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The effect of fibroblast activation on vascularization in transplanted pancreatic islets

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ABSTRACT

Background: Insufficient revascularization of transplanted pancreatic islets is an important reason why the long-term effects of pancreatic islet transplantation on type I diabetes patients have been so limited. The goal of this study was to investigate the role of fibroblasts (FBs) activated by tumor cell supernatants on the vascularization of transplanted pancreatic islets.
Materials and methods: Pancreatic islets and activated or inactivated FBs were used for subrenal capsule transplantation. Mouse melanoma cell supernatants were used to activate FBs; the tests of the purity of the pancreatic islet cells of the donor, survival rate, and function of insulin secretion were performed to ensure high-quality transplants. Mice receiving the allogeneic transplantation were given tacrolimus and sirolimus to prevent rejection. The diabetic model was induced by streptozotocin.
Results: Conditioned medium made of tumor cell supernatants was found to stimulate the expression of α-smooth muscle actin and vascular endothelial growth factor A to an extent notably greater than that of pancreatic islet transplantation alone or pancreatic islet transplantation combined with inactivated FBs. FBs from the recipient were associated with capillary density in the transplanted pancreatic islet most closely to that observed in isogenically transplanted pancreatic islets and the original pancreatic islet. In this way, activated FBs derived from the recipient combined with pancreatic transplantation were able to treat diabetes, and long-term survival was achieved.
Conclusions: The current research sheds new light on the revascularization of transplanted pancreatic islets: activated FBs derived from the recipients, when transplanted alongside pancreatic tissue, can promote revascularization inside the transplanted pancreatic islet.

1. Introduction

Currently, two large problems for clinically successful pancreatic islets transplantation are delayed and insufficient vascularization and the resulting hypoxic damage to the implanted pancreatic islet [1,2]. Studies have shown that even after revascularization, the density of blood vessels of transplanted islets is significantly lower than that of normal islets. Insufficient blood supply may be a leading cause of the large amount of deaths of transplanted islets and their impaired function [3]. Currently, most scholars believe that insufficient vascularization is the most important factor affecting the long-term effects of pancreatic islet transplantation. Vascular endothelial growth factor A (VEGF-A) plays an important role in blood vessel formation and in improving the function of islet revascularization after transplantation [4,5].

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Tumor studies have shown that because stromal fibroblasts (FBs) are activated by many growth factors secreted by tumor cells, they upregulate the expression of cytokines that stimulate blood vessel growth, such as VEGF-A, thus promoting the growth of capillaries surrounding the tissue [6–8].

First, we hypothesize that FB can be activated *in vitro*, and the activation of FBs upregulates VEGF-A expression. Next, we hypothesize that transplantation of pancreatic islets with activated FB increases islet revascularization and the capillary density of the transplants, thus increasing the blood and oxygen supplies of the transplanted islet and alleviating the problem of difficult long-term survival.

2. Materials and methods

2.1. Experimental animals

SPF male BALB/c ($H2^d$) mice and C57BL/6($H2^b$) mice were all purchased from the animal center of Chongqing Medical University. All experimental procedures conformed to the principle of animal experimentation and were performed under the supervision of the Animal Ethic Committee of Chongqing Medical University.

2.2. Preparation of FBs

For details, see SDC, Materials and methods.

2.3. Preparation of conditioned medium

For details, see SDC, Materials and methods.

2.4. Examination of the expression of α-smooth muscle actin (α-SMA) and VEGF at different points in time using real-time reverse transcription–polymerase chain reaction and western blot analysis

All procedures were performed following the instructions (SDC, Materials and methods).

2.5. Examination of α-SMA levels using immunohistochemical methods

For details, see SDC, Materials and methods.

2.6. Examining the potential tumor activity of activated FBs

A total of 1×10^6 FBs, activated at the optimal period, were added and mixed with 200 μL of saline before subcutaneous implantation into nude mice.

2.7. Isolation and purification of the pancreatic islets

Donor islets came from C57BL6 or BALb/C mice. Isolation and purification procedures were the same as reported before [9] (SDC, Materials and methods).

2.8. Observation of islet morphology and purity calculation, survival rate of pancreatic islets, pancreatic islet count, and test of islet secretion function

For details, see SDC, Materials and methods.

2.9. Transplantation of mouse islet cells combined with FBs

For details, see SDC, Materials and methods.

2.10. Transplantation groups

There were in total six groups. The six groups are the following: group A, transplantation of pancreatic islet ($H2^d$) alone; group B, transplantation of pancreatic islet ($H2^d$) combined with donor FB ($H2^d$); group C, transplantation of pancreatic islet ($H2^d$) combined with recipient FB ($H2^b$); group D, transplantation of pancreatic islet ($H2^d$) combined with activated donor FB ($H2^d$); group E, transplantation of pancreatic islet ($H2^d$) combined with activated recipient FB ($H2^b$); and group F, transplantation of homogenic islet ($H2^b$). In each group, there were 12 recipient mice.

2.11. Application of immunosuppressive drugs

Application of immunosuppressive drugs was performed as in the SirTac method [10] (SDC, Materials and methods).

2.12. Relevant tests performed after pancreatic islet transplantation

The relevant tests performed are frequency of blood sugar testing, histologic hematoxylin and eosin (HE) staining of the transplants, and examination of the transplant tissue using confocal immunofluorescence microscopy (SDC, Materials and methods).

2.13. Statistical methods

SPSS17.0 software was used for statistical analysis. The experimental data were expressed as $\bar{x} \pm s$. Analysis of variance for factorial design was used for comparison between two factors. The values of $P < 0.05$ were considered significant.

3. Results

3.1. Morphological changes of FBs cultured in normal and conditioned media

The cell morphology of FBs cultured in conditioned media containing tumor cell supernatants was not notably different from that of FBs cultured in primary culture (SDC, Fig. 1).