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Impact of source tissue and *ex vivo* expansion on the characterization of goat mesenchymal stem cells

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Abstract

Background: There is considerable interest in using goats as models for genetically engineering dairy animals and also for using stem cells as therapeutics for bone and cartilage repair. Mesenchymal stem cells (MSCs) have been isolated and characterized from various species, but are poorly characterized in goats.

Results: Goat MSCs isolated from bone marrow (BM-MSCs) and adipose tissue (ASCs) have the ability to undergo osteogenic, adipogenic and chondrogenic differentiation. Cytochemical staining and gene expression analysis show that ASCs have a greater capacity for adipogenic differentiation compared to BM-MSCs and fibroblasts. Different methods of inducing adipogenesis also affect the extent and profile of adipogenic differentiation in MSCs. Goat fibroblasts were not capable of osteogenesis, hence distinguishing them from the MSCs. Goat MSCs and fibroblasts express CD90, CD105, CD73 but not CD45, and exhibit cytoplasmic localization of OCT4 protein. Goat MSCs can be stably transfected by Nucleofection, but, as evidenced by colony-forming efficiency (CFE), yield significantly different levels of progenitor cells that are robust enough to proliferate into colonies of integrants following G418 selection. BM-MSCs expanded over increasing passages *in vitro* maintained karyotypic stability up to 20 passages in culture, exhibited an increase in adipogenic differentiation and CFE, but showed altered morphology and amenability to genetic modification by selection.

Conclusions: Our findings provide characterization information on goat MSCs, and show that there can be significant differences between MSCs isolated from different tissues and from within the same tissue. Fibroblasts do not exhibit trilineage differentiation potential at the same capacity as MSCs, making it a more reliable method for distinguishing MSCs from fibroblasts, compared to cell surface marker expression.

Keywords: Adipose, Bone marrow, Characterization, Differentiation, Goat, Mesenchymal stem cells

Background

Mesenchymal stem cells (MSCs), also known as multipotent stromal cells, are one of the most studied adult stem cells for their ease of culture *ex vivo* and their multipotentiality, as well as supportive functions *in vivo*. Believed to reside in virtually all post-natal tissues [1], MSCs have been isolated and characterized from a variety of tissue types, most commonly from bone marrow [2,3] and adipose tissue [4]. Even though MSCs isolated

from different tissues appear similar in morphology and their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages [5], they can display phenotypical differences, including in differentiation potential [6-9] and cell surface proteins [10,11]. Bone marrow-derived MSCs (BM-MSCs) have a higher capacity to differentiate into osteogenic and chondrogenic lineages [6,7], whereas adipose-derived MSCs (ASCs) are better at differentiating into adipocytes [8,9]. The influence of source tissue, along with variations due to species, donor, and culture methods, have challenged the consistency of reports in the literature and complicated the understanding of MSC biology.

Goats are widely used as large animal models for bone tissue engineering as they have knee joints that are

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Authors' contributions

NM conceived of the study, carried out all the experiments and drafted the manuscript. PJR assisted with the immunofluorescence experiment and the interpretation of its results. EAM and JDM participated in the study's design and coordination. All authors contributed to, read, and approved the final manuscript.

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