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Short Communication

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Tissue Engineering 2019- Amniotic membrane mapping discloses novel promising features of amniotic membrane epithelial cells for regenerative medicine purposes

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The amniotic membrane (AM) is the innermost part of the placenta, in direct contact with the amniotic fluid. In recent years the interest toward placenta stem cells has been increasingly growing, due in part to the absence of any ethical issues concerning their isolation. At present, two main stem cells populations have been identified in AM: amniotic epithelial cells (AECs) and amniotic mesenchymal stromal cells (AMSCs). Albeit AM is an excellent source of cells for regenerative medicine, additionally due to its immune-modulatory properties and low immunogenicity, only a few papers have studied its sub-regions. Thus, our focus was to map the human AM under physiological conditions to identify possible differences in morpho-functional features and regenerative capacity of its components. Human term placentas were amassed from salubrious women after vaginal distribution or caesarean section at Fondazione Poliambulanza-Istituto Ospedaliero of Brescia, University Hospital of Cagliari and SS. Annunziata Hospital of Chieti. Samples of AM were isolated from four different regions according to their position relative to umbilical cord (central, intermediate, peripheral, reflected). By designates of immunohistochemistry, morphometry, flow cytometry, electron microscopy, CFU assays, RT-PCR and AECs in vitro differentiation we demonstrated the esse of different morpho-functional features in the different regions of AM, highlighting that AECs are a heterogeneous cell population. This should be considered to increment efficiency of amniotic membrane application within a therapeutic context.

Introduction

One of the most astronomically immense challenges of regenerative medicine is to find a safe and efficacious source of stem cells that can be utilized for treatment of different diseases. Albeit embryonic and adult stem cells represent a promise for the remedy or rectification of structural, congenital or acquired diseases, there are inhibitions in their utilization in clinical practice. They have ethical and licit obstacles, they are arduous to obtain and tumorigenicity has been associated. In recent years much attention has been given to the diverse cell types that can be isolated from the placenta, which is now apperceived as an opulent and plentiful source of pluripotent and multipotent stem cells. Gestational tissue offers considerable advantages over other sources of stem cells: the illimitable potential supply of, the facile access to such tissues, minimal ethical and licit barriers associated with their utilization, they can be obtained without the desideratum of invasive procedure and more importantly, they are not tumorigenic . Furthermore, placental stem cells have unique and valuable immunomodulatory properties

Amniotic epithelial cells have been reported to differentiate into a broad spectrum of cell types. They are capable to inception insulin secreting pancreatic β-islet like-cells, functional neurons and glia and surfactant engendering alveolar epithelial cells. Several laboratories have withal reported hepatic, cardiac , osteogenic, chondrogenic and adipogenic differentiation of hAECs. Among others alluring features for the clinic, the amniotic membrane promotes re-epithelization, inhibits angiogenesis, decreases inflammation and is utilized in ocular surface reconstruction and in wounds rejuvenating .

Liver diseases affect millions of people all over the world. The only currently available curative treatment for end stage liver disease arising from chronic exposure to viruses, extortionate alcohol use, metabolic diseases and acute liver failure is orthotropic liver transplantation. However, due to the astringent shortage of felicitous donor organs and the desideratum for perennial immune suppression following transplantation, alternative therapies are being actively investigated. In the last years, the ascension of cognizance concerning not only the biology of the stem cells but additionally the processes for liver repair has unraveled incipient ways for the utilization of stem cells in liver regenerative medicine. Despite the esse of several types of stem cells that can differentiate into hepatic like cells, hAECs seem to be ideal candidates.

Materials and Methods

Amniotic epithelial cells isolation and culture

Human placentas were obtained after cesarean section following mundane term pregnancies (37–40 weeks gestation) and immediately suspended in frozen dihydrogen monoxide-cold PBS and conveyed to the laboratory. The amnion membrane was manually divested from the chorion membrane, cut and placed in sterile physiological solution. The amnion was washed two or three times to plenarily abstract bloody or torn pieces and cut into equal 4 pieces. All pieces were sterilized in a laminar permeate successive washings in PBS containing 100 U/ml penicillin and 100 μg/ml streptomycin. Pieces from amnion were digested at 37°C with 50 ml trypsin-EDTA 0.25% to relinquish epithelial cells, during two successive incubations of 20 min. Then, the amnion membrane pieces were abstracted and the remaining cell solutions were centrifuged 10 min at 1300 rpm. The cell pellet was resuspended and filtered through a 100 μm cell strainer (BD Falcon). Determinately, the cell solution was centrifuged (10 min, 1300 rpm) and gently resuspended in IMDM 10% FBS. Cells were stained with trypan blue and counted in a Neubauer chamber. Cell viability and quantity were tenacious. Sterility

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of the cultures was tenacious by seeding 10 µl of cells in a LB plate and incubating them at 37°C. Cells were frozen in 90% FBS or plated for experiments.

Flow cytometry analysis

For evaluation of cell phenotype, cell suspensions were incubated for 20 minutes at 4°C with fluorescein isothiocyanate (FITC) or phycoerythrin (Phy) conjugated antibodies specific for human CD166, CD90, CD326, CD13, SSEA-4, CD45 and Gly-A, or isotype control IgG1. All monoclonal antibodies were obtained from BD Biosciences. Samples were analyzed with a FACSCalibur instrument and the CellQuest software (BD Biosciences).

Western Blot

Cells were incubated in 6-wells plates for the times designated, with or without differentiation medium. Then, they were washed with 1X PBS. Total cell lysates were yare in lysis buffer and centrifuged to abstract cellular debris. The protein concentration of the supernatant (5µl) was resolute by the Bradford staining method with BSA as standard. Lysates were commixed with Laemmli's sample buffer containing 2% sodium dodecyl sulfate and 30 mM β-mercaptoethanol, boiled for 5 min in cracking buffer, resolved by SDS-PAGE on a 12% gel, and electrophoretically transferred to a nitrocellulose membrane (Hybond; Amersham Pharmacia) thereafter. Membranes were equilibrated in 1X PBS, and nonspecific binding sites were blocked by 5% nonfat milk in PBS at room temperature for 1 h. Then they were immunoblotted with the concrete antibody anti-albumin (mouse, 1:1000, Santa Cruz Biotechnology), anti-CYP3A4 (goat, 1:1000, Santa Cruz Biotechnology), anti-CYP7A1 (rabbit, 1:1000, Santa Cruz Biotechnology), anti-Cyclin D1 (mouse, 1:1000, Santa Cruz Biotechnology), anti-p53 (mouse, 1:1000, Santa Cruz Biotechnology), and anti-P-ERK 1/2 (Thr202/Tyr204) (rabbit, 1:1000, Cell Signaling). The antibodies were detected utilizing horseradish peroxidase-linked goat anti-mouse IgG, anti-rabbit IgG or anti-goat IgG (1:1000, Sigma and co.), visualized by the Amersham Pharmacia ECL Chemiluminescence signaling system and a Bio-Imaging Analyzer G-Box Chemi XT4 (Syngene). Control for total ERK 1/2 expression was performed utilizing anti-ERK 1/2 antibody (mouse, 1:1000, Cell Signaling). Control for equal gel loading was carried out by GAPDH detection (mouse, 1:5000, Cell Signaling). Quantification of protein bands was tenacious by densitometry utilizing Image J ink 1.45 program (National Institute of Health, Bethesda, MD, USA).

Data Analysis

For Western blots analysis, representative images of at least three independent experiments are shown along with the quantification of immunoreactive bands. Quantitative RT-PCR, MTT and [H3]-thymidine incorporation assays were reiterated discretely at least three times to ascertain reproducible results. For immunofluorescence analysis, representative images of at least three independent experiments are shown along with quantification of positive fluorescent cells. Results are expressed as the mean ± standard deviation (S.D.). The statistical consequentiality was assessed by ANOVA followed by Bonferroni's multiple comparisons post hoc test and was calculated utilizing the GraphPad Instat computer program (GraphPad, San Diego, CA). A p value less than 0.05 was considered statistically consequential.

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