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ORIGINAL ARTICLE

Engraftment of mouse amniotic fluid-derived progenitor cells after *in utero* transplantation in mice

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Background/Purpose: Amniotic fluid-derived progenitor cells (AFPCs) are oligopotent and shed from the fetus into the amniotic fluid. It was reported that AFPCs express stem cell-like markers and are capable of differentiating into specific cell type in *in vitro* experiments. However, no study has fully investigated the potentiality and destiny of these cells in *in vivo* experiments.
Methods: Ds-red transgenic mice (on Day 13.5 of pregnancy) were transplanted *in utero* with enhanced green fluorescent protein-labeled mouse AFPC (EGFP-mAFPCs). After birth, baby mice were euthanized at 3-week intervals beginning 3 weeks postnatally, and the specimens were examined by polymerase chain reaction, histology, and flow cytometry.
Results: Our results demonstrate the transplantability of mAFPCs into all three germ layers and the potential of mAFPCs in the study of progenitor cell homing, differentiation, and function. Engraftment of EGFP-mAFPCs was detected in the intestine, kidney, muscle, skin, bladder, heart, stomach, etc., at 3 weeks after delivery.
Conclusion: This model using EGFP-mAFPCs injected *in utero* may provide an ideal method for determining the fate of transplanted cells in recipients and these findings may justify a

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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clinical trial of *in utero* transplantation during gestation for patients who have inherited genetic disorders.

Introduction

Somatic stem cells (SSCs) and embryonic stem cells (ESCs) are currently the principal stem cells used in regenerative medicine.[1,2,3] Despite their therapeutic potential, both SSCs and ESCs have limited clinical application. SSCs are very difficult to isolate and cell yield for transplantation is insufficient. Furthermore, the number of SSCs decreases dramatically with age. ESCs can give rise to three germ layer lineages and proliferate rapidly. However, these cells have limited use in clinical trials due to ethical concerns, allograft rejection problem, and risk of teratoma formation. It has been verified that induced pluripotent stem (iPS) cells can be produced in humans from terminally differentiated cells using transfection factors such as c-myc, Oct4, Klf4, and Sox-2.[4,5] However, virus vectors are required to maintain the transfection efficiency needed to produce iPS cells, risking carcinoma and teratoma formation due to the virus vector or mutation accumulation. Although a nonvirus transfection method has been reported, its efficiency remains low.[6]

It was reported that amniotic fluid-derived stem cells (AFSCs) are multipotent stem cells, and are intermediate between ESCs and SSCs. AFSCs can express pluripotent markers such as Oct4, Sox-2, Nanog, specific stage embryonic antigen-3 (SSEA-3), and SSEA-4,[7,8] share surface markers with bone marrow-derived stem cells (BMSCs),[9] and cannot form tumors.[10] It has been revealed that stem cells can be acquired from amniotic fluid and these can give rise to specific cell lineages under appropriate differentiation conditions.[10] As a result, these cells can be autotransplanted pre- or postnatally without any immune rejection.[10]

However, it was also revealed that amniotic fluid-derived cells are heterogeneous and can be classified into pluripotent stem cells, committed progenitor cells, and completely differentiated cells.[11] Compared with the earlier period of gestation, abundant committed progenitor cells can be clearly found after 18 weeks in humans. These cells are believed to be a brand new source for regenerative medicine research.[11] Therefore, based on current evidence, amniotic fluid-derived progenitor cells (AFPCs) can avoid ethical concerns, avoid the risks of tumorigenicity and immune rejection, and provide a suitable alternative cell source for *in utero* transplantation (IUT).

The IUT technology was developed to treat inherited genetic defects in the fetus during the gestation period. Mesenchymal stem cells were used to successfully treat diseases such as osteogenesis imperfecta and muscular dystrophy.[12,13] Because the fetus has a tolerance for allogeneic or xenogeneic donor cells, the mechanism of hematopoietic stem cell migration and differentiation could be investigated thoroughly. However, no proof could be obtained *in vivo* that the donor cells had given rise to specific cells, had undergone cell fusion, or were secreting mediators needed to recover from the illness. Whether donor cells fuse with recipient cells, give rise to specific lineages, or remain undifferentiated remains a controversial issue and is still unexplored. The purpose of this study was to investigate the engraftment and cell fusion potential of enhanced green fluorescent protein-labeled mouse AFPCs (EGFP-mAFPCs) after IUT in Ds-red transgenic mice.

Materials and methods

Animals

ICR mice were acquired from the Laboratory Animal Center of National Taiwan University College of Medicine (Taipei, Taiwan). The mice were raised under standard laboratory conditions and treated according to principles established by the Institutional Animal Care and Use Committee.

Generation of GFP- and red fluorescent protein (Ds-red)-expressing transgenic mice

To trace donor cell distribution after IUT and to verify donor cell fate, the EGFP- and red fluorescent protein (RFP; Ds-red)-expressing transgenic mice were produced by pronuclear microinjection of ICR strain zygotes with *ScaI—PstI* DNA fragments consisting of β-actin promoter-driven EGFP or RFP from pCX-EGFP or pCX-RFP plasmids. All tissues of the transgenic mice (including AFPCs) uniformly expressed high levels of GFP or RFP.

Procedure to collect the AFPCs from EGFP-bearing mice

EGFP-bearing mice on Day 13.5 of pregnancy were anesthetized by carbon dioxide and then euthanized by cervical dislocation. The whole uterus was removed, washed with 70% alcohol, and then placed into phosphate-buffered saline (PBS) with 2% fetal bovine serum (FBS). The uterus was dissected and each fetus was separated from its amniotic sac and checked for EGFP expression. After the amniotic membrane was pierced, the amniotic fluid was collected and centrifuged at 12,000 $\times$ *g* for 10 minutes. The pellet was resuspended in minimum essential medium (MEM) alpha with 10% FBS. Cells were cultured in 32-well plates at a density of 1.25×10^4 cells/cm^2 and colonies with fibroblast-shaped cells were lifted after incubation with trypsin/EDTA (Invitrogen, Grand Island, NY, USA) for 5 minutes at 37°C and cultured to confluence. After discarding the spent culture medium, the cells were subcultured at a dilution of 1:2.