Differential Expression Patterns of Gangliosides in the Liver and Heart of NIH-miniature Pigs

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Gangliosides are a major component of the plasma membrane of mammalian cells, which are directly involved in a variety of immunological events, including cell-to cell or cell-to-protein interactions. In this study, we investigated whether gangliosides, sialic acid-containing glycosphingolipids, are related to rejection during the xenotransplantation of NIH-miniature pig livers and hearts to humans. Both high performance thin-layer chromatography and immunohistochemistry analyses revealed that the expression of gangliosides in the liver tissue of NIH-miniature pigs was higher than that in the heart. Gangliosides GD3, GD1a, GD1b, GT1b and GQ1b were observed in both the liver and heart, whereas GQ1b was detected only in the liver, indicating that the ganglioside expression profiles are tissue specific. Moreover, other ganglio-series gangliosides, including GM3, were not detected in the livers and hearts of NIH-miniature pigs. Taken together, these results suggest that gangliosides may play important roles in immune responses in clinical xenotransplants of pig livers and hearts.

Key words : Xenotransplantation, gangliosides, NIH-miniature pig

Introduction

Because of their similar anatomy and physiology, swine have been used in biomedical applications for many decades as a model for human diseases, as a genetically defined model for surgery and xenotransplantation, and as a source of human disease therapeutics [22]. Several pig models that have potential applications in basic life science research or in the study of human diseases have been used to make such as enhanced green fluorescent protein (GFP)-expressing pigs [20], alpha-1,3-galactosyltransferase knockout pigs [15], and cystic fibrosis transmembrane conductance regulator knockout pigs [23,24]. Specifically, the National Institutes of Health (NIH)-miniature pig was developed by Sachs [26], more than 30 years ago specifically for xenotransplantation.

Xenotransplantation has the potential to resolve the chronic shortage of organs for transplanatation. However, the major obstacle in pig to human xenotransplantation is hyperacute rejection (HAR) triggered by human antibodies to Galα1,3Gal (Gal)-terminated carbohydrate determinants in porcine tissues [20]. To gain further information regarding this response, several studies have evaluated alpha-1,3-galactosyltransferase (α1,3GalT) knock-out pigs [13,19]. However, pig to human xenotransplantations were still not possible because acute humoral xenograft rejection and cell mediated immune responses remained. Kim et al. [12] demonstrated that immune responses were mediated by various glycan-binding proteins showing highly diverse ligand specificities using miniature pig kidney N-glycan immobilized beads. In addition, several studies revealed that glycosphingolipids were expressed in a pig kidney and aorta and demonstrated the expression of α-Gal-terminated glycosphingolipid pig organs [2,7-9].

Eukaryotic cell surfaces are very rich in glycoconjugates, such as glycoproteins and glycolipids [23]. Glycosphingolipids, specifically gangliosides, are characterized by the presence of one or more sialic acid moieties in the oligosaccharide chains [6] that are localized on the outer leaflet of the plasma membrane. Gangliosides have recently been shown to modulate epithelial cell proliferation, adhesion, migration, differentiation, survival and immunosuppressive activity [1,5,14, 16,18,21-29].

In this study, we investigated the ganglioside expression

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patterns in the liver and hearts of NIH-miniature pigs using high performance thin layer chromatography (HPTLC) and immunocytochemistry analysis to solve the acute rejection and cell mediated immune response.

Materials and Methods

Animals

The Korean Rural Development Administration approved the three miniature pigs (3 years old) were used in this study. The pigs were bred and maintained in compliance with the principals of laboratory animal care stated in the "Guide for the care and use of laboratory animals" produced by the National Institute of Health (NIH).

Tissue preparation and histological examination

The animal was sacrificed and the liver and heart were immediately removed and mixed with OCT compound (Sakura Finetek, Torrance, CA), after which they were frozen in liquid nitrogen and stored at -80°C until use. These samples were sectioned to a thickness of 7 μm, after which they were fastened to slides and stained with Hematoxylin and eosin. The stained cells were then observed under a universal research microscope (Model Axioskop 2 plus, Carl Zeiss Zena GMBH).

Preparation of Gangliosides from the Liver and Heart in NIH-miniature Pigs

Gangliosides were extracted using a previously established method [15]. The extracted total lipids were then applied to a DEAE sephadex A25 column (Sigma) that was subsequently washed with 20 ml of chloroform/MeOH/ H_2O (30:60:8, v/v/v). The acidic lipids were then eluted with 15 ml of chloroform/MeOH/0.8 M sodium acetate (30:60:8, v/v/v). The eluted samples were dried at 30°C under N_2 for 5 hr dissolved in chloroform/MeOH (1:1, v/v), and then alkalized in 12 N ammonia hydroxide solution overnight at room temperature. Next, the dried samples were dissolved in distilled water and applied to a Sep-Pak C18 cartridge (Millipore) to eliminate salts, after which they were eluted with 2 ml of MeOH and 4 ml of chloroform/MeOH (2:1, v/v). Finally, the eluted gangliosides were dried at 30°C under N_2 gas for 3 hr and then stored at -80°C until further analysis.

High performance Thin-layer chromatography (HPTLC)

HPTLC analysis of the gangliosides was conducted using a 10×10 cm HPTLC 5651 plate (Merck) as previously described [15]. Purified gangliosides were run into the HPTLC plates, which were subsequently developed with chloroform/MeOH/0.25% $CaCl_2$•H_2O (50:40:10, v/v/v). The gangliosides were then visualized with 0.2% resorcinol stain, after which the density of the ganglioside bands was quantified by HPTLC densitometry analysis (Image Quant TL v2005). Rat and bovine brain gangliosides were used as markers of the individual ganglioside species.

Immunocytochemistry

The cells were washed with PBS and then fixed with 4% paraformaldehyde at 4°C for 1 hr, after which they were and permeabilized in 0.25% Triton X-100 for 10min at 37°C. Next, the fixed cells were blocked for 20 min in 5% BSA/PBS and then incubated with a monoclonal mouse anti-ganglioside antibody (GD3, GD1a, GD1b, GT1b and GQ1b; Seikagaku Corporation, Tokyo, Japan) in 0.5% BSA/PBS overnight at 4°C. The cells were then washed with 1% BSA/PBS. A fluorescence-conjugated secondary antibody, goat anti-mouse IgM-FITC specific for gangliosides (Sigma), was then applied at dilutions of 1:500 and 1:800 and Hoechst33342 reagent (Sigma) was used to stain the nuclei. The stained cells were observed under a confocal scanning laser fluorescence microscope (Model FV300, Olympus).

Statistical analysis

All data are presented as the mean±SD. Comparisons of multiple groups were conducted by two-way analysis of variance (ANOVA) followed by pairwise comparisons with a Bonferroni post-hoc test. Differences were considered to be statistically significant at $p<0.001$. All data were analyzed using the GraphPad Prism software, version 5.00 (GraphPad software).

Results

Characterization of liver and heart tissue

Histological analysis of liver and heart tissue in NIH-miniature pigs was conducted using Hematoxylin and Eosin stain (Fig. 1). The results revealed the presence of hepatocytes in the liver tissue (Fig. 1A and B), as well as liver lobles (Fig. 1A, arrow). Furthermore, cardiac muscles were primarily observed in the heart tissue (Fig. 1C and D).

Ganglioside expression in the livers and hearts of NIH-miniature pigs

Ganglioside expression patterns in the livers and hearts of

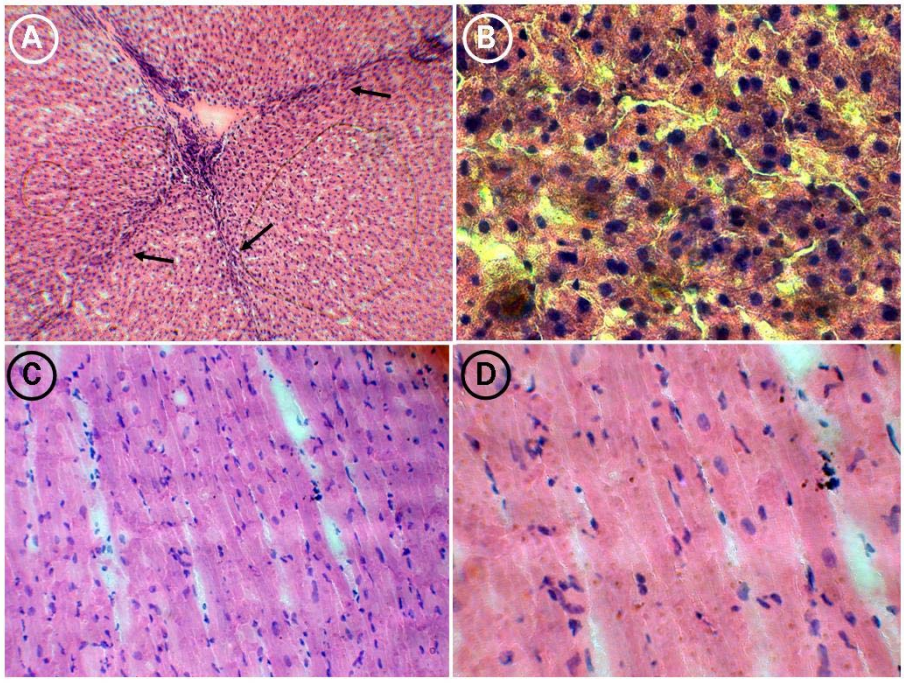

Fig. 1. Cryosection and Hematoxylin & Eosin staining of NIH-miniature pig livers and hearts. Serial sections of NIH-miniature pig livers (A and B) and hearts (C and D) showing the vertical section. (A and C) Photograph at ×200. (B and D) Photograph at ×400.

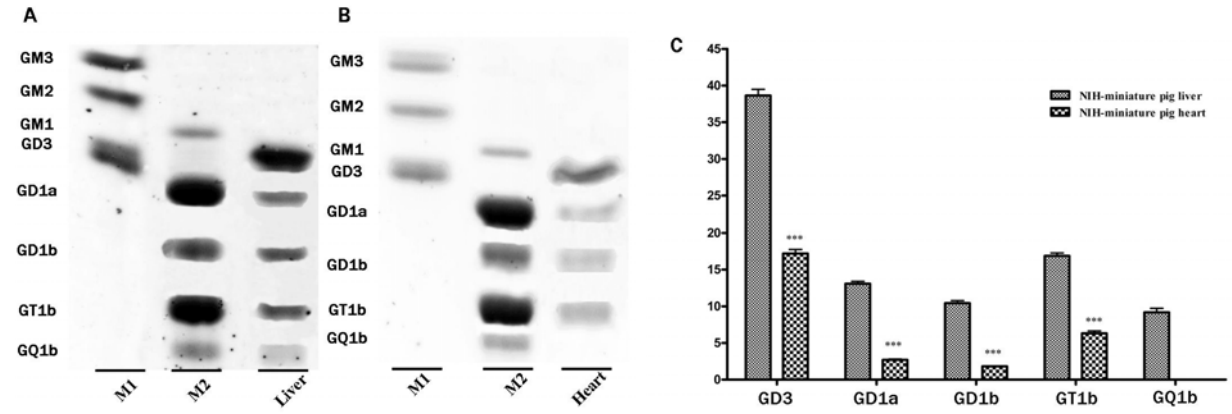

Fig. 2. High performance thin-layer chromatography (HPTLC) analysis of ganglioside expression in NIH-miniature pig livers and hearts. (A) Expression of gangliosides in NIH-miniature pig livers. M1; ganglioside standard marker from bovine brain, M2; ganglioside standard marker from rat brain, Liver; NIH miniature pig liver. (B) Expression of gangliosides in NIH-miniature pig hearts. M1; ganglioside standard marker from bovine brain, M2; ganglioside standard marker from rat brain, Heart; NIH miniature pig heart. (C) Quantitative analysis of ganglioside expression in NIH-miniature pig livers and hearts. The quantitative values generated using the densitometry program (Image Quant TL v2005) represent the mean values obtained from three separate experiments.

NIH-miniature pigs were analyzed by HPTLC (Fig. 2A and B). Gangliosides GD3, GD1a, GD1b, GT1b and GQ1b were the major components in the NIH-miniature pig livers (Fig. 2A), whereas gangliosides GD3, GD1a, GD1b and GT1b were primarily expressed in the NIH-miniature pig heart (Fig. 2B). Interestingly, there was a significant difference in the ganglioside expression level and concentration between the livers and hearts of NIH-miniature pigs (Fig. 2C and Table 1).

Immunocytochemistry of NIH-miniature pig livers and hearts

To confirm the results shown in Fig. 2 and to evaluate the distribution pattern of gangliosides GD3, GD1a, GD1b, GT1b and GQ1b in NIH-miniature pig livers and hearts, we performed immunofluorescence labeling followed by scanning confocal microscope analysis. To accomplish this, the tissues were labeled with anti-GD3, GD1a, GD1b, GT1b and

Table 1. Distribution of gangliosides in the liver and heart of NIH-miniature pigs

Tissue type	Degree of expression[a]				
	GD3	GD1a	GD1b	GT1b	GQ1b
Liver	+++	++	++	++	++
Heart	++	+	+	+	-

Gangliosides GD3, GD1a, GD1b, GT1b and GQ1b were detected by HPTLC.
[a] +++, strong; ++, moderate; +, weak; -, negative
Scores were from one experiment that was representative of five similar repeats.

GQ1b monoclonal antibodies. As shown in Fig. 3, NIH-miniature pig livers strongly expressed gangliosides GD3, GT1b and GQ1b, but weakly expressed gangliosides GD1a and GD1b (Fig. 3). Conversely, immunostaining revealed that NIH-miniature pig hearts weakly expressed gangliosides GD3, GD1a, GD1b and GT1b (Fig. 4). These results are consistent with those obtained by HPTLC (Fig. 2).

Discussion

In this study, we explored the expression of gangliosides while focusing on NIH-miniature pig livers and hearts. Our results indicate a difference in the pattern of ganglioside expression between the livers and hearts of NIH-miniature pigs. Several studies have shown that gangliosides are ex-

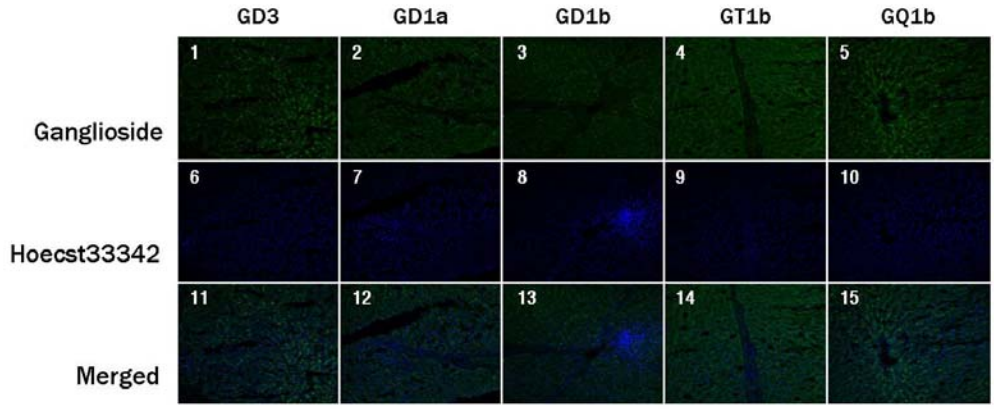

Fig. 3. Immunofluorescence staining of gangliosides expressed in NIH-miniature pig livers. The expression of gangliosides was analyzed using confocal microscopy. The expression of gangliosides GD3, GD1a, GD1b, GT1b and GQ1b (1, 2, 3, 4 and 5) was detected by FITC (green). Hoechst 33342 (6, 7, 8, 9 and 10) showed an equal cell number (blue) for all tests. Immunofluorescence images were obitained from three separate experiments.

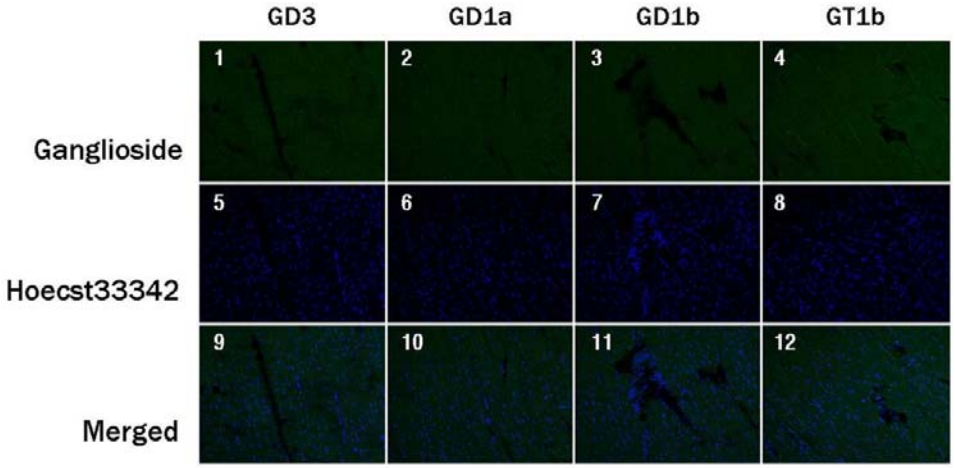

Fig. 4. Immunofluorescence staining of ganglioside expression in NIH-miniature pig hearts. The expression of gangliosides was analyzed by confocal microscopy. The expression of gangliosides GD3, GD1a, GD1b, GT1b and GQ1b (1, 2, 3 and 4) was detected by FITC (green). Hoechst 33342 (5, 6, 7, and 8) showed an equal cell number (blue) for all tests. Immunofluorescence images were obitained from three separate experiments.

pressed in the peripheral nerve, small intestine, pancreas, kidney, spleen, lung and liver in miniature-pigs [3,4,17]. Additionally, Fredman et al. [4] investigated the expression of gangliosides GM3, GD3 and GM2 in the livers of miniature pigs. However, our results showed that the major ganglioside GD3 and the minor gangliosides GD1a, GD1b, GT1b and GQ1b were expressed in the livers of NIH-miniature pigs. In addition, we showed here for the first time that the gangliosides GD3, GD1a, GD1b and GT1b are expressed in the pig hearts of NIH-miniature pigs.

Kolber-Simonds et al. [13] and Phelps et al. [19] produced α1,3galactosyltransferase (α1,3GalT), primary factor responsible for hyperacute rejection of pig organs transplanted into human, knock-out pigs for xenotransplantation of organs to humans. However, acute humoral xenograft rejection and cell mediated immune response still remained for xenotransplantation of α1,3GalT knock-out pig to human. Kim et al. [12] investigated several glycan-binding proteins that were involved in xenograft rejection. Additionally, Kim et al. [10,11] showed that 60 and 47 glycosphingolipids derived glycans and N-glycans were present in miniature pig endothelial cells and islets.

In summary, expression of various gangliosides in the liver and heart of NIH-miniature pigs can activate both the innate and adaptive immune response; therefore, the targeting of these gangliosides will be an attractive avenue in the development of techniques to control graft rejection. Therefore, these results suggest that gangliosides play a role in the immune response for successful clinical xenotransplantation. Further studies should be conducted to demonstrate the expression of gangliosides in α1,3GalT knock-out pig tissues or cells.

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References

1. Bergelson, L. D., E. V. Dyatlovitskaya, T. E. Klyuchareva, E. V. Kryukova, A. F. Lemenovskaya, V. A. Matveeva, and E. V. Sinitsyna. 1989. The role of glycosphingolipids in natural immunity. Gangliosides modulate the cytotoxicity of natural killer cells. *Eur. J. Immunol.* **19**, 1979-1983.
2. Bouhours, D., J. Liaigre, J. Naulet, D. Maume, and J. F. Bouhours. 1997. A novel glycosphingolipid expressed in pig kidney: Gal alpha 1-3Lewis(x) hexaglycosylceramide. *Glycoconj. J.* **14**, 29-38.
3. Diswall, M., J. Angström, H. J. Schuurman, F. J. Dor, L. Rydberg, and M. E. Breimer. 2007. Studies on glycolipid antigens in small intestine and pancreas from alpha1,3-galactosyltransferase knockout miniature swine. *Transplantation* **84**, 1348-1356.
4. Fredman, P., G. W. Klinghardt, O. Nilsson, and L. Svennerholm. 1982. Lipid accumulation in liver, spleen, lungs and kidneys of miniature-pigs after chloroquine treatment. *Biochem. J.* **201**, 581-588.
5. Grayson, G. and S. Ladisch. 1992. Immunosuppression by human gangliosides. II. Carbohydrate structure and inhibition of human NK activity. *Cell Immunol.* **139**, 18-29.
6. Hakomori, S. I. and Y. Igarashi. 1993. Gangliosides and glycosphingolipids as modulator of cell growth, adhesion, and transmembrane signling. *Adv. Lipid. Res.* **25**, 147-162.
7. Hallberg, E. C., J. Holgersson, and B. E. Samuelsson. 1998. Glycosphingolipid expression in pig aorta: identification of possible target antigens for human natural antibodies. *Glycobiology* **8**, 637-649.
8. Hendricks, S. P., P. He, C. L. Stults, and B. A. Macher. 1990. Regulation of the expression of Gal alpha 1-3Gal beta 1-4GlcNAc glycosphingolipids in kidney. *J. Biol. Chem.* **265**, 17621-17626.
9. Jalali-Araghi, K. and B. A. Macher. 1994. Characterization of porcine kidney neutral glycosphingolipids: identification of a carbohydrate antigen recognized by human natural antibodies. *Glycoconj. J.* **11**, 266-271.
10. Kim, Y. G., D. J. Harvey, Y. H. Yang, C. G. Park, and B. G. Kim. 2009. Mass spectrometric analysis of the glycosphingolipid-derived glycans from miniature pig endothelial cells and islets: identification of NeuGc epitope in pig islets. *J. Mass Spectrom.* **44**, 1489-1499.
11. Kim, Y. G., G. C. Gil, K. S. Jang, S. Lee, H. I. Kim, J. S. Kim, J. Chung, C. G. Park, D. J. Harvey, and B. G. Kim. 2009. Qualitative and quantitative comparison of N-glycans between pig endothelial and islet cells by high-performance liquid chromatography and mass spectrometry-based strategy. *J. Mass Spectrom.* **44**, 1087-1104.
12. Kim, Y. G., D. S. Shin, Y. H. Yang, G. C. Gil, C. G. Park, Y. Mimura, D. K. Cooper, P. M. Rudd, R. A. Dwek, Y. S. Lee, and B. G. Kim. 2008. High-throughput screening of glycan-binding proteins using miniature pig kidney N-glycan-immobilized beads. *Chem. Biol.* **15**, 215-223.
13. Kolber-Simonds, D., L. Lai, S. R. Watt, M. Denaro, S. Arn, M. L. Augenstein, J. Betthauser, D. B. Carter, J. L. Greenstein, Y. Hao, G. S. Im, Z. Liu, G. D. Mell, C. N. Murphy, K. W. Park, A. Rieke, D. J. Ryan, D. H. Sachs, E. J. Forsberg, R. S. Prather, and R. J. Hawley. 2004. Production of alpha-1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. *Proc. Natl. Acad. Sci. USA* **101**,

7335-7340.
14. Ladisch, S., B. Gillard, C. Wong, and L. Ulsh. 1983. Shedding and immunoregulatory activity of YAC-1 lymphoma cell gangliosides. *Cancer Res.* **43**, 3808-3813.
15. Lai, L., D. Kolber-Simonds, K. W. Park, H. T. Cheong, J. L. Greenstein, G. S. Im, M. Samuel, A. Bonk, A. Rieke, B. N. Day, C. N. Murphy, and D. B. Carter. 2002. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science* **295**, 1089-1092.
16. Lee, D. H., D. B. Koo, K. Ko, K. Ko, K., S. M. Kim, J. U. Jung, J. S. Ryu, J. W. Jin, H. J. Yang, S. I. Do, K. Y. Jung, and Y. K. Choo. 2007. Effects of daunorubicin on ganglioside expression and neuronal differentiation of mouse embryonic stem cells. *Biochem. Biophys. Res. Commun.* **362**, 313-318.
17. Lu, P. and F. J. Sharom. 1996. Immunosuppression by YAC-1 lymphoma: role of shed gangliosides. *Cell Immunol.* **173**, 22-32.
18. Magnusson, S., V. Strokan, L. Svensson, J. E., Månsson, L. Rydberg, and M. E. Breimer. 2005. Expression of carbohydrate xenoantigens on porcine peripheral nerve. *Xenotransplantation* **12**, 49-58.
19. Paller, A. S., S. L. Arnsmeier, G. J. Fisher, and Q. C. Yu. 1995. Ganglioside GT1b induces keratinocyte differentiation without activating protein kinase C. *Exp. Cell Res.* **217**, 118-124.
20. Park, K. W., H. T. Cheong, L. X. Lai, G. S. Im, B. Kuhholzer, A. Bonk, M. Samuel, A. Rieke, B. N. Day, C. N. Murphy, B. D. Carter, and R. S. Prather. 2001. Production of nuclear transfer-derived swine that express the enhanced green fluorescent protein. *Anim. Biotechnol*. **12,** 173-181.
21. Phelps, C. J., C. Koike, T. D. Vaught, J. Boone, K. D. Wells, S. H. Chen, S. Ball, S. M. Specht, I. A. Polejaeva, J. A. Monahan, P. M. Jobst, S. B. Sharma, A. E. Lamborn, A. S. Garst, M. Moore, A. J. Demetris, W. A. Rudert, R. Bottino, S. Bertera, M. Trucco, T. E. Starzl, Y. Dai, and D. L. Ayares. 2003. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science* **299**, 411-414.
22. Prather, R. S., R. J. Hawley, D. B. Carter, L. Lai, and J. L. Greenstein. 2003. Transgenic swine for biomedicine and agriculture. *Theriogenology* **59**, 115-123
23. Rogers, C. S., D. A. Stoltz, D. K. Meyerholz, L. S. Ostedgaard, T. Rokhlina, P. J. Taft, M. P. Rogan, A. A. Pezzulo, P. H. Karp, O. A. Itani, A. C. Kabel, and C. L. Wohlford-Lenane. 2008. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* **321**, 1837-1841.
24. Rogers, C. S., Y. Hao, T. Rokhlina, M. Samuel, D. A. Stoltz, Y. Li, E. Petroff, D. W. Vermeer, A. C. Kabel, Z. Yan, L. Spate, and D. Wax. 2008. Production of CFTR-null and CFTR-DeltaF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. *J. Clin. Invest.* **118,** 1571-1577.
25. Rydberg, L., J. Holgersson, B. E. Samuelsson, and M. E. Breimer. 1999. alpha-Gal epitopes in animal tissue glycoproteins and glycolipids. *Subcell Biochem.* **32**, 107-125.
26. Sachs, D. H., G. Leight, J. Cone, S. Schwarz, L. Stuart, and S. Rosenberg. 1976. Transplantation in miniature swine, I: fixation of the major histocompatibility complex. *Transplantation* **22,** 559-567.
27. Sun, P., X. Q. Wang, K. Lopatka, S. Bangash, and A. S. Paller. 2002. Ganglioside loss promotes survival primarily by activating integrin-linked kinase/Akt without phosphoinositide 3-OH kinase signaling. *J. Invest. Dermatol.* **119**, 107-117.
28. Sung, C. C., E. A. O'Toole, B. J. Lannutti, J. Hunt, M. O'Gorman, D. T. Woodley, and A. S. Paller. 1998. Integrin alpha 5 beta 1 expression is required for inhibition of keratinocyte migration by ganglioside GT1b. *Exp. Cell Res.* **239**, 311-319.
29. Varki, A., R. Cummings, J. Esko, H. Freeze, G. Hart, and J. Marth. 1999. Essentials of Glycobiology. Cold Spring Harber Laboratory Press, New York.
30. Wang, X., Z. Rahman, P. Sun, E. Meuillet, D. George, E. G. Bremer, A. Al-Qamari, and A. S. Paller, 2001. Ganglioside modulates ligand binding to the epidermal growth factor receptor. *J. Invest. Dermatol.* **116**, 69-76.
31. Wang, X., P. Sun, A. Al-Qamari, T. Tai, I. Kawashima, and A. S. Paller. 2001. Carbohydrate-carbohydrate binding of ganglioside to integrin alpha(5) modulates alpha(5)beta(1) function. *J. Biol. Chem.* **276**, 8436-8444.
32. Wang, X. Q., P. Sun, M. O'Gorman, T. Tai, and A. S. Paller. 2001. Epidermal growth factor receptor glycosylation is required for ganglioside GM3 binding and GM3-mediated suppression [correction of suppresion] of activation. *Glycobiology* **11**, 515-522.
33. Wang, X. Q., P. Sun, and A. S. Paller. 2001, Inhibition of integrin-linked kinase/protein kinase B/Akt signaling: mechanism for ganglioside-induced apoptosis. *J. Biol. Chem.* **276**, 44504-44511.
34. Wang, X. Q., P. Sun, and A. S. Paller. 2002. Ganglioside modulation regulates epithelial cell adhesion and spreading via ganglioside-specific effects on signaling. *J. Biol. Chem.* **277**, 40410-40419.
35. Wang, X. Q., P. Sun, and A. S. Paller. 2002. Ganglioside induces caveolin-1 redistribution and interaction with the epidermal growth factor receptor. *J. Biol. Chem.* **277**, 47028-47034.

초록 : NIH-미니돼지의 간과 심장에서 갱글리오시드의 서로 다른 발현 패턴

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갱글리오시드는 포유동물 세포막의 중요한 구성요소로서 세포와 세포 혹은 세포와 단백질간의 상호작용을 포함한 다양한 면역학적 역할을 수행하고 있다. 이 연구는 NIH-미니돼지의 간과 심장을 인간에게 이식할려고 할 때 예측되어지는 거부 반응과 관련된 구성성분들 중 시알산을 함유하고 있는 스핑고당지질인 갱글리오시드에 대해 조사하였다. 얇은막크로마토그래피와 면역조직화학적분석을 실시한 결과 NIH-미니돼지의 간은 갱글리오시드의 발현이 심장보다 높게 나타났다. 갱글리오시드 GD3, GD1a, GD1b, GT1b는 간과 심장의 두 기관에서 발견되었다. 그러나 GQ1b는 간에서만 발견되었고 심장에서는 검출되지 않았다. 이러한 결과는 갱글리오시드의 발현양상은 간과 심장에서 조직특이적이라는 것을 의미한다. 한편, GM3를 포함한 다른 갱글리오 시리즈인 갱글리오시드들은 NIH-미니돼지의 간과 심장에서 검출되어지지 않았다. 이와 같은 연구결과로부터 갱글리오시드는 미니돼지의 장기중 특히, 간과 심장의 이종장기이식과 관련된 면역거부반응에서 어떤 역할을 수행하고 있다고 여겨진다.