

CANINE AMNION-DERIVED STEM CELLS CAUSES TUMOR IN MICE.

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Introduction Fetal stem cells are isolated from tissues normally discarded at birth. They are attractive for clinical applications because avoid ethical concerns that plague the isolation embryonic stem cells. The amniotic membrane (AM), or amnion, delineates the gestational sac, a highly resilient, transparent, fluid-filled cavity that encompasses a developing fetus during gestation. The amnion is an avascular structure consisting of three discrete layers: an inner epithelial layer, an interposing, acellular basement membrane, and an outer layer of mesodermal cells. Mix of multipotent cell population, including amniotic epithelial and amniotic mesenchymal cells, denominated amnion-derived stem cells (ADSC) can be derived from the amnion (Miki et al., 2005). The goal of present work was isolation and characterization of ADSC from canine fetuses (C-ADSC).

Methods AM was obtained from the dog fetus at 35 days of gestation. The cells were isolated from amnion using a tissue explant methodology and cultured according to Marcus *et al* 2008. Differentiation of the ADSC towards mesodermal lineage was performed following routine protocols. Analysis of C-ADSC morphology was performed by transmission electron microscopy (TEM). Before inoculation into mice C-ADSC were transduced with retrovirus vectors carrying reporting genes LacZ in order to facilitate the cell tracking after implantation. 1×10^6 cells of normal and Lac Z - cells were injected in right limb of each mice Nude (n=8) and Swiss mice (n=2) of both strains. Histological analyses have been performed.

Results C-ADSC showed high proliferative rate after isolation and present both embryonic stem (ES) cell-like and epithelial-like cells phenotypes. They were positive for both anti-vimentin e anti-*nestin* antibodies, suggesting that isolated mixed C-ADSC population was composed by both amniotic epithelial and mesenchymal stem cells. TEM analysis showed the cells, which present ES cells – like morphology, with a large nucleus and with cytoplasm poor in organelles. The cells demonstrated tight contact and gap junction formation. Tumor formation was observed in right limb of all animals one month after cell implantation. Histological analysis confirms formation of teratocarcinomas composed by undifferentiated and differentiated cells.

Conclusion Our data suggest that mixed C-ADSC population was composed mainly by the cells of epithelial phenotype, which were able to produce teratocarcinomas in mice. Although culture conditions could promote isolation of cells with teratogenic potential, caution is needed in respect of fetal stem cell use in the cell therapy. On the other hand C-ADSC could present interesting model for cancer research.