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Research paper

Regulation of neural stem cells by choroid plexus cells population

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HIGHLIGHTS

- The choroid plexus is heterogenic cell population tissue.
- Choroid plexus tissue has pluripotent, primordial neuronal, telocytes and hematopoietic cells.
- Choroid plexus can help on neural stem cell regulation and brain balance.

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ABSTRACT

The choroid plexus is a tissue on the central nervous system responsible for producing cerebrospinal fluid, maintaining homeostasis and neural stem cells support; though, all of its functions still unclear. This study aimed to demonstrate the niches of choroid plexus cells for a better understanding of the cell types and functions, using the porcine as the animal model. The collected material was analyzed by histology, immunohistochemistry, and cell culture. The cell culture was characterizated by immunocytochemistry and flow cytometry. Our results showed OCT-4, TUBIII, Nestin, CD45, CD73, CD90 positive expression and GFAP, CD105 negative expression, also methylene blue histological staining confirmed the presence of telocytes cells. We realized that the choroid plexus is a unique and incomparable tissue with different niches of cells as pluripotent, hematopoietic, neuronal progenitors and telocyte cells, which provide its complexity, differentiated functionality and responsibility on brain balance and neural stem cells regulation.

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1. Introduction

The choroid plexus (CP) is a specialized tissue on the central nervous system (SNC) located in the ventricular zone, in a cubical epithelium cells, originated from neural plate epithelium [1–8] and bone morphogenetic proteins from neural tube [5]. Therefore, choroid plexus has origin from different embryonic structures, forming a unique tissue into the brain with incredible multifunction responsibilities.

The CP is responsible for: cerebrospinal fluid production [6], CNS homeostasis [7,8], connection between CNS with peripheral nervous system, detoxifying [9], mediation of the immune and inflammatory responses [1,10], regulation of nutrients [11], transport of the proteins for brain balance [12], neurotrophins synthesis [13], pre inflammatory modulation such as TNF-α (tumor necrosis factor alpha) and IL-1β (interleukin 1 beta) [1], lipids, hormones and glucose source [14], and production of several neurogenesis molecules [14–17].

One of the first choroid plexus cells isolation and culture study was conducted using bovine cells [18], and since then other animal models as murine [19] and porcine [20] were used. Choroid plexus cells culture systems are very useful for intramembrane transport, kinetic parameters and vector transportation researches, which can help to determine the flow of the nutrients and chemical substances between blood and brain cells [21].

Recently studies with neural stem cells have demonstrated their development in the cerebral cortex following the ventricu-

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Table 1
Antibodies used for the characterization of cells in the region of the choroid plexus of fetuses.

Primary antibody	Company	Code	Host	mono/poly	Specificity
Oct-4	Santa Cruz	sc365509	Mouse	Monoclonal	Mouse, Rat, Human
Nanog	Abcam	Ab80892	Mouse	Monoclonal	Mouse, Rat, Human
Beta Tubulin III	Abcam	ab18207	Rabbit	Polyclonal	Mouse, Rat, Human, Pig
Nestin	Abcam	ab105389	Rabbit	Monoclonal	Human
GFAP	Abcam	ab10062	Mouse	Monoclonal	Mouse, Rat, Human
CD105 (Endoglin) IgG2a	Santa Cruz	sc-71042	Rat	Monoclonal	Mouse
CD90 (Thy-1)	Santa Cruz	sc6071	Goat	Policlonal	Human/mouse
CD73(Ecto-5′-nucleotidase)	Santa Cruz	sc14682	Goat	Policlonal	Human/mouse
CD45 (lymphocyte common antigen)	Santa Cruz	sc101839	Mouse	Monoclonal	Human/mouse

Secondary antibody	Company	Code	Specificity
Anti-mouse IgG	Dako		Goat
Anti-rat IgG	Invitrogen	a21210	Rabbit
Anti-goat IgG	Invitrogen	a11075	Rabbit
Anti-rabbit (igG)-alexa fluor 488	Invitrogen	a11034	Rabbit
FICT goat anti-rabbit (IgG)	Abcam	ab6717	Rabbit
FICT anti-mouse (igG2B)	Abcam	ab97249	Mouse

lar region in contact with cerebrospinal fluid [5,17,22–27], nearby choroid plexus region. In addition, they have shown the relationship between cerebrospinal fluid and neural stem cells due to components contained on it, giving the capacity to induce the proliferation or/and survival of these stem cells niche [17,22]. These researches lead us to think about choroid plexus origins and its correlation with neural stem cells [5].

Hence, there are studies with telocytes cells (TC), previously called interstitial cajal-like cells [28], present in the stroma tissues on the whole body, can help on the stem cells regulation as neural stem cell; once the telocytes have podocytes which improve cell to cell contact. However it was not established the main function of these cells, but, probably they are part of maintain tissue, and a supportive cell correlated with stem cells [22,29].

Therefore, this study aims to increase the knowledge about the choroid plexus cells, using porcine as an animal model, across the cells niche characterization using immunology and morphological techniques analyses, its correlation with the maintenance and regulation of neural stem cells, and also localization of the telocytes into the tissue. Thus, we believe that a better understanding of CP can be useful to improve the knowledge of the disorders and pathological situations in the brain, where the neurogenesis and neural stem cells are the major keys.

2. Material and methods

The study was conducted at Laboratory of Stem Cells Culture and Gene Therapy (GDTI), Faculty of Animal Science and Food Engineering, University of Sao Paulo, Brazil. In addition all procedures performed involving animals were in accordance with the ethical standards of the institution where studies were conducted and respecting the Ethical Committee of Faculty of Animal Sciences and Food Engineering, University of Sao Paulo (FZEA/USP). It was used 15 swine stillbirth (dystocic parturition),collected at the Center for Research in Swine at Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, Brazil, the skulls were isolated from the body and cut in sagittal and transversal sections to access the studied region. The CPs were isolated and processed for immunohistochemistry, cell culture, immmunocitochemistry and flow cytometry.

2.1. Histology

The choroid plexus tissue was fixed in paraformaldehyde 4% (pH 7.4–0.1 M) and routinely processed through the paraffin and frozen embedding techniques. Sections of 4–5 μm were placed in previously silanized slices, deparaffinized and rehydrated on paraffin method. The sections were stained in H&E and the material was analyzed, mapped and photographed through a light microscope (AXIOVISION 4.7.1. Carl Zeiss).

2.2. In vitro cell characterization

The primary cell culture followed Gath et al. protocol [20] with modifications, the choroid plexus was exposed and transferred to a falcon tube with trypsin for enzymatic digestion at 37 °C for 30 min, then centrifuged for 5 min at 1000 rpm. The pellet was ressuspended in DMEM/F12 (Dulbecco's Modified Eagle Medium/Ham's F-12; Gibco®) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, Utah, USA), and penicillin/streptomycin (1%; PAA), or DMEM (Dulbecco's Modified Eagle Medium; Gibco®) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were plated in 25 cm^2 culture dish (TPP, Switzerland) and incubated under standard conditions 5% CO_2, 37 °C. After 72 h of incubation, non-adherent cells were removed and media was replaced every 3 days.

The cell plating density was set at 1×10^4 and the cells were cultivated during 3–4 days in DMEM/F12 until 70% confluence [30]. Each passage was performed using trypsin and the recovered cells were replated. Cell number at each passage was quantified by hemocytometer [31] and performed the replating until the cells start the plateau on the grow curve.

The colony forming units (CFU) assay was performed according to Wenceslau et al. [32] protocol, 1×10^3 cells were suspended in DMEM/F12 and plated on 90 mm dish, and the medium was replaced every 3 days until the presence of minimum 50-cells colonies. Microscopic analyses were performed daily the material was fixed and stained with Crystal violet solution (15 min) and after rinsing the material with distilled water, it was possible to determine the number of colonies formed.

2.3. Immunohistochemistry

Choroid plexus sections were dipped in citrate buffer and heated for 15 min to recover the antigen following Roballo et al. [33] protocol with modifications. Then the material was cooled in room temperature and treated with 3% hydrogen peroxide in buffer solution (Tris–HCl 1 M, pH7.5) for 30 min. Non-specific binding block was performed by 10% goat serum in DPBS (Dulbecco's Phosphate Buffered Saline) for 30 min. After slides were incubated with primary antibodies OCT-4 (octamer-binding transcription factor 4) and GFAP (glial fibrillary acidic protein) overnight (Table 1). Sec-

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