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Phenotypic characterization of canine bone marrow stromal cells

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Introduction:

Bone marrow stromal cells (BMSCs) are the major cellular component of the marrow stroma and include the mesenchymal progenitors. Their in vitro adherence ability allows isolation and expansion of multipotent colonies (CFU-F). BMSCs display osteogenic commitment, which can be modulated by culture conditions. The aim of this study was to evaluate the expression of osteogenic phenotypic traits in BMSCs to assess their potential use in tissue-engineering strategies.

Material and Methods:

BMSCs were isolated from canine marrow aspirates after plating at a density of 6-9 x 10^7 cells per 150-cm^2 flasks. After 24 hours medium was replaced and changed three times per week until cultures became ~90% confluent. The number of progenitors was quantified on day 14 by counting CFU-Fs with > 50 cells. The master gene of osteogenic commitment CBFA1, type I collagen, alkaline phosphatase (ALP) and gene products characterizing the osteoblastic phenotype, such as osteonectin were quantified by RT-PCR. The expression of fibronectin, type I collagen, decorin, bigican, osteonectin, osteopontin and bone sialoprotein (BSP) was investigated on days 7, 14 and 21 by immunocytochemistry and ALP by cytochemistry. BMSCs were also incubated for 21 days under osteogenic conditions to induce Ca deposition that was further analyzed by nuclear fast red staining, scanning electron microscopy (SEM) and by energy dispersive X-ray analysis (EDXA).

Results:

BMSCs obtained from CFU-F expansion displayed spindle shape morphology with ~30% of the colonies positive for ALP on day 14. On passaging, the levels of mRNA expression of osteoblast-related markers decreased while CBFA-1 levels remained constant. The expression of osteopontin, BSP, type I collagen and fibronectin did not change substantially over time in culture. Decorin and biglycan were variably expressed. Calcium deposits, detected by nuclear fast red staining as amorphous structures, were confirmed by EDXA analysis and were identified on SEM as 10 µm spheroid structures encased by fibers and extracellular matrix.

Discussion and Conclusions:

A number of in vitro methods are employed to determine the differentiation ability and commitment of BMSCs with the osteogenic
phenotype. Our data demonstrate that canine BMSCs, after ex-vivo expansion express CBFA-1 and other osteoblast-related gene products that can be taken as evidence of the osteogenic commitment. This biological property strongly supports the use of BMSCs for the reconstruction of bone defects as well as in general veterinary regenerative medicine.