BRAZILIAN JOURNAL OF MICROBIOLOGY

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Veterinary Microbiology

Goat umbilical cord cells are permissive to small ruminant lentivirus infection *in vitro*

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ARTICLE INFO

Article history:
Received 6 January 2016
Accepted 16 August 2016
Available online 19 November 2016
Associate Editor: João Pessoa Araújo Junior

Keywords:
CAEV
MVV
Cell culture
MSC
Flow cytometry

ABSTRACT

Small ruminant lentiviruses isolated from peripheral blood leukocytes and target organs can be propagated *in vitro* in fibroblasts derived from goat synovial membrane cells. These cells are obtained from tissues collected from embryos or fetuses and are necessary for the establishment of the fibroblast primary culture. A new alternative type of host cells, derived from goat umbilical cord, was isolated and characterized phenotypically with its main purpose being to obtain cell monolayers that could be used for the diagnosis and isolation of small ruminant lentiviruses in cell culture. To accomplish this goal, cells were isolated from umbilical cords; characterized phenotypically by flow cytometry analysis; differentiate into osteogenic, chondrogenic and adipogenic lineage; and submitted to viral challenge. The proliferation of goat umbilical cord cells was fast and cell monolayers formed after 15 days. These cells exhibited morphology, immunophenotype, growth characteristics, and lineage differentiation potential similar to mesenchymal stem cells of other origins. The goat umbilical cord derived cells stained positive for vimentin and CD90, but negative for cytokeratin, CD34 and CD105 markers. Syncytia and cell lysis were observed in cell monolayers infected by CAEV-Cork and MVV-K1514, showing that the cells are permissive to small ruminant lentivirus infection *in vitro*. These data demonstrate the proliferative competence of cells derived from goat umbilical cords and provide a sound basis for future research to standardize this cell lineage.

Introduction

The small ruminant lentivirus (SRLV) comprises two types of viruses, the Caprine Arthritis Encephalitis virus (CAEV) and the Maedi Visna virus (MVV); both of which are widely distributed throughout the world. CAEV and MVV share genetic similarities, molecular mechanisms of replication, morphology and similar biological interactions in their hosts. These lentiviruses cause persistent infections, including

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http://dx.doi.org/10.1016/j.bjm.2016.11.002

encephalitis, arthritis, progressive pneumonia, and mastitis in goats and sheep. Like other lentiviruses, CAEV and MVV infect cells of the monocyte/macrophage lineage and dendritic cells.[1,2]

CAEV and MVV can replicate in primary fibroblasts derived from synovial membranes or from choroid plexus cultures,[3,4] and can also replicate in the immortalized TIGEF cell line (T Immortalized Goat Embryonic Fibroblast).[5] These cells are obtained from the tissues collected from embryos or fetuses and are necessary for the establishment of the fibroblast primary culture.

An alternative method would involve using cells derived from goat umbilical cords, which is an invaluable tool that does not use embryos or fetuses. Generally, the umbilical cord would be discarded after calving. However, it is an excellent source material, rich in cells that are able to originate several cell types. The umbilical cord is a well-known source of mesenchymal stem cells (MSCs), which have been isolated and characterized in umbilical cord samples from canine,[6] equine[7] and ovine[8] species. MSCs are a multipotent adult stem cell.[9]

Undifferentiated MSCs exhibit fibroblast-like morphology and are characterized phenotypically by the expression of surface markers; however, the characterization of these cells has not yet been fully defined. There are several positive markers described, and there is a consensus that undifferentiated MSCs should be positive for CD29, CD44, CD90, CD105, CD73 and negative for CD34, CD45, CD14 and CD3.[10,11] MSCs are multipotent stromal cells capable of differentiating to mesenchymal lineages, including tissues such as adipose tissue, bone, cartilage and muscle.[9,12]

The aims of the experiments in this study were to isolate, phenotypically characterize and investigate the differentiation potentials of cells from goat umbilical cords (cGUCs), with the main purpose being to obtain cell monolayers for use in the diagnosis of small ruminant lentiviruses *via* isolation in cell culture.

Materials and methods

Samples

All experiments were approved by the Ethics Committee for Animal Use at the State University of Ceará, protocol number 127.769.79-0. For this study, mongrel pregnant goats were used, aged between two and three years and free of infection by SRLV.

Three umbilical cord samples were collected during calving. After birth, the goat umbilical cords were clamped at both ends, *i.e.*, next to the fetus and the goat, and then cut and packed in the transport medium. The solutions used during transport were low glucose DMEM (Dulbecco's Modified Eagle Medium) (Gibco®, Grand Island, NY, USA), MEM-E (Minimum Essential Medium with Earle's salt and L-glutamine) (Gibco®, Grand Island, NY, USA), PBS (phosphate buffered saline) and saline solution 0.9% (SS), supplemented with 300 U/mL penicillin, 300 μg/mL streptomycin, 0.5 mg/mL amphotericin and 10% FBS. The samples were transported within 2 h to the laboratory, packed in an isothermal box with ice.

Isolation and culture of cGUCs

The tissue samples were processed in a laminar flow cabinet. The umbilical cord was washed with PBS to remove blood. The amniotic epithelium surrounding the umbilical cord was dissociated using sterile scissors and forceps. Tissue samples of umbilical cord matrix were cut into pieces of 0.5–1 cm (explants) and were plated in A25 cell culture flasks and 6-well culture plates using DMEM-low glucose or MEM-E supplemented with 300 U/mL penicillin, 300 μg/mL streptomycin, 0.5 mg/mL amphotericin and 10% FBS and cultured at 37 °C in a CO_2 incubator (5% CO_2).

The cell cultures were observed in an inverted microscope every day, until proliferation of the first cells from the explants had occurred. The media was changed after 5 days to avoid any mechanical stress, and thereafter, it was replenished every third day. At 80% confluence, cells were trypsinized (0.25% Trypsin-EDTA, Gibco®, Grand Island, NY, USA) and reseeded in new cell culture flasks.

Virus infectivity assay

The cells from the confluent monolayer after the third passage were infected with either the CAEV-Cork or MVV-K1514 strains, provided by Dr. Roberto Soares de Castro (Federal Rural University of Pernambuco – UFRPE). These strains originated from the Institut National de la Recherche Agronomique, Lyon-France. The virus infectivity assay was performed in duplicate for each of three separate experiments. The cells were incubated with viral suspension 10^5 TCID50 at a MOI of 1 for 1 h at 37 °C in a 5% CO_2 incubator. Thereafter, the supernatant was discarded and the growth medium was added (DMEM supplemented with 300 U/mL penicillin, 300 μg/mL streptomycin, 0.5 mg/mL amphotericin and 2% FBS).

The cell monolayers were observed in an inverted microscope every day until the cytopathic effect caused by the virus was evident, proving that the cells were permissive for infection by SRLV.

FACS analysis

FACS analysis was performed to investigate the expression of the surface markers CD90, CD105, CD34, and the intracellular markers vimentin and cytokeratin in cGUCs after the third passage. The cells were harvested and aliquoted at a density of 10^6 cells/mL for each marker assay. First, these cells were incubated with antibodies against surface markers [mouse anti-Human CD90 FITC, CD105 APC and CD34 APC (BD Biosciences®, USA)] for 30 min at 4 °C in the dark. Then, the cells were permeabilized with fixation/permeabilization solution (BD Cytofix/Cytoperm Fixation/Permeabilization Kit, BD Biosciences®, USA) for 20 min at 4 °C in the dark. Permeabilized cells were incubated with antibodies against intracellular markers [mouse anti-Human vimentin PE and cytokeratin PE (BD Biosciences®, USA)] for 30 min at 4 °C in the dark. Thereafter, the cells were washed twice with BD perm/wash buffer (BD Biosciences®, USA), fixed with formaldehyde buffer (PBS with 1% formaldehyde) and analyzed using a flow cytometer (FACS Calibur BD, BD Biosciences®). A gate was defined and