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ENDOTHELIAL CELLS

Establishment, characterization and long-term culture of human endocrine pancreas-derived microvascular endothelial cells

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Abstract

Background. *In vitro* primary cultures of microvascular endothelial cells from endocrine pancreas are difficult to obtain, but can be a very helpful tool for studies of islet biology, transplantation and regenerative medicine. *Methods.* We applied a protocol recently described for the isolation and culture of brain microvascular endothelial cells (EC) on human pancreatic islets. EC obtained were characterized in terms of morphological (light and transmission electron microscopy), phenotypical (by immunofluorescence and flow cytometry) and functional (cord formation assay and protein secretion by multiplex bead-based assay) characteristics. *Results.* EC were obtained from 25% of islet preparations processed. Two primary endothelial cell lines showed high proliferative potential and were deeply characterized: they presented endothelial cell morphology and expressed CD31, CD49a, CD49e, CD34, von Willebrand Factor (vWF), Vascular Endothelial CAdherin (VE-CAD), Tyrosine Kinase with Ig and EGF Homology Domains-2 (TIE2), Vascular Endothelial Growth Factor Receptor 1 (VEGFR1), Ulex lectin and the endothelium endocrine-specific marker nephrin. Besides, they were able to form cordons *in vitro* and secreted factors involved in the process of angiogenesis such as Vascular Endothelial Growth Factor (VEGF), Monocyte Chemotactic Protein 1 (MCP-1), interleukin (IL)-8 and Melanoma Growth Stimulatory Activity Alpha (GROα). These cell lines were termed Human Islet Microvascular Endothelial Cells (HIMEC). *Discussion.* This study establishes a simple and effective strategy for isolation and long-term culture of EC derived from human pancreatic islet. HIMEC in culture preserve phenotype and functional properties and are, therefore, a useful tool for future experiments of *in vitro* pancreas modelling, co-transplantation with pancreatic islets, re-vascularization of scaffold or matrix for regenerative medicine purposes.

Key Words: *endothelial cell line, islets, pancreas, primary culture*

Introduction

Pancreatic islet is one of the most vascularized organs of the body [1–3]. Islet microcirculation is characterized by a dense network of sinusoidal capillaries branching from arterioles entering the islet and the fenestrated endothelium has a dual role of allowing a rapid release of insulin into the circulation, but also of fine-tuning blood glucose sensing [4]. In pancreatic islets, endothelial cells (EC) are essential for pancreas differentiation, endocrine specification and function. In fact, EC produce factors that are essential to maintain insulin secretion in β cells [5]. Moreover, some studies support the possibility that the islet microvasculature participates in sensing the environment of the islets and affects adult β-cell func-

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tion, promotes β-cell proliferation and produces a number of vasoactive, angiogenic substances and growth factors [6,7]. Once assembled, a cross talk between endocrine cells and EC contributes to the maintenance of islet function during the whole life of the adult individual [8]. Specific marker of the islet microvasculature is nephrin, a highly specific barrier protein, which is absent in other microvascular endothelial districts [9].

Most of our knowledge on the active participation of EC in pathophysiological processes of pancreatic islet comes from studies on EC derived from umbilical vein, which are easily isolated and cultured. However, these large-vessel EC are likely not the best model to study the phenotypic and functional characteristics of the relevant microvasculature. It is well known, in fact, that phenotype and function of microvascular EC derived from different vascular beds are heterogeneous [10,11], supporting the evidence that EC have tissue-specific specialized functions [12,13]. The current limitations to the study of pancreatic islet microvascular EC are mainly related to the difficulty in isolating and culturing large numbers of pure cells and to the limited life span of *ex vivo* culture. Only a few studies with immortalized pancreatic endothelial cell lines have been reported until now [14–16] but the process of cell immortalization deviates necessarily the cell from the authentic physiological condition. For these reasons, the capability to set up an adequate method to isolate and expand human islet microvascular EC would be extremely useful to enhance our understanding of the role of the micro-endothelium in pathophysiological processes, allowing us to address *in vitro* questions that are difficult or impossible to address *in vivo* [15]. We recently published a protocol for isolation and expansion of human and mouse microvascular EC from the brain [17]. Taking advantage of this, we succeeded in isolating microvascular EC from the pancreas and we report here the establishment of primary cultures of human islet microvascular endothelial cells (HIMEC). HIMEC expanded rapidly, exhibited all the features of endothelium and were long-term expanded for approximately more than 25 passages, without signs of senescence. HIMEC are the first non-immortalized culture of EC that retains the morphological, phenotypic and functional characteristics of normal human microvascular EC.

Materials and methods

Human pancreatic islet isolation and purification

The use of human specimens (islet preparations discarded from clinical use) was approved by the Institutional Review Board under the European Consortium for Islet Transplantation (ECIT) human islet distribution program supported by Juvenile Diabetes Research Foundation (JDRF) (3-RSC-2016-160-I-X) [18]. Human islets were isolated as previously described [19–21] from January 16, 2013 to June 14, 2015 from the Pancreatic Islet Processing Unit of Diabetes Research Institute (SR-DRI) at the San Raffaele Scientific Institute in Milan, Italy. Briefly, the pancreatic duct was cannulated with 14–20 G catheter and distended by intra-ductal infusion of a cold collagenase solution (Collagenase NB1 Premium Grade, Serva). After digestion at 37°C in a modified Ricordi chamber, islets were purified on a Cobe 2991 (Terumo BCT) using a continuous HBSS-Ficoll (Biochrom) gradient. Purified islet fractions were cultured in Final Wash (Mediatech Cellgro) plus 1% penicillin/streptomycin and 1% Glutamine (Lonza).

EC isolation and culture

Pancreatic islet preparations from 10 multi-organ donors (7 males and 3 females, with a mean age of 49 ± 13 years), with a percentage of endocrine tissue ranging from 10%–50% and not used for transplantation, were processed for EC isolation. Briefly, the islet preparation was washed with phosphate buffer saline (PBS) without Ca^{2+} and Mg^{2+} (Euroclone) and centrifuged at 300*g* for 10 minutes; the pellet obtained was then re-suspended in a Collagenase solution (0.1% weight/volume (w/v); Sigma) and incubated for 60 minutes at 37°C, under gentle tilting and rotation, to obtain a single cell suspension. After enzymatic digestion, the suspension was washed with PBS and centrifuged at 300*g* for 10 minutes and the cells seeded in a T25 culture flask pre-coated with Bovine Collagen (1 μg/cm^2, BD) and Human Fibronectin (1 μg/cm^2, Chemicon) and then incubated at 37°C 5% CO_2. For EC growth, a specific endothelial growth medium called Endothelial Proliferation Medium (EndoPM) [17], developed and patented in our laboratory (MI2011A000201, Patent Cooperation Treaty [PCT]), was used.

Immunomagnetic selection

EC were purified by immunomagnetic selection, using the CELLection™ Pan Mouse IgG Kit (DYNAL BIOTECH), using the Direct Technique of cell isolation. In detail, cells cultured in EndoPM were detached, the pellet was resuspended in 1 mL PBS + 0.1% bovine serum albumin (BSA; Sigma) and incubated, with gentle tilting, for 45 minutes at 4°C with anti-mouse CD31 pre-coated dynabeads. The solution was then placed in a magnetic grid, the cells washed three times and re-suspended in Roswell Park Memorial Institute (RPMI) + 1% fetal bovine serum (FBS; Gibco). To maximize bead releasing, cells were incubated for 15 minutes Room Temperature (RT) with DNase I under tilting and rotation and subse-