7th Annual Meeting of the International Society for Stem Cell Research, 8 a 11 de junho de 2009, Barcelona, Espanha

Engraftment potential of human Amniotic Fluid and Bone Marrow Stem Cells cultured in expansion and osteogenic conditions in a Fetal Sheep Model

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Introduction: The role of stem cells in tissue engineering and regenerative medicine is evolving rapidly, namely the use of mesenchymal stem cells (MSCs) due to their putative immune privilege. These cells can be isolated from different sources such as bone marrow, adipose tissue, umbilical cord and more recently from amniotic fluid. Although the amniotic fluid cells have been used for prenatal diagnosis since 1950s in a well established routine technique, little is known about the origin and properties of these cells. In this study, we investigated the effect of culture conditions (expansion versus osteogenic media) over the *in vivo* potential of adult stem cells derived from human amniotic fluid (hAFSCs) and bone marrow (hBMMSCs) in a large non-injury animal model: the fetal sheep model.
Materials and Methods: We isolated hAFSCs from day 6 supernatant of the cultures of amniotic fluid obtained from amniocentesis, and hBMMSCs were obtained from aspirates from the iliac crest of healthy donors. hAFSCs were characterized for their "stemness" by flow cytometry, stability by karyotype analysis and differentiative potential *in vitro.* Cells were maintained in culture until confluence either in expansion or in osteogenic media (7 days), and then transplanted into 58 to 62 day-old fetal sheep at a concentration of $1x10^6$ cells/fetus. Pregnant ewes were fasted for 24 hours. General anaesthesia was induced with thiopental sodium and maintained by inhalation anaesthesia with isoflurane and oxygen. After general anaesthesia, the ewes were positioned in dorsal recumbency and prepared in a sterile surgical environment for a ventral midline celiotomy. The abdomen was exposed through a ventral midline incision and the gravid uterus located. After the identification of the fetus inside the uterus and their gentle handling contention against the inner epithelium layer of the uterus, cells were transplanted into the intraperitoneal fetus cavity by injection through the intact uterus walls. The animals were euthanized sixty days after transplant, and samples from various tissues were collected. The engraftment and phenotype of human-derived cells was evaluated by immunocytochemistry and Real Time-PCR analysis.
Results: The phenotype of hAFSCs is identical to MSCs isolated from bone marrow ($CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD117^+$, $CD34^-$ and $CD45^-$), the cell populations demonstrated karyotype stability up to passage 22, and osteogenic and chondrogenic potential *in vitro*. The hBMMSCs are stable populations, isolated using GMP protocols by Biopredic®. Two months post-transplant, samples from several tissues, namely muscle, bone and skin, were evaluated for the presence of human-derived cells by immunohistochemistry using human specific antibodies (e.g. anti-MyoD1, anti-osteopontin, anti-osteocalcin, anti-involucrin, anti-cytokeratin 14, anti-fibroblast surface protein).
Discussion and Conclusions: Amniotic fluid represents an attractive alternative source to embryonic and adult stem cells because of their apparent advantage of accessibility and multipotentiality over embryonic and adult stem cells, respectively. Animals transplanted with hAFSCs show higher percentages of engraftment then the hBMMSCs counterparts. Human-derived cells are presented in muscle and bone samples as well as in tissues such as skin. In the future, hAFSCs could represent a noncontroversial source of stem cells for regenerative medicine.