Morphology and morphometry of feline adipose-derived mesenchymal stem cells in culture

Bruno B. Maciel[a], Carmen Lúcia K. Rebelatto[b], Paulo Roberto S. Brofman[b], Lia F. L. Patricio[a], Harald F. V. Brito[a], Marúcia A. Cruz[c], Patrícia Y. Montaño[a], Rosangela Locatelli-Dittrich[a]

[a] Postgraduate Program in Veterinary Science, Federal University of Paraná (UFPR), Curitiba, PR – Brazil
[b] Experimental Laboratory of Cell Culture, Pontifical Catholic University of Paraná (PUCPR), Curitiba, PR – Brazil
[c] "Mania de Gato" Veterinary Clinic, Curitiba, PR – Brazil

Abstract

The mesenchymal stem cells (MSC) are a very promising subpopulation of adult stem cells for cell based regenerative therapies in veterinary medicine. The MSC have been isolated from adipose tissue (AT), however, there are very few data on the morphology of these cells and no data were found about the morphology and morphometry of feline AT-derived mesenchymal stem cells (AT-MSC). The aim of this study was to isolate, cultivate and differentiate AT-MSC into osteocytes, chondrocytes, and adipocytes and conduct clonogenic and morphological assays. The cats ranged in age from five months to eight years. Adipose tissue was collected from subcutaneous fat depots of cats. For differentiation of AT-MSC into adipocytes, osteoblasts and chondrocytes, we used cells at third or fourth passage. It was assessed the morphology of 50 cells from the same animal at first passage (P1) and third (P3) and measured the length and width of the cells and of their nuclei. Cells were measured at 24, 48, 72 and 120 hours of culture. The measurement results were submitted to ANOVA and the means were compared by the Tukey's test. The number of isolated cells ranged from 12,857 to 510,204 cells/g of fat, with an average of 283,793 (±219,946). In the clonogenic assays, we obtained colonies which varied in size and morphology. The mean CFU-F observed after the culture of 470 and 752 cells/cm2 was 20.62 ± 16.38 colonies at P1 and 21.87 ± 17.64 colonies at P3. In qualitative assays, we showed the in vitro differentiation of AT-MSC of cats into osteoblasts, chondrocytes and adipocytes. We observed the predominance of spindle-shaped and widespread cells with abundant cytoplasm. Spindle-shaped cells are longer, have less cytoplasm and a round central nucleus, with varying shapes of cytoplasmic extensions. We also found slender cells with long cytoplasmic extensions at two ends, with Y-shaped cytoplasmic extensions and smaller cells with larger abundance of cytoplasm only at one end, in addition to rare multinucleated cells. Widespread cells also varied in shape: some were rectangular while others were round. In terms of morphometry, we observed a significant increase in the mean length of cells during culture, both at first and third passages. The cell lengths were 109.61±56.86 µm and 155.47±74.68 µm, respectively, at first and third passages (24h). The cell widths were 24.72±7.05 µm and 34.86±20.45 µm, respectively, at first and third passages (24 h). The nucleus length of the feline AT-MSCs increased from 17.92 µm (24h) to 23.06 µm (120h) and from

22.13 µm (24h) to 32.17 µm (120h), respectively, at P1 and P3. This study has demonstrated the isolation of feline AT-MSC and the proliferation potential. Cultures of feline MSCs cultures undergo changes as they are expanded, as observed in cultures of human MSCs. This information will be important for future qualitative investigations of feline AT-MSC.