EBioMedicine 2 (2015) 109–119

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Original Article

3-D Imaging Reveals Participation of Donor Islet Schwann Cells and Pericytes in Islet Transplantation and Graft Neurovascular Regeneration

Jyuhn-Huarng Juang [a,b], Chien-Hung Kuo [a,c], Shih-Jung Peng [d,e], Shiue-Cheng Tang [d,e,f,*]

[a] Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan 33302, Taiwan
[b] Department of Medicine, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan
[c] Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hsinchu 31040, Taiwan
[d] Connectomics Research Center, National Tsing Hua University, Hsinchu 30013, Taiwan
[e] Institute of Biotechnology, National Tsing Hua University, Hsinchu 30013, Taiwan
[f] Department of Medical Science, National Tsing Hua University, Hsinchu 30013, Taiwan

ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 25 January 2015
Accepted 25 January 2015
Available online 28 January 2015

Keywords:
3-D histology
Islet transplantation
Schwann cells
Pericytes
Revascularization
Reinnervation

ABSTRACT

The primary cells that participate in islet transplantation are the endocrine cells. However, in the islet microenvironment, the endocrine cells are closely associated with the neurovascular tissues consisting of the Schwann cells and pericytes, which form sheaths/barriers at the islet exterior and interior borders. The two cell types have shown their plasticity in islet injury, but their roles in transplantation remain unclear. In this research, we applied 3-dimensional neurovascular histology with cell tracing to reveal the participation of Schwann cells and pericytes in mouse islet transplantation. Longitudinal studies of the grafts under the kidney capsule identify that the donor Schwann cells and pericytes re-associate with the engrafted islets at the peri-graft and perivascular domains, respectively, indicating their adaptability in transplantation. Based on the morphological proximity and cellular reactivity, we propose that the new islet microenvironment should include the peri-graft Schwann cell sheath and perivascular pericytes as an integral part of the new tissue.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The goal of islet transplantation is to use the donor β-cells to restore the insulin production and glycemic regulation in patients with type 1 diabetes to avoid serious complications (Barton et al., 2012; Goland and Egli, 2014). For long-term graft survival, neurovascular regeneration in the new islet microenvironment is essential for the engraftment process (Jansson and Carlsson, 2002; Persson-Sjogren et al., 2000; Reimer et al., 2003). The graft–host integration through the neurovascular networks is important for grafts to receive nutrients and stimulations from the circulation and nerves to maintain survival and respond to physiological cues. Thus, identification of the mechanisms and cellular players that participate in islet neurovascular regeneration holds the key to improving the outcome of transplantation.

In the pancreas, the endocrine islets receive rich neurovascular supplies, which consist of not only the nerves (sympathetic, parasympathetic, and sensory nerves; Ahren, 2012; Borden et al., 2013; Tang et al., 2014) and blood vessels, but also the Schwann cells (the glial cells of the peripheral nervous system) and pericytes residing at the exterior and interior boundaries of the islet, facing the exocrine pancreas and endothelium, respectively (Donev, 1984; Hayden et al., 2008; Richards et al., 2010; Sunami et al., 2001). Morphologically, the islet Schwann cell network resides in the islet mantle, forming a mesh-like sheath (with apparent openings) surrounding the islet. The Schwann cells also release neurotrophic factors such as the nerve growth factor and glial cell line-derived neurotrophic factor (GDNF) to the islet microenvironment to support the "neuroendocrine" tissue (Mwangi et al., 2008; Teitelman et al., 1998).

The pericytes, also known as the mural cells, reside on the abluminal side of the blood vessels, guarding the vascular network. The pericyte and endothelium interactions are important for the angiogenesis and survival of endothelial cells (Armulik et al., 2005; Lindahl et al., 1997; von Tell et al., 2006). Particularly in angiogenesis, the release of platelet-derived growth factor from the endothelial cells recruits pericytes to establish their physical contact for paracrine signaling to stabilize the vascular system.

While sheathing the islets and blood vessels, in the pancreas the Schwann cells and pericytes also react to the islet injury and lesion formation in experimental diabetes (Tang et al., 2013; Teitelman et al., 1998; Yantha et al., 2010). For example, in the rodent model of islet injury induced by the streptozotocin injection, both Schwann cells and pericytes become reactive in response to the islet microstructural and

Abbreviations: 2-D, 2-dimensional; 3-D, 3-dimensional; GFP, green fluorescence protein; GFAP, glial fibrillary acidic protein; NG2, neuron-glial antigen 2.

* Corresponding author at: Department of Medical Science, National Tsing Hua University, Hsinchu 30013, Taiwan.
E-mail address: sctang@life.nthu.edu.tw (S.-C. Tang).

http://dx.doi.org/10.1016/j.ebiom.2015.01.014
2352-3964/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

vascular damages (Tang et al., 2013; Teitelman et al., 1998). In the non-obese diabetic (NOD) mouse model, the islet lesion induced by lymphocytic infiltration leads to peri-lesional and perivascular Schwann cell activation occurring at the front of lymphocytic infiltration in insulitis (Tang et al., 2013). Regarding the pericytes, their cellular responses were also found in the islet injuries induced by streptozotocin injection and lymphocytic infiltration with global (streptozotocin injection) and localized (NOD mice) changes of pericyte density (Tang et al., 2013). Importantly, the plasticity of Schwann cells and pericytes in response to islet injury suggests their potential reactivity in islet transplantation, in which the injuries occur both to the donor islets and at the transplantation site of the recipient.

To elucidate the ability of Schwann cells and pericytes in the participation of islet graft neurovascular regeneration, in this research we transplanted the mouse islets under the kidney capsule and prepared transparent graft specimens by tissue clearing (or optical clearing, use of an immersion solution of high refractive index to reduce scattering in optical microscopy; Chiu et al., 2012; Fu et al., 2009; Fu and Tang, 2010; Liu et al., 2015) to characterize the 3-dimensional (3-D) features of the Schwann cell and vascular networks, which otherwise cannot be easily portrayed by the standard microtome-based histology. We also transplanted the labeled islets with the nestin (the marker of neurovascular stem/progenitor cells; Dore-Duffy et al., 2006; Mignone et al., 2004; Treutelaar et al., 2003) promoter-driven green fluorescence protein (GFP) expression in the Schwann cells and pericytes (Alliot et al., 1999; Clarke et al., 1994; Frisen et al., 1995) to trace their locations and activities in the recipient.

Taking advantage of the 3-D features of the islet graft neurovascular network, in this research we demonstrate: (i) the regeneration of the islet Schwann cell sheath and the perivascular pericyte population after islet transplantation under the kidney capsule and (ii) the contribution of the donor islet Schwann cells and pericytes to the regeneration process. In this article, we present the morphological and quantitative data of the islet graft Schwann cell network and pericytes and discuss the implications of their participation in the islet graft neurovascular regeneration.

2. Materials & Methods

2.1. Animals and Islet Transplantation

Male inbred C57BL/6 (B6) mice, age 8–12 weeks, were used as the donors and recipients for islet transplantation. The nestin-GFP transgenic donor mice used in this research have been developed previously (Mignone et al., 2004). In these animals, the GFP expression is under the control of the 5.8-kb promoter and the 1.8-kb second intron of the nestin gene, which encodes a type VI intermediate filament protein. The nestin-GFP$^+$ mice and their nestin-GFP$^-$ littermates, age 8–12 weeks, were used as the donors and recipients, respectively, to avoid rejection. The Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital approved all procedures with these mice.

All recipient mice used in this research were diabetic prior to islet transplantation. The diabetic B6 and nestin-GFP$^-$ mice were induced by a single intra-peritoneal injection of streptozotocin (STZ, Sigma, St. Louis, MO, USA, 200 mg/kg body weight). Before transplantation, diabetic recipients were confirmed by hyperglycemia, weight loss, and polyuria; only the mice with blood glucose levels higher than 350 mg/dl two weeks after the STZ injection were transplanted. Blood glucose concentration was measured from the tail tip with a portable glucose analyzer (One Touch II, LifeScan Inc., Milpitas, CA, USA).

Islet isolation was performed under sodium amobarbital-induced anesthesia with the donor pancreases distended with 2.5 ml of digestion solution (ductal injection of RPMI-1640 medium supplemented with 1.5 mg/ml of collagenase; RPMI: Invitrogen, Carlsbad, CA, USA; collagenase: Sigma, from *Clostridium histolyticum*, type XI), excised, and incubated in a water bath at 37 °C. Afterward, the islets were purified by a density gradient (Histopaque-1077, Sigma) and then handpicked under a stereo microscope. Islets with a diameter between 75 and 250 μm were collected for transplantation. Three hundred islets were syngeneically transplanted under the left kidney capsule on the same day of isolation. Mice with reversal of diabetes to normoglycemia two weeks post-transplantation were defined as mice with engrafted islets and included in the study (Juang et al., 2014).

2.2. Tissue Labeling

Blood vessels of the kidney and islet graft were labeled by vessel painting (Fu et al., 2010; Juang et al., 2014) via cardiac perfusion of the lectin-Alexa Fluor 488 conjugate (30 μg/g of body weight, Invitrogen, Cat No. W11261) followed by 4% paraformaldehyde perfusion fixation. Afterward, grafts under the kidney capsule were harvested and the vibratome sections of the tissue (~400 μm) were post-fixed in 4% paraformaldehyde solution for 1 h at 25 °C. The fixed tissues were then immersed in 2% Triton X-100 solution for 2 h at 25 °C for permeabilization.

Three different primary antibodies were used to immunolabel the tissues following the protocol outlined below. The antibodies used were polyclonal rabbit anti-glial fibrillary acidic protein (GFAP, Schwann cell marker) antibody (DAKO, Z0334; Carpinteria, CA, USA), a rabbit anti-neuron-glial antigen 2 (NG2, pericyte marker) antibody (AB5320; Millipore, Billerica, MA, USA), and polyclonal guinea pig anti-insulin (Gene Tex, Irvine, CA, USA) antibody. Before applying the antibody, the tissue was rinsed in phosphate-buffered saline (PBS). This was followed by a blocking step, incubating the tissue with the blocking buffer (2% Triton X-100, 10% normal goat serum, and 0.02% sodium azide in PBS). The primary antibody was then diluted in the dilution buffer (1:50, 0.25% Triton X-100, 1% normal goat serum, and 0.02% sodium azide in PBS) to replace the blocking buffer and incubated for one day at 15 °C.

Alexa Fluor 647 conjugated goat anti-rabbit secondary antibody and Alexa Fluor 546 conjugated goat anti-guinea pig secondary antibody (1:200, Invitrogen) were used to reveal the immunostained structures. Nuclear staining by propidium iodide (50 μg/ml, Invitrogen) was performed at room temperature for 1 h to reveal the nuclei, if necessary. The labeled specimens were then immersed in the optical-clearing solution (FocusClear™ solution, CelExplorer, Hsinchu, Taiwan or RapiClear 1.52 solution, SunJin Lab, Hsinchu, Taiwan) before being imaged via confocal microscopy.

2.3. Confocal Microscopy

Imaging of the tissue structure was performed with a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss, Jena, Germany) equipped with an objective of 25× LD 'Plan-Apochromat' glycerine immersion lenses (working distance: 570 μm) (optical section: 5 μm; Z-axis increment: 2.5 μm) and an objective of 40× LD 'C-Apochromat' water immersion lenses (working distance: 620 μm) (optical section: 3 μm; Z-axis increment: 1.5 μm) under a regular or tile-scan mode with automatic image stitching. The laser-scanning process was operated under the multi-track scanning mode to sequentially acquire signals, including the transmitted light signals. The Alexa Fluor 647-labeled structures were excited at 633 nm and the fluorescence was collected by the 650- to 710-nm band-pass filter. The propidium iodide-labeled nuclei and the Alexa Fluor 546-labeled structures were excited at 543 nm and the signals were collected by the 560- to 615-nm band-pass filter. The lectin-Alexa Fluor 488-labeled blood vessels were excited at 488 nm and the fluorescence was collected by the 500- to 550-nm band-pass filter.

2.4. Image Projection and Analysis

The LSM 510 software (Carl Zeiss, Jena, Germany) and the Avizo 6.2 image reconstruction software (VSG, Burlington, MA, USA) were used

Download English Version:

https://daneshyari.com/en/article/2121316

Download Persian Version:

https://daneshyari.com/article/2121316

Daneshyari.com