Abstract The potential of mesenchymal stem and progenitor cells (MSC) to replicate undifferentiated and to mature into distinct mesenchymal tissues suggests these cells as an attractive source for tissue engineering. The objective was to establish a protocol for the isolation of porcine MSC from bone marrow and to demonstrate their ex vivo differentiation into various mesenchymal tissue cells. MSC from passage 2 were selected for differentiation analysis. Differentiation along the osteogenic lineage was documented by deposition of calcium, visualization of alkaline phosphatase activity, and by analysis of osteogenic marker genes. Adipocytes were identified morphologically and by gene-expression analysis. Deposition of type II collagen and histological staining of proteoglycan indicated chondrogenic differentiation. Therefore, porcine MSC may be introduced as a valuable model system with which to study the mesenchymal lineages for basic research and tissue engineering.

Keywords Porcine mesenchymal stem cells · Isolation · Osteogenesis · Chondrogenesis · Adipogenesis · Tissue engineering · Cell culture

Introduction

Adult bone marrow contains mesenchymal stem and progenitor cells (MSC), which give rise to different mesenchymal tissues. MSC can be isolated and grown in vitro (Haynesworth et al. 1992). Important characteristics of human MSC are their ability to proliferate in culture with an attached fibroblastic morphology, the presence of specific marker proteins on their surface, and their in vitro multilineage potential (Pittenger et al. 1999).

Purified and expanded MSC from various species have been shown to differentiate along osteogenic (Jaiswal et al. 1997), chondrogenic (Johnstone et al. 1998; Mackay et al. 1998), adipogenic (Pittenger et al. 1999), tenogenic (Young et al. 1998), and marrow stromal lineages (Cheng et al. 2000).

In the present study, we established a protocol for the isolation of porcine MSC to compare their phenotype and their multilineage potential with human MSC. Here we report that the techniques used for purification, expansion, osteogenic, chondrogenic, and adipogenic differentiation of human MSC can be adopted for analysis of porcine MSC, which may serve the increasing demand for stem and progenitor cells in tissue engineering. As a result of the presented similarities between porcine and human MSC, porcine MSC are likely to be considered as a valuable model system for skeletal research.

Materials and methods

Isolation, culture, and differentiation of MSC

Femur and tibia bones from 6- to 8-month-old porcine donors (n=4, 100–130 kg) were sawn and gelatinous bone marrow was extracted under sterile conditions. Porcine MSC were isolated according to modifications of a method developed for human MSC (Haynesworth et al. 1992). Briefly, gelatinous bone marrow (3–4 g per sample) was resuspended in PBS and dispersed mechanically by passing through syringes fitted with a series of 16-, 18-, and 20-gauge needles. Cells were centrifuged, resuspended, and plated in complete DME medium (Biochrom), containing 10% fetal bo-
Vine serum (lot 228U; Biochrom) from selected lots (Lennon et al. 1996). The differentiation potential was demonstrated by culture of porcine MSC (passage 2) under conditions that were favorable for osteogenic (Bellows et al. 1986; Maniatopoulos et al. 1988; Jaiswal et al. 1997), chondrogenic (Johnstone et al. 1998), or adipogenic (Gimble et al. 1992; Pittenger et al. 1999) development.

Polymerase chain reaction

Total RNA was isolated as described previously (Chomczynski 1993). Subsequently, total RNA (5 µg) was reverse transcribed after annealing with 500 ng oligo-(dT)12–18 primers (Gibco) and 5 U Superscript reverse transcriptase (Gibco) in 70 µl (Gubler and Hoffmann 1983). The relative expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize marker gene expression in each sample in different concentrations.

Real-time PCR using the i-Cycler PCR system (BioRad) was performed with 1 µl of the single-stranded cDNA sample, using the SYBR Green PCR Core Kit (Applied Biosystems). Relative quantitation of marker genes (Table 1) was performed according to the ∆∆Ct method as described (Winer et al. 1999).

Histological methods and immunohistochemistry

Osteoblasts exhibit high levels of alkaline phosphatase, which were visualized by staining with Sigma fast BCIP/NBT (Sigma). Von Kossa staining identified deposition of mineralized bone matrix. Proteoglycan secreting chondrocytes were stained with alcian blue 8GS (Roth) at pH 2.5. Presence of collagen was analyzed by azan staining. Adipocytes were identified morphologically and by staining with oil red O (Sigma). For immunohistochemistry of type II collagen, cryosections (6 µm) were incubated for 1 h with primary antibodies (rabbit anti-human type II collagen; DPC-Bier-