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Sialo-Xenoantigenic Glycobiology

Molecular Glycobiology of Sialylglycan-Xenoantigenic Determinants in Pig to Human Xenotransplantation

Springer

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Preface (Summary)

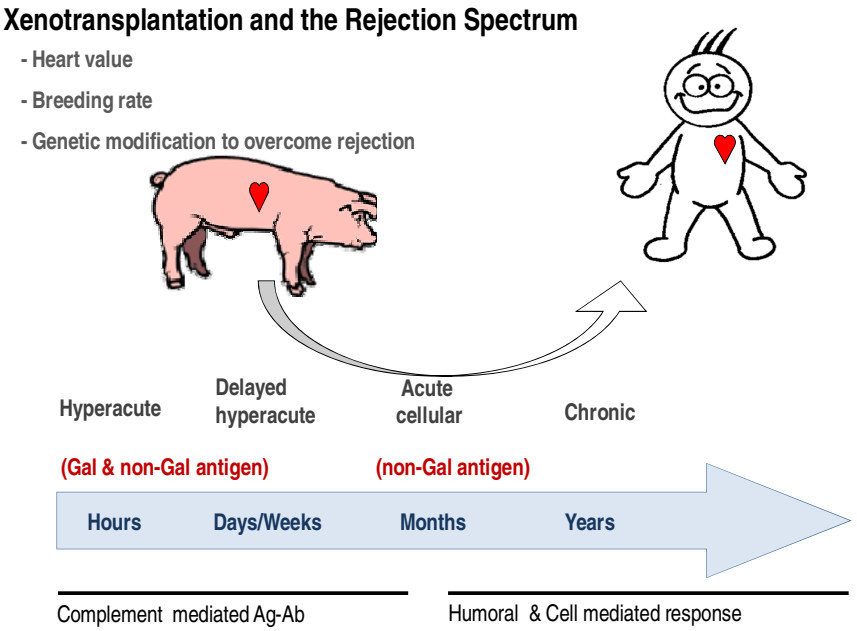
During the past two decades, glycobiology has hugely been developed in its biological significance and biological diversity. The glycobiology is specifically involved in cell-cell interaction, cell differentiation, receptor-mediated targeting, molecular recognition and transplantation. The scope of the present new book is narrow, focusing on carbohydrate antigens including sialic acids as xenoantigenic determinant in human.

In xenotransplantation, the pig has been identified as a suitable organ donor candidate for humans because of its compatible organ size and short breeding time (Scheme 1). However, exposure of pig organs to human blood results in hyper acute rejection (HAR) in pig to human xenotransplantation. The rejection is caused by differences in carbohydrate epitopes on the human and pig vascular endothelia (Table. 1). When pig organs or tissues are transplanted into the human body, the human IgM isotype of anti-Gal binds to Gal antigens on the pig tissues, which causes activation of the complement cascade resulting in cell lysis. The Gal antigen was eliminated by knocking out the α-1,3 galactosyltransferase, but the remaining so-called non-Gal antigens are considered to be xenoantigens subsequently involved in the rejection phenomenon.

Carbohydrate antigens, present on glycoconjugates of all mammalian cells, play crucial roles in various biological processes and are epitopes recognized by the immune system. Among them, carbohydrate antigens containing sialic acid, such as sialosyl-Tn or Hanganutziu-Deicher (HD), are non-Gal antigens against which humans are suggested to have naturally occurring antibodies (Table. 2).

To overcome rejection responses such as HAR in xenotransplantation, studies of genes involved in carbohydrate antigens that cause xenoantigenicity are necessary. Knowledge of pig glycosyltransferases would be useful to apply to xenoantigen masking or identification of the xenoantigenic sialylglycan(s). However, most pig glycosyltransferase genes have not yet been isolated. Therefore, in the first chaprer of the present study, we screened for pig glycosyltransferase genes involved in generating xenoantigens. In the chpter II to IV, we cloned, functionally characterized, and investigated the regulatory mechanism of the pig CMAH gene in NeuGc biosynthesis. Lastly, we investigated the effects of an alteration of pig glycosylation pattern on human serum-mediated cytotoxicity, caused by human sialyltransferases including hST6GalNAc IV.

Keywords: *glycosyltransferase, sialyltransferase, xenoantigen, N-glycolylneuraminic acid*



Scheme 1. Xenotransplantation and the rejection spectrum

Table 1. Structure of the known carbohydrate epitopes exposed at the surface of human and pig vascular endothelia

Human	Pig
Galβ 1,4GlcNAcβ 1-R	Galβ 1,4GlcNAcβ 1-R
ABH-Galβ 1,4GlcNAcβ 1-R[a]	**Galα 1,3**Galβ 1,4GlcNAcβ 1-R[b]
NeuAcα 2,3Galβ 1,4GlcNAcβ 1-R[c]	NeuAcα 2,3Galβ 1,4GlcNAcβ 1-R[c]
	NeuGcα 2,3Galβ 1,4GlcNAcβ 1-R[d]

Modified from Oriol et al.
The epitopes represented by bold type are different between two species.
R is glycolipid or glycoprotein carrier molecules anchored in the cell membrane.
[a] A,B,H or AB blood group antigen. [b] Gal antigen. [c] N-acetylneuramic acid.
[d] N-glycolylneuramic acid.

Table 2. Known carbohydrate antigens against which humans may have naturally occurring antibodies

1.	**Hanganutziu-Deicher : NeuGc**
2.	**Thomsen-Friedenreich (T or TF) : Galβ1,3GalNAcα1-R**
3.	**Tn(TF precursor) : GalNAcα1-R**
4.	**Sialosyl-Tn : NeuAcα2,6GalNAcα1-R**
5.	**Forssman : GalNAcα1,3GalNAcβ1,3Galα1,4Galβ1,4Glcβ1-R**
6.	**αRhamnose-containing oligosaccharides**
7.	**Sulphatide I : SO_4-3Gal-R**

Modified from Ezzelarab et al. R is glycolipid or glycoprotein carrier molecules anchored in the cell membrane

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Chapter 1
Screening of Pig Glycosyltransferase Genes Related to Xenoantigens and Their Masking

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Abstract. In this study, to screen for pig glycosyltransferase genes involved in xenoantigen synthesis or masking, pig EST sequences were collected from the TIGR and NCBI databases, and local BLAST was performed using a known human sialyltransferase gene. Sequence fragments of pig sialyltransferase genes were obtained from the BLAST results, and from these, full ORF sequences of three pig sialyltransferase genes, ST3Gal III, ST3Gal IV, and ST8Sia IV, were isolated. A partial fragment of the pig iGb3 synthase gene was also isolated by PCR, using primers based on the conserved sequence information of known iGb3S genes.

1 Introduction

Glycoconjugates are generated by the sequential and coordinated action of many glycosyltransferases (Milland, et al., 2005). Glycosyltransferases, located in the endoplasmic reticulum (ER) and Golgi apparatus, catalyze the sequential transfer of monosaccharides from nucleotide sugars to saccharide acceptors, resulting in mature oligosaccharides (Milland et al., 2005). Many glycosyltransferases are involved in xenotransplantation, related to the expression or masking of xenoantigens. Among these, the most important glycosyltransferases in xenotransplantation are the α1,3 galactosyltransferases including the α1,3GT and iGb3 synthase (iGb3S).

α1,3GT and iGb3S both transfer UDP-Gal but differ in the acceptor utilized: N-acetyllactosamine (NacLac, protein and lipid) or lactosylceramide (LacCer, lipid), respectively (Fig. 1). A terminal galactose-α1,3-galactose (Gal) residue is acommon structure of carbohydrate antigens presented on most mammalian cell surfaces, well characterized because of their importance in blood transfusion and

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organ allo- and xeno-transplantation (Heissigerova et al., 2003). The Gal-α1,3-Gal antigen (Gal antigen) is a major xenoantigen in pig-to-human xenotransplantation. The pig α1,3GT gene, was isolated and targeted to develop α1,3GT deficient pigs. However, the Gal-α1,3-Gal antigen is still present in α1,3GT deficient pigs and possibly synthesized on lipids by iGb3S. Therefore, removal of the pig iGb3S gene is the next step necessary to achieve successful pig-to-human xenotransplantation without rejection responses. Although iGb3S has been cloned and characterized from mouse and rat (Taylor et al., 2003; Milland et al., 2006), the pig iGb3S gene has not yet been cloned.

Sialyltransferases are a family of more than 18 members that catalyze the transfer of sialic acid from CMP-Neu5Ac forming an α2,3-, an α2,6-, or an α2,8-linkage, depending on the acceptor sugar chain. In pig-to-human xenotransplantation, sialyltransferases such as ST3Gal III and ST6Gal I reduce the levels of Gal antigen by competing with α1,3-galactosyltransferase for the common acceptor substrate (Ezzelarab and Cooper, 2005; Koma et al., 2000; Tanemura et al., 1998). Almost all members of the sialyltransferase gene family of human and mouse have been isolated and characterized. However, most pig sialyltransferase genes have not yet been isolated, even though pig sialyltransferases would be useful to apply to xenoantigen masking or identification of xenoantigenic sialylglycan(s).

In this study, to screen for pig glycosyltransferase genes involved in xenoantigen synthesis or masking, pig EST sequences were collected from the TIGR and NCBI databases, and local BLAST was performed using a known human sialyltransferase gene. Sequence fragments of pig sialyltransferase genes were obtained from the BLAST results, and from these, full ORF sequences of three pig sialyltransferase genes, ST3Gal III, ST3Gal IV, and ST8Sia IV, were isolated. A partial fragment of the pig iGb3 synthase gene was also isolated by PCR, using primers based on the conserved sequence information of known iGb3S genes.

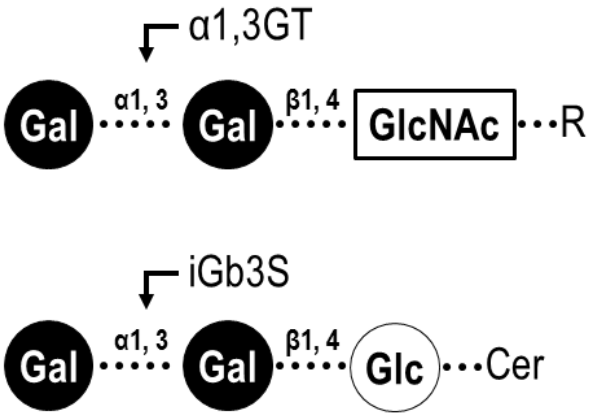

Fig. 1. Two types of α1, 3 galactosyltransferase involved in biosynthesis of Gal antigen.
R is glycolipid or glycoprotein carrier molecules anchored in the cell membrane. Gal; Galactose, Glc; Glucose, GlcNAc; N-acetylglucosamin, Cer; Ceramide.

2 Materials and Methods

2.1 Local BLAST

Pig expressed sequence tag (EST) sequences were collected from the institute of genome research (TIGR, http://www.tigr.org) and national center for biotechnology information (NCBI, www.ncbi.nlm.nih.gov) databases. Local BLAST searches were performed using BioEdit software to obtain the sequence information of pig sialyltransferase genes corresponding to the following human sialyltransferase genes (accession number in parentheses) : ST6Gal I (X17247), ST6GalNAc I (Y11339), ST6GalNAc II (AJ251053), ST6GalNAc IV (AJ271734), ST3Gal I (L29555), ST3Gal II (U63090), ST3Gal III (L23768), ST3Gal IV (L23767), ST3Gal V (AB018356), ST3Gal VI (AB022918), ST8Sia Ⅰ (D26360), ST8Sia Ⅱ (U33551), ST8Sia Ⅲ (AF004668), ST8Sia Ⅳ (L41680), and ST8Sia Ⅴ (U91641).

2.2 Cell Culture

A pig kidney cell line (PK15) was obtained from the Korean Cell Line Bank (KCLB) and pig ear fibroblast (PEF) was kindly provided by the National Livestock Research Institute, RDA. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; WelGENE, Korea) containing 100 units/ml of penicillin-streptomycin and 10% fetal bovine serum (FBS; WelGENE, Korea) at 37°C in a 5% CO_2 incubator/humidified chamber.

2.3 Isolation of Pig Sialyltransferases and iGb3S Gene

Total RNA was isolated from PK15 cells, PEF cells, or fresh pig tissues using TRIZOL reagent (Invitrogen, Carlsbad, CA), and cDNAs were synthesized by reverse transcriptase (RT) with an oligo dT-adaptor primer using AccuPower® RT-PreMix (Bioneer Co., Korea). For cloning the pig sialyltransferase, PCR was performed on PK15 orPEF cDNA using the following primer sets: ST3Gal I, 5'-ACCATGGCCCCCATGAGGAAGAAG A-3' (sense) and 5'-ACCTCATCTGCCCTTGAAGATCCGGA-3' (antisense); ST3Gal III, 5'-ACCATGGGACTCTTGGTATTTGTACG-3' (sense) and 5'-ACCTCAGATGCCGCTGGTCAGGTCA-3' (antisense) ST3Gal IV, 5'-ACCATGATCAGCAAGTGCCGCTGGA-3' (sense) and 5'-ACCTCAGAAGTATGTGAGGTTCTTG-3' (antisense); ST6GalNAc IV, 5'-ACCATGAAGCCTCCGGGTCGGCTCC-3' (sense) and 5'-ACCCTACTGGGTCTTCCAGGAGGGG-3' (antisense); ST8Sia IV, 5'-ACCATGCGCTCCATTAGGAAGAGGTG-3' (sense) and 5'-ACCTTATTGCTTTACGCACTTTCCTG-3' (antisense). The pig iGb3 gene fragment was amplified by PCR using the following primer set: iGb3S-S,

5'-GTGTTCTGCCTGGACGTG-3' and iGb3S-AS, 5'-CCAGAAGAACTTGTTAAGGTGG CT-3'. The amplified DNA fragments were subcloned into the EcoRV site of pSTBlue-1 AccepTorTM vector (Novagen). Multiple independent clones were isolated and sequenced to confirm the sequence data.

2.4 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from various pig tissues and cDNA was synthesized with oligo dT primer using 1 ug of total RNA as template. cDNA from various pig tissues was amplified by PCR with the above primer sets using EF-Taq polymerase (SolGent, Korea). The use of equal amounts of mRNA in the RT-PCR assay was confirmed by assessing β-actin expression levels.

Pig EST sequences were collected from the TIGR and NCBI data base, and Local blast was performed using a known human sialyltransferase gene. Black bar indicates sequence data from TIGR and gray bar indicates sequence data from NCBI. Overlapped region between TIGR and NCBI sequence data was represented by black bar. The number indicates sequence length.

3 Results

3.1 Screening and Cloning of Pig Sialyltransferase Genes

The nucleotide sequences of 17 human sialyltransferase genes were used to perform a local BLAST analysis of pig EST sequences in the TIGR and NCBI data base. As a result, pig EST sequences corresponding to each query sequence were collected. The retrieved sequences were aligned with the query sequence, and partially overlapping sequences were assembled to build a larger fragment or ORF (Table 1). From this analysis, we obtained full ORF sequences of five pig sialyltransferase genes, ST3Gal I, ST3Gal III, ST3Gal IV, ST6GalNAC IV, and ST8Sia IV, and sequence fragments for 9 pig sialyltransferases. We did not find pig ESTs for ST6GalNAc I, ST6GalNAc III, and ST8Sia VI.

Next, to isolate the complete gene sequences of the five pig sialyltransferase genes, ST3Gal I, ST3Gal III, ST3Gal IV, ST6GalNAC IV, and ST8Sia IV, we performed PCR using PK15 or PEF cDNA as template. As a result, the pig ST3Gal III and ST3Gal IV gene were isolated from PK15 cells, and the ST8Sia IV gene was isolated from PEF cells (Fig. 2). The pig ST3Gal III gene (Fig. 3) is comprised of 1077 bp encoding 358 amino acids, pig ST3Gal IV (Fig. 4) has 1002 bp encoding 333 amino acids, and pig ST8Sia IV (Fig. 5) has 1080 bp encoding 359 amino acids. Multiple alignment analysis revealed that three sialylmotifs were highly conserved in these 3 sialyltransferase genes (Fig. 6).