Isolation and characterization of bone marrow-derived equine mesenchymal stem cells for future therapeutic applications in horses.

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**Introduction**

Diseases of the musculoskeletal system are a main reason for early retirement and euthanasia of race, pleasure, and working horses. Mesenchymal stem cells (MSCs) have been recently investigated for their potential use in regenerative medicine. MSCs, in particular, have great potential, as in various reports they have shown pluripotency for differentiating into many different cell types. Despite the controversy of what defines a ‘mesenchymal stem cell’, there is general agreement that MSCs lack typical hematopoietic antigens, namely, CD45, CD34 and CD14. The undifferentiated cells express molecular markers for "stemness" namely: Nanog, Oct4, Sox-2 the expression of these genes is required for self-renewal and demonstrates multilineage differentiation potential.

The gold standard assay utilized to identify MSCs is the colony forming unit-fibroblast (CFUF assay which, at least, identifies adherent, spindle-shaped cells). The objective of this study was to isolate and characterize bone marrow-derived equine mesenchymal stem cells for possible future therapeutic applications in horses.

**Materials and Methods**

Bone marrow aspirates were obtained from sternebra of 5 horses, 3-5 years old. The aspirates were obtained by Jamshidi bone marrow aspirate needles and were collected into syringes containing heparin to a final concentration of 1,000 UI/ml. The aspirates were diluted in Phosphate Buffered Saline (PBS) to a final solution 1:1. The fraction of mononuclear cells was separated with Ficoll (Amersham Bioscience Ficoll- Paquete Plus) and washed with PBS. The pelleted stromal cells were resuspended in DMEM F12 medium containing 20% fetal bovine serum and 100 UI/ml of penicillin and 100 µg/ml of streptomycin and cells were incubated. Part of the sample was utilized for flow cytometry tested with human antibodies (CD90, CD34, CD14, CD73, CD45, CD 117 and CD 166.) The presence of Sox-2 and Nanog amplicons in BMMSCs was assessed by PCR at passage1, 2 and chondrocytes like cells negative control.

**Results**

We found that cells from equine bone marrow express stem cell markers, negative for CD14, CD34 and CD 45, and positive for CD73, CD 90, CD117 and CD166. Morphologically cells appeared in clusters of large stellate cells growing outward in a swirling pattern as they multiplied, large, spindloid, fibroblastic-appearing cells. PCR analysis showed that Nanog and Sox-2 genes were expressed by the stem cells but not in chondrocytes.

**Discussion and Conclusions**

Bone marrow aspirates yielding mesenchymal stem cells were easily,atraumatically and aseptically obtained from the sternebra of standing adult horses under sedation. Populations of adherent equine MSC’s that had similar morphologic characteristics to human MSC’s were successfully isolated. Our data indicate that bone marrow-derived stromal cells of horses can be characterized as MSC’s. As an autologous cell population, equine MSC’s can be regarded as a promising cell population for tissue engineering in lesions of the musculoskeletal system in horses.

**References**

5 Violini Stefania, Ramelli Paola, Pisani Laura, Chiara Gorni and Paola Mariani Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by in vitro exposure to BMP-12 BMC Cell Biology 2009, 10:29.