymal stromal cells isolated from cord blood in chondrogenic differentiation potential

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Abstract

Background aims. Cord blood (CB) and amniotic fluid (AF) could represent new and attractive mesenchymal stromal cell (MSC) sources, but their potential therapeutic applications are still limited by lack of standardized protocols for isolation and differentiation. In particular, chondrogenic differentiation has never been deeply investigated. *Methods.* MSCs were obtained from CB and AF samples collected during cesarean sections at term and compared for their biological and differentiation properties, with particular interest in cartilage differentiation, in which quantitative real-time polymerase chain reaction and immunohistochemical analyses were performed to evaluate the expression of type 2 collagen, type 10 collagen, SRY-box9 and aggrecan. *Results.* We were able to isolate MSCs from 12 of 30 (40%) and 5 of 20 (25%) CB and AF units, respectively. Fluorescence *in situ* hybridization analysis indicated the fetal origin of isolated MSC strains. Both populations expressed mesenchymal but not endothelial and hematopoietic markers, even though we observed a lower expression of human leukocyte antigen (HLA) I in CB-MSCs. No differences in proliferation rate and cell cycle analysis could be detected. After osteogenic induction, both populations showed matrix mineralization and typical marker expression. Under chondrogenic conditions, pellets derived from CB-MSCs, in contrast with AF-MSCs pellets, were significantly larger, showed cartilage-like morphology and resulted positive for chondrocyte-associated markers, such as type 2 collagen, type 10 collagen, SRY-box9 and aggrecan. *Conclusions.* Our results show that CB-MSCs and AF-MSCs collected at term differ from each other in their biological and differentiation properties. In particular, only CB-MSCs showed a clear chondrogenic potential and thus could represent an ideal candidate for cartilage-tissue engineering.

Key Words: *amniotic fluid, chondrogenic differentiation, cord blood, mesenchymal stromal cells*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent non-hematopoietic progenitors characterized by plastic adhesion, expression of a specific panel of surface antigens and capacity to differentiate into mesenchymal lineages, specifically bone, cartilage and adipose tissue (1–3). For these reasons, MSCs are considered a promising therapeutic tool for tissue repair and regenerative medicine (4,5). MSCs were initially isolated from bone marrow (BM) by Friedenstein *et al.* (6). Although it has been considered for years the main source of MSCs for both experimental and clinical applications, the use of BM-MSCs has disadvantages. First, the procedure for

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collecting BM from patients and donors is highly invasive and painful. Furthermore, frequency, proliferation and differentiation potential of BM-MSCs decrease with increasing donor age (4). As a consequence, alternative sources from adult and, more importantly, perinatal tissues, have been considered. Among these sources, cord blood (CB) and amniotic fluid (AF), usually regarded as medical waste, could represent new and attractive MSC sources because of their advantages over BM. A large amount of samples can be easily obtained at delivery, with no risk to the donor. Moreover, perinatal tissue–derived MSCs have many advantageous features, such as high proliferation capacity and differentiation potential as the result of their ontogenically younger origin, and no donor age-dependent variations (7). These cells could also be useful for development of *in utero* stem cell transplantation strategies. Several studies have demonstrated that fetal MSCs harvested in the prenatal period can be processed during the remainder of gestation to obtain autologous tissues for the repair of congenital anomalies soon after birth or *in utero* (8–10). Despite this evidence, clinical application of AF-MSCs and CB-MSCs has not yet been achieved, mainly because of the low success rate of isolation. Moreover, the application of these cells for transplant purposes requires extensive characterization, standardization of reproducible differentiation protocols and functional characterization of the differentiated cells. Interestingly, recent studies have shown that MSCs isolated from several prenatal sources represent heterogeneous populations, whose principal differences depend on the source and the donor (11–15).

MSCs are a promising cell source for cartilage tissue engineering, given their chondrogenic potential. Recently, an increasing number of studies have described that specific properties of MSCs, including chondrogenic capability, are dependent on their origin. In fact, it has been previously demonstrated that MSCs isolated from different tissues do not represent identical cell populations but vary in the expression profile of some growth factors relevant for chondrogenesis (12,16–22). Considering these aspects, we hypothesized that the frequency of fetal MSCs correlates with clinical parameters and that term CB-MSCs and AF-MSCs may display differences in their differentiation potential. Therefore, the objectives of this study were to analyze a possible correlation between clinical parameters and the success rate of fetal MSCs isolation, to compare the biological properties of fetal MSCs isolated from term CB and AF samples and, more specifically, to evaluate their capability into cartilage differentiation. Chondrogenic differentiation was induced by means of a three-dimensional pellet culture system submitted to specific stimuli and investigated through histology, immunohistochemistry and gene expression studies of cartilage-associated markers.

Methods

Specimen collection

Both CB and AF samples were collected from volunteer donors during elective cesarean sections at term (37–42 weeks of gestation) at the San Gerardo Hospital (Monza, Italy). Exclusion criteria for collection were presence of clinical chorioamnionitis, prenatally diagnosed chromosome abnormalities and severe oligohydramnios. AF was collected by means of amniotic puncture after transversal incision of the myometrium layer of the lower uterine segment and stored in sterile tubes. CB was collected immediately after delivery of the infant and cord clamping, before the delivery of placenta, and stored in bags (MacoPharma, Rho, Italy) with anticoagulant (citrate-phosphate-dextrose buffer). Collections were performed after maternal consent was given and in accordance with the ethical standards of the hospital ethics committee. Clinical information from each donor was prospectively collected and included date of delivery, gestational age, maternal characteristics, sex and birth weight of the infant and pregnancy details.

Isolation and culture of human MSCs from CB and AF

Mononuclear cells (MNCs) were isolated from whole CB by means of density gradient centrifugation with the use of Ficoll-Hypaque-Plus solution (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). The colony-forming unit fibroblast (CFU-F) assay was performed as follows. MNCs were counted with the use of an automated cell counter (Coulter AcT Diff, Beckman Coulter, Brea, CA, USA) and placed in culture at 37°C in a humidified atmosphere containing 5% CO_2 at a density of 2×10^6 cells/cm^2 into culture dishes (100-mm diameter; Nunc, Rochester, MN, USA) in Dulbecco's modified Eagle's medium (DMEM)–low glucose (Invitrogen, Carlsbad, CA, USA) supplemented with 20% fetal bovine serum (FBS) (Biosera, Ringmer, United Kingdom), 1% penicillin-streptomycin (Invitrogen) and 1% L-glutamine (Invitrogen). Dexamethasone (DEX; 10^{-7} mol/L) (Sigma-Aldrich, St Louis, MO, USA) was added in the primary culture medium for 1 week. Non-adherent cells were removed 48 h after initial plating. After 17 days of culture in medium, CFU-F colonies were scored at ×20 magnification. The cultures, consisting of adherent cells, were maintained in basal