Isolation, characterization and differentiation of mesenchymal stem cells obtained from bovine umbilical cord blood

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Abstract

Bone marrow and adipose tissue are the most common sources of mesenchymal stem cells, although their frequency and differentiating capacity decrease with age. Therefore, extra-embryonic tissues, including umbilical cord blood, amniotic membrane, amniotic fluid and umbilical cord matrix are alternative sources in stem cell therapy. Furthermore, the umbilical cord blood (UCB) cells show low levels of HLA antigens and no evidence of teratoma formation after transplantation, and have a high in vitro proliferative capacity. In cattle, there is little data concerning UCB cells, although this tissue can be considered a promising alternative for bovine regenerative medicine. The aim of this study was to isolate, characterize and differentiate the mesenchymal stem cells obtained from bovine UCB cells. For the UCB isolation, three samples were obtained from slaughterhouse, in the half of the gestational period. The samples were collected in sterile syringe containing heparin, and maintained cooled until been processed in the laboratory. Then, they were centrifuged, the supernatant was removed and a culture medium containing DMEM/F12 (Gibco®) and 20% of FCS (Gibco), 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma) and 3 µg/ml amphotericin B (Sigma) was added. The blood was diluted with the same amount of medium and added to the same proportion of Ficoll Histopaque before centrifugation to remove the mononuclear cell layer. The resulting mononuclear cells were centrifuged and then submitted to culture in incubator with 5% CO_2 at 37,5 ºC. At the third passage, the UCB samples (each aliquot contained 1 x 10^6 cells) were reserved to flow cytometry. The antibodies used to detect cell surface antigens were MHC II, vimentin and CD34. The other aliquot was reserved for in vitro differentiation into osteogenic and adipogenic lineage. The UCB samples were capable of differentiating into osteogenic and condrogenic lineages in 10 days. When submitted to flow cytometry, 37% of the cells were positive for vimentin, indicating its mesenchymal origin. However, 16% of the cells showed positive staining for CD34, confirming its hematopoietic origin. The expression of MHC II was considered negative (< 2%). The UCB cells are an alternative source of stem cells, since they can be obtained noninvasively, have the ability to proliferate and differentiate in vitro and thereby could be used to develop a stem cell bank. However, more information is necessary to improve the characterization of bovine mesenchymal stem cells and their immunomodulatory and remodeling ability.

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