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Characterization of teratogenic potential and gene expression in canine amniotic membrane-derived stem cells

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The biosafety of innovative procedures that utilize stem cells in regenerative medicine has been addressed in several studies. Previous work has showed no teratoma formation following the use of feline (1) and human amniotic membrane-derived stem cells (AMSCs). In contrast, tumor formation was observed when canine AMSCs were utilized (2). These findings suggested that feline and human, but not canine, AMSCs are suitable for cell transplantation trials. The present study aimed to further evaluate the feasibility of utilizing canine AMSCs for transplantation purposes. The study protocol was approved by the Faculty of Animal Science and Food Engineering research ethical committee (13.1.2823.74.2). The canine and feline AMSCs used in this study were isolated from fetal membranes collected after routine ovariohysterectomy in cats and dogs at veterinary clinics in Pirassununga city, SP, Brazil. Pregnant uteri (35 to 45 days) were collected and fetal membranes were separated and washed using sterile phosphate-buffered saline. The amnion was mechanically separated from the allantoic sac, washed repeatedly with sterile PBS, and then minced using a scalpel blade. Subsequently, the minced pieces of amnion were plated in 35-mm culture dishes containing Alpha MEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. We utilized cells sub-cultured 3 times (passage-3 cells, P3) due to the optimal growth and differentiation potential exhibited by these cells (3). We tested teratoma formation following cell injection into Balb/c-Nude mice, and then assessed expression of hematopoietic, mesenchymal, tumorigenic, pluripotency, and cellular regulation markers using flow cytometry and qPCR. The use of canine AMSCs did not result in macroscopic tumor formation as determined 60 days after transplantation. However, immunohistochemical assays revealed low expression of mesenchymal markers (CD73 and CD90) and high expression of the pluripotent marker OCT4. Moreover, gene expression was heterogeneous when cell isolates from siblings were compared, and expression of CD30, a marker of tumorigenesis, was evident. Similar sources from amniotic stem cells among anine and felines differs when comparing gene expression.

