Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

A lectin microarray study of glycoantigens in neonatal porcine islet-like cell clusters

Akira Maeda, MD,[a] *Takehisa Ueno, MD,*[a] *Shino Nakatsu, MS,*[a] *Dandan Wang, BS,*[a] *Noriaki Usui, MD,*[a] *Shunsaku Takeishi, PhD,*[b] *Teru Okitsu, MD,*[c] *Masafumi Goto, MD,*[d] *Hiroshi Nagashima, PhD,*[e] *and Shuji Miyagawa, MD*[a,*]

[a] *Department of Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan*
[b] *Glycomics Research Laboratory, Moritex Corporation, Yokohama, Kanagawa, Japan*
[c] *Department of Mechanical and Biofunctional System, Institute of Industrial Science, University of Tokyo, Tokyo, Japan*
[d] *Department of Life Science, New Industry Creation Hatchery Center, Tohoku University, Sendai, Miyagi, Japan*
[e] *Department of Life Science, Meiji University, Kawasaki, Kanagawa, Japan*

ARTICLE INFO

Article history:
Received 6 October 2012
Received in revised form
19 November 2012
Accepted 17 December 2012
Available online 8 January 2013

Keywords:
NPCC
Glycoantigen
Xenotransplantation
Gal-knockout pig
Lectin array

ABSTRACT

Background: Besides α-Gal expression, the differences of glycosylation and antigenicity between adult pig islets (APIs) and neonatal porcine islet-like cell clusters (NPCCs) are altogether unclear. In this study, lectin microarray analyses of NPCCs were performed and the results compared with the corresponding values for wild-type APIs and NPCCs from α-Gal transferase knockout (GalT-KO) pig.

Methods: NPCCs were isolated from 1–3-d-old neonatal wild-type pigs and cultured for 1 d, 5 d, and 9 d, using a previously described technique. Alternatively, the isoration of APIs were isolated based on the method for human islets.

Results: In a comparison between NPCCs and APIs, all of the NPCCs showed higher signals for *Sambucus nigra*, *Sambucus sieboldiana*, and *Trichosanthes japonica I* and the binding of α2,6 sialc acid, whereas the APIs showed stronger signals for *Lotus tetragonolobus*, *Aleuria aurantia*, *Narcissus pseudonarcissus*, and *Galanthus nivalis*, suggesting that APIs contain high levels of high-mannose forms. Among the NPCCs, NPCC (day1) appeared to be richer than the others in *Lotus tetragonolobus*, *Narcissus pseudonarcissus*, *Galanthus nivalis*, and *Urtica dioica*, implying the presence of high-mannose forms. However, as a whole, the signals for many lectins for NPCCs were very similar. The NPCCs from a GalT-KO pig indicated not only the down-regulation of α-Gal expression but α-GalNAc as well, and α2-6 sialic acid was upregulated.

Conclusions: The results reported herein contain useful information for the future production of immunomodified pigs with less antigenicity than GalT-KO pigs toward clinical applications of NPCCs.

1. Introduction

The increasing problem of the worldwide shortage of donor organs has led to a revival of interest in xenotransplantation. Xenografting can be classified as either discordant or concordant, such as pig to human or monkey to human, respectively, based on the severity and pattern of the graft rejection. The pig represents an ideal animal for discordant

* *Corresponding author*. Department of Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3062; fax: +81 6879 3069.
E-mail address: miyagawa@orgtrp.med.osaka-u.ac.jp (S. Miyagawa).
0022-4804/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jss.2012.12.037

xenografts for a variety of reasons, including anatomical, physiological, and ethical considerations. The exposure of pig cells, tissues, and organs to human blood, however, results in a hyperacute rejection, which is mediated by naturally occurring high-titer antibodies and complements that are produced by humans [1]. The major xenoantigen responsible for this type of rejection is a single carbohydrate structure, the α-Gal epitope (Galα1-3Galβ1-4GlcNAc-R) [2], which is expressed by most mammalian cells, including the pig. Old World monkeys, apes, and humans lack this enzyme activity because the α1,3-galactosyltransferase gene is inactivated, and, in contrast, produce large amounts of antibodies, designated as anti-Gal, against the α-Gal epitope. Human anti-pig antibodies are not only IgG, but also include IgM and IgA, and these bind most strongly to oligosaccharides that contain this alpha galactose terminal residue [3].

On the other hand, using somatic cell nuclear transfer technology [4,5], we successfully produced genetically engineered pigs by heterozygous knockout (KO) of the α1-3galactosyltransferase (GalT) gene (GalT-KO). However, even after GalT-KO pigs were produced, most antigens, including the so-called non-Gal antigen, were still present on the surface of the pig cells, and this fact represents a significant obstacle to successful xenotransplantation. That is, although the production of GalT-KO pigs appeared to eliminate the problem of hyperacute rejection, acute vascular rejection/acute humoral xenograft rejection, which involves the production of xenoreactive antibodies related to glycoantigens and subsequent activation of the graft endothelium, containing the system that controls activation of the complement and coagulation, was defined as a new obstacle for clinical xenotransplantation [6].

The pig pancreas is now considered to be the most suitable source of islets for clinical xenotransplantation, especially for patients with type I diabetes, which is a very common disease (approximately one million people have been diagnosed in the United States). Two types of islet transplantations are under consideration. One is the transplantation of adult pig islets (APIs) [7]. The other is the transplantation of neonatal porcine islet-like cell clusters (NPCCs) [8]. Several clinical trials [9] and preclinical experiments [10] using either type of islets are currently in progress. However, in addition to the α-Gal epitope, our knowledge of the extent of glycosylation and xenoantigenicity of pig islets is far from clear.

Regarding the extent of α-Gal expression on APIs, the results of a number of published studies indicate that the expression of α-Gal in adult pig islet cells is negligible. However, APIs have non-Gal antigenicity, including the Hanganutziu-Deicher (H-D) antigen, especially in terms of *N*-linked sugars. Evidence collected in our previous study showed that human natural antibodies IgG and IgM react with adult islets. Neuraminidase-sensitive sialic acid antigens other than H-D antigens are related to the binding of non-Gal antibodies. On the other hand, concerning the expression of α-Gal on NPCCs, NPCCs clearly express α-Gal, and the existence of the H-D antigen on NPCCs was also demonstrated. NPCCs have a strong antigenicity derived from *N*-linked sugars, but at the same time the cell surface glycolipids of these cells are also antigenic. Available data indicate that pig sialic acids, besides the H-D antigen, are clearly antigenic to human serum [7,8].

In this study, we took advantage of the fact that lectins are available that bind to unique glycostructures. Lectin microarray analyses [11] for NPCCs cultured for 1, 5, and 9 d were performed and the results compared with the corresponding values for islets from wild-type APIs. In addition, changes in the glycoantigens of the NPCCs from a GalT-KO pig that was produced as a pig line by our group were also analyzed.

2. Materials and methods

2.1. *Preparation of NPCC*

Pancreases were isolated using the technique described by Korbutt *et al.* [12]. Pancreases were obtained from 1–3-d-old neonatal pigs (Large White/Landrace × Duroc) of either sex. A pig cloned from a homozygous GalT-KO pig and wild-type pigs were used. The pancreas was dissected and stored in cold Hanks' balanced saline solution (HBSS; GIBCO Labs, Grand Island, NY) supplemented with 0.25% bovine serum albumin (Nakalai Tesque, Kyoto, Japan), 10 mm N-2-hydroxyethylpiperazine-N1-2-ethane-sulfonic acid (HEPES, Nakalai Tesque), and antibiotics until processed. In a typical preparation, a pancreas was minced into 1–2 mm^3 fragments and then digested with type V collagenase (2.5 mg/mL; Sigma, St Louis, MO) at 37°C with gentle shaking. After filtration through a nylon screen (500 μm), the tissue was washed in HBSS three times and cultured at 37°C in a humidified atmosphere with 5% CO_2 for 7–9 d in Ham's F10 Medium (GIBCO) containing 10 mmol/L glucose, 50 μmol/L isobutylmethylxanthine (Nakalai Tesque), 0.5% bovine serum albumin (Nakalai Tesque), 2 mmol/L l-glutamine (Nakalai Tesque), 10 mmol/L nicotinamide (Sigma), and antibiotics. The culture medium was changed on the first day after isolation and every other day thereafter [8].

2.2. *Adult pig islet isolation*

Porcine islet isolation and cultures were performed as previously described [13], with minor modifications. In brief, the duodenal-splenic lobe and connective lobe were distended intraductally by treatment with a cold enzyme solution of LiberaseHI (Roche Molecular Biochemicals, Indianapolis, IN) diluted with HBSS. The distended glands were digested by the semi-automated continuous-filtration method at 37°C.The cleaved islets were purified from non-islet tissue with a continuous density gradient in a COBE 2991 cell processor (COBE Blood Component Technology, Lakewood, CO). Isolated islets were cultured overnight at 37°C in a humidified atmosphere with 5% CO_2 in Tohoku culture bags (Nipro, Osaka, Japan) [14] with medium 199 (Gibco) supplemented with 10% heated inactivated porcine serum, 2 mM N-acetyl-l-alanyl-l-glutamine, 10 mM HPEPES (Nakarai Tesque), 100 IU/mL penicillin, 100 μg/mL streptomycin (Biochrom), and 20 μg/mL ciprofloxacin.

2.3. *Preparation of the samples*

Each sample was washed with phosphate-buffered saline and sonicated in PBST (phosphate-buffered saline containing 1.0%