Acta Med. Nagasaki 46 : 21－25

Upregulation of CA19-9 in the Mouse Kidney Following Unilateral Ureteral Obstruction

Shengwen LI[1], Koichiro NOMATA[1], Tomayoshi HAYASHI[2], Daiyu AOKI[1], Shigeru KANDA[1], Hiroshi KANETAKE[1]

1) Department of Urology, Nagasaki University School of Medicine
2) Department of Pathology, Nagasaki University School of Medicine

High serum levels of carbohydrate antigen19-9 (CA19-9) have been detected in patients with pancreatic cancer and described in several recent case reports of patients with hydronephrosis. However, the mechanism of high serum levels of CA19-9 among hydronephrosis cases remains to be elucidated. In this study, we established a mouse unilateral ureteral obstruction (UUO) model to investigate the expression of CA19-9 protein in renal tissue. To investigate the progression of hydronephrosis following UUO, MR urography and pathological analysis were performed. CA19-9 expression was examined by immunohistochemistry and western blot analysis. MR urography revealed that the grade of pelvic dilatation increased in a time dependent manner. Pathologically, both interstitial cellular infiltration and fibrosis were detected from the second to the fourteenth day after surgery in UUO mice. CA19-9 was detected in the UUO kidney after the second day. The immunoblot analysis revealed that the elevated expression of CA19-9 was demonstrated at an early stage of obstructive nephropathy. Our study shows that the ureteral obstructed kidney is dominated by cell infiltration and induced fibrosis. The selective expression of CA19-9 was detected in renal fibrous tissue. Based on these findings, the level of CA19-9 might be a good indicator for onset of renal fibrosis induced by obstruction.

ACTA MEDICA NAGASAKIENSIA 46 : 21－25, 2001

Key Words: CA19-9, hydronephrosis

Introduction

A series of specific interactions between adhesion molecules expressed on circulating leucocytes and their ligands on endothelial and other cell types are involved in the development of interstitial renal fibrosis.[1] One of the selectin family, L-selectin, is thought to play an important role in the pathogenesis of this disorder.[2]

A carbohydrate antigen (CA19-9) is a high molecular weight glycoprotein, which is a ligand for L-selectin,[3] and is detected by a monoclonal antibody that is produced by immunizing mice with human colon cancer cells.[4] The antigenic determinant is a sialylated derivative of the Lewis^a (Le^a) blood group antigen.[5] CA19-9 is known to be elevated in the serum of patients with intraabdominal malignancies, particularly in those who suffer from pancreatic cancer.[6] In contrast, patients diagnosed as having non-malignant disease, such as pulmonary fibrosis, and hemochromatosis which is pathologically noted as hepatic fibrosis, were found to have associated marked elevations of serum CA19-9.[7, 8] These data support the idea that fibrotic disease may be associated with high serum CA19-9 level. The CA19-9 serum level is also elevated in hydronephrosis.[9-12] Unilateral ureteral obstruction (UUO) is a well established experimental model of renal hydronephrosis leading to interstitial fibrosis.[13] Previous studies have investigated the molecular and cellular mechanisms of interstitial fibrosis in UUO,[14] and the accumulation of leucocytes within the interstitium of the kidney is well recognized in the course of hydronephrosis.[15]

In the present study, we examined the relevance of CA19-9 expression in the progression of interstitial fibrosis caused by unilateral ureteral obstruction in mice and found that CA19-9 expression was elevated in fibrotic renal tissue.

Materials and methods

Unilateral obstruction of ureter

Five-weeks-old male ddy mice in each experiment were used and underwent unilateral obstruction of the ureter. Briefly, mice were anesthesized by an intraperitoneal injection of pentobarbital (5.0 μ g/kg body wt) and right

Address Correspondence: Koichiro Nomata, M.D.
Department of Urology, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
TEL: +81-95-849-7340, FAX: +81-95-849-7343
E-mail: knomata@net. nagasaki-u.ac.jp

ureter was identified through a small dorsal lumbar incision and was ligated by using 5-0 silk at the point of upper the ureter. The sham operation to provide control kidneys involved a similar incision. After indicated days, obstructed kidneys were morphologically examined by MR urography.

MRI examination

Experiments were performed on the 1st day (n=5), 2nd day (n=5), 3rd day (n=5), 7th day (n=5) and 14th day (n=5) after UUO surgery. The sham operated groups (n=5) acted as controls. Magnetic resonance imaging (MRI) analysis was performed by a 9.41 unit DMX400WB (Bruker, Rheinstetten, Germany) to study the morphological change in UUO.[16] The repetition time (TR) and echo time (TE) were determined for T1-and T2-weighted images. Multiple coronal T2-weighted images were obtained with a 5cm field of view, 512 x 512 matrix, 6 acquisitions, and 1 mm slice thickness.

Tissue preparation and pathological experiment

Kidney specimens were fixed in 10% buffered formalin and embedded in paraffin. The 4 μm thick sections were stained with hematoxylin-eosin and for pathological examination. For protein assay and western blotting, the kidney specimens were removed and snap-frozen in liquid nitrogen until use.

Immunohistochemical analysis of CA19-9

Immunohistochemical analysis of CA19-9 expression was performed using the Histo mouse plus kit® (Zymed Laboratories Inc, San Francisco, CA) and a mouse monoclonal antibody to human CA19-9 (DAKO, Kyoto, Japan). Briefly, paraffin-embedded specimens were sectioned and placed on silan coated glass slides (Matsunami, Osaka, Japan). Deparaffinized sections were then placed in 0.1M citrate buffer (PH6.0) and treated with 0.3% H_2O_2 in methanol for 20 min to block endogeneous peroxidase activity. After washing with phosphate-buffered saline (PBS), sections were incubated with the blocking solution (Zymed). The primary antibody, mouse monoclonal antibody CA19-9, was applied to the sections at a dilution of 1:50. These sections were then incubated for one hour at room temperature. After washing in PBS, the sections were incubated with the second antibody of the Histo mouse plus kit (Zymed) for 30 min at room temperature. After appropriate washings, the sections were incubated in streptoavidin-peroxidase (Zymed) for 15 min. The act of peroxidase were visualized by the addition of Regent3 (Zymed).[17] Finally, the sections were counterstained with methyl green.

Western blot of CA19-9

A whole kidney was homogenized by a Polytron homogenizer (Kinematica, Littau/luzern, Switzerland), then lysed in 10% SDS, protease inhibitor cocktail tablets (Roche Diagnostics Gmbh, Mannheim, Germany) in PBS buffer and sonicated for 30 sec on ice. Human colon cancer cells (colo205) acting as a positive control[18] were cultured in RPMI1640 supplemented with 10% FBS, and lysed with bromomix buffer (Wako, Osaka Japan). Then the protein concentration was determined in the supernatant by using the Bio Rad detergent compatible protein assay kit® (Bio Rad Laboratories, Tokyo, Japan). Next, 80μg of protein in each lysate was separated by 5% SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Millipore Japan, Tokyo, Japan) affixed with the blocking solutions of the Histo mouse plus kit® (Zymed). The membranes were then incubated with anti CA19-9 monoclonal antibodies (DAKO Japan) diluted 50-fold with blocking solution (Zymed) at room temperature overnight. After washing with PBS containing Tween20, the membranes were incubated with the second antibody of the Histo mouse plus kit® (Zymed) for two hours at room temperature, and streptoavidin-peroxidase (Zymed) for one hour at room temperature. After extensive washing, the proteins were visualized using the ECL-chemiluminescence detection kit (Amersham Pharmacia Biotech Inc, Piscataway, NJ).[19]

Results

MRI study of UUO mouse kidney

Unilateral ureteral obstruction was performed and morphological change was monitored by MR urography (Fig. 1). It was found that the renal pelvis was dilated time-dependently, and the intensity of renal parenchyma was increased and volume of renal parenchyma was decreased as well.

Histopathological change in the obstructed kidney

Sections obtained from the kidneys of UUO mice were histological examined. Fig 2 B shows that sections at day 1 revealed a slight dilatation of distal convoluted tubules (DCTs), a minimal change in proximal convoluted tubules (PCTs), and a slight cellular infiltration of the interstitium when compared with sections

of sham operated kidney (Fig. 2A). Renal tissue at day 2 revealed dilatation of PCT and DCT, and mild inflammatory cell infiltration was observed in the interstitium (Fig. 2C). A day 3 dilatation of PCTs and inflammatory cells were more evident than that of the day 2 specimens (Fig. 2D). Thin and atrophic parenchyma with partial coagulation necrosis and dilatation of the pelvis at 7 post operative days. On the 14th day increased necrosis of the pyramidal area, widened interstitial fibrosis and atrophied parenchyma were observed (Fig. 2F).

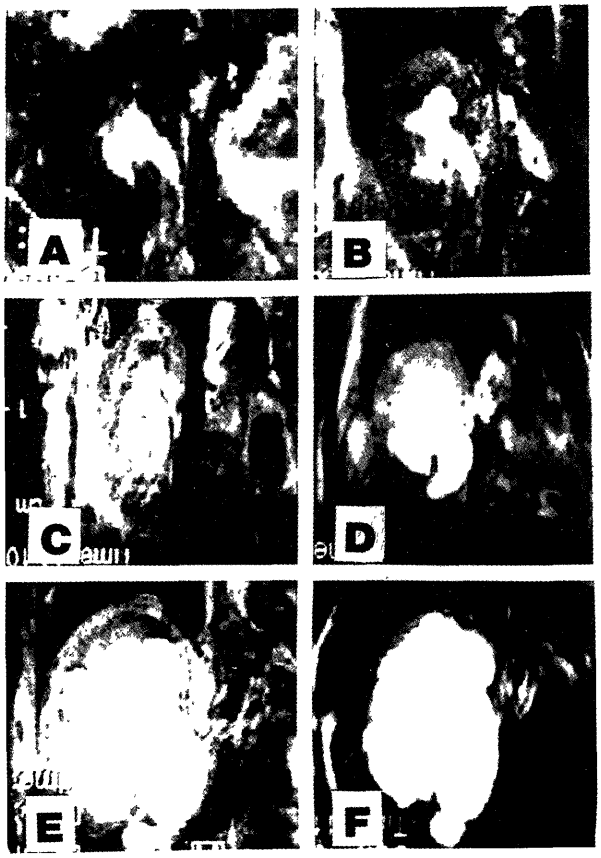

Figure 1. MR urography T2-weighted representative unilateral ureteral obstruction (UUO) in the mouse kidney. (A) Sham operated mouse kidney. (B, C, D) Renal pelvis was extended on the 1st day, 2nd day and 3rd day after UUO respectively. (E, F) Renal parenchyma was thin on the 7th day after UUO and was thinner on the 14th day.

Immunohistological study of CA19-9

It has been reported that serum CA19-9 is elevated in the patients with hydronephrosis. These reports suggest that CA19-9 may be produced in obstructive kidney. To clarify the localization of CA19-9 protein expression in renal tissue, immunohistochemical staining

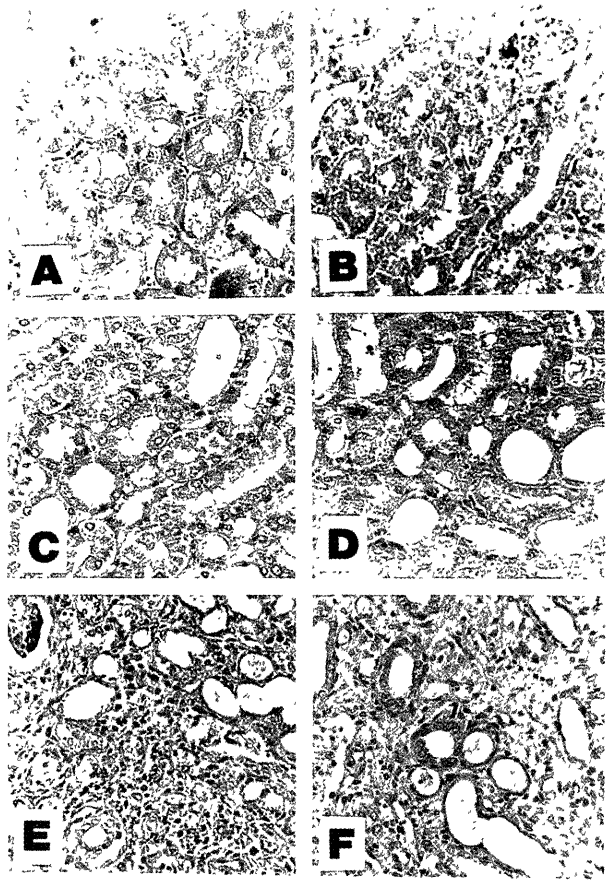

Figure 2. Histopathological change in HE staining observed in the unilateral ureteral obstruction (UUO) of the mouse kidney. (A) Representative sections of a kidney from a sham operated mouse. (B) On the 1st day after UUO, there was slight dilatation of the DCT but minimal change in the PCT, and slight cellular infiltration of the interstitium. (C) Dilatation of the PCT and DCT, and mild inflammatory cell infiltration in the interstitial tissue was observed on the second day after UUO. (D) Dilatation of the PCT became more evident on the 3rd day after UUO. (E) Atrophy of the parenchyma, and interstitial fibrosis are evident on the 7th day after UUO. (F) Widening of interstitial fibrosis and atrophy of tubular are revealed on the 14th day after UUO. (×100)

of CA19-9 was performed. Figure 3 shows that almost no staining was observed in sham operated (Fig. 3A) or UUO renal tissue at day 1 (Fig. 3B). At day 2 the representative appearance of the staining of CA19-9 in the UUO kidneys (Fig. 3C). CA19-9 immunoreactivity was observed in the periglomerular and peritubular interstitium of the obstructed kidney. In the sections of renal tissue obtained at day 7 (Fig. 3E) and day 14 (Fig. 3F), CA19-9 immunoreactivity was observed in the majority of expanded peritubular spaces. Without anti CA19-9 Antibody, no staining was observed (data not shown).

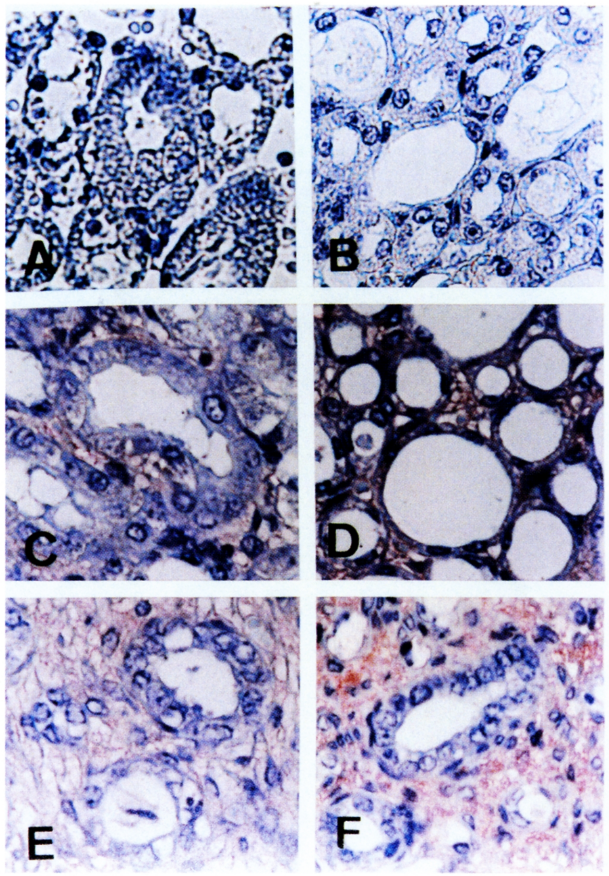

Figure 3. Immunostaining of CA19-9. (A, B) No staining was detected in the sham operated mouse kidney or on the 1st day after surgery in the UUO mouse kidney. (C, D) Immunolocalization of the peritubular interstitial areas on the 2nd and 3rd day after UUO. (E) Immunolocalization of the peritubular interstitial fibrotic area on the 7th day after UUO. (F) Tubular interstitial fibrotic area was stained on the 14th day after UUO. (×200)

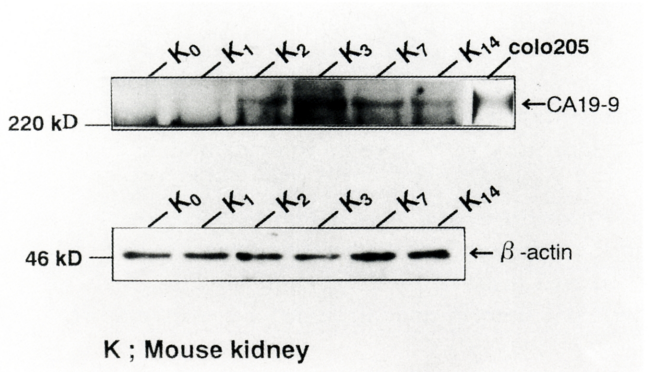

Figure 4. Western blotting of CA19-9 in the unilateral ureteral obstructed mouse kidney and the colo205 cells acting as a positive control. Expression of CA19-9 at approximately 250Kd immunoreactive band was detected on the 2nd, 3rd, 7th, and 14th day after UUO in the mouse kidney and colo205 cells. β-actin was expressed in all specimens.

Western blot analysis of CA19-9

To quantify the expression levels of CA19-9 in the renal tissue, immunoblot analysis was performed with total protein extracts of renal tissues. Figure 4 shows that CA19-9 protein was observed in the extract of obstructed kidney from 2 to 14 days after UUO. The extract from colo205 cells was used as a positive control and (-actin bands served as an indicator of loaded protein amounts. No band was detected in the extract of sham operated kidney.

Discussion

In the present study, the authors demonstrated that the protein expression of CA19-9 in the obstructed kidneys of UUO mice was localized in the peritubular interstitial space. Dilatation of PCT and DCT became significant 2 days after ureteral ligation and thereafter, renal interstitial fibrosis progressed. The expression of CA19-9 was observed in renal interstitial tissue. These results are consistent with the previous reports of pulmonary and hepatic fibrosis.

L-selectin is a member of selectin family and is involved in site-specific lymphocyte adhesion.[20] Selectins bind to sialyl Le^x (sLe^x), sialyl Le^a (sLe^a) and related car bohydrates. During kidney allograft rejection, peritubular capillary endothelium reacts strongly with the anti-sLex antibody. Turunen et al reported that the ligand for L-selectin was induced during kidney rejection characterized by lymphocyte infiltration.[21] This pathological feature was similar to that of the acute obstructed kidney.

Moriyama et al reported that fibrotic changes in the interstitium became significant 4 days after obstruction was induced in the kidneys, and a significant increase in type I collagen mRNA also appeared 4 days after the onset of ureteral obstruction.[22] Hence, CA19-9 up-regulated expression was induced two days after obstruction. These data suggest that the onset of CA19-9 expression might be related to early events of interstitial renal fibrosis.

The site of CA19-9 production in fibrous renal tissue is not clarified yet. There are possibllities that either infiltrated monocytes or peritubular endothelial cells, or both might produce CA19-9. Theoretically, two major mechanisms have been suggested for the induction of endothelial cells to become sLe^x and sLe^a positive. Firstly, they could synthesize sialyl Lewis epitopes directly, which would require the presence of functional α 1,3 fucosyltransferase (α 1,3 Fuc-Ts) or α 1,4 fucoslt-

ransferase (α 1,4 Fuc-Ts) and α 2,3 sialyltransferase activities in endothelial cells. Secondly, these epitopes could be absorbed passively from the blood. The genes encoding α 1,3 Fuc-Ts form a family and 5 human and 3 mouse α 1,3 Fuc-Ts genes have been cloned.[23] The mouse α 1,3 Fuc-Ts can synthesize the Le^x epitope and mouse α 1,3 Fuc-Ts transcripts were abundant in the mouse kidney.[24] However, whether mouse α 1,3 Fuc-Ts has the ability of α 1,4 fucosylation remains to be confirmed. In the present study, the expression of CA19-9 protein was elevated in hydronephrotic mouse kidney. The Fuc-Ts activity might be increased in the kidney after hydronephrosis. Alternatively, infiltrated monocytes may produce CA19-9 in a similar way that has been described above.

To date, the CA19-9 producing cells in obstructed renal tissue has not yet been identified. It may be possible that CA19-9 levels as an indicator of renal fibrosis induced by upper urinary tract obstruction. However, the elevated expression of CA19-9 in transitional cell carcinoma has recently been reported. Thus, careful examination must be taken whether urothelial carcinoma is associated or not.

In conclusion, the present study showed that the ureteral obstructed kidney is dominated by cell infiltration and induced fibrosis. The selective expression of CA19-9 was detected in renal fibrotic area and the expression of CA19-9 protein was higher in the early stage of fibrosis. Given these results, CA19-9 might be an effective marker for the onset of renal fibrosis induced by urinary obstruction.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education and Culture of Japan (No. 1167560). We thank Mr. Takumi Shimogama and Mrs. Miki Yoshimoto for outstanding technical assistance.

References

1. Roy-Chaudhury P, Wu B, King G et al. Adhesion molecule interactions in human glomerulonephritis: importance of the tubulointerstitium. *Kidney Int.* **49**: 127-34, 1996
2. Roy-Chaudhury P, Hillis G, McDonalds S, Simpson JG, Power DA. Importance of the tubulointerstitium in Human glomerulonephritis. Distribution of integrin chains beta 1, alpha 1 to 6 and alpha V. *Kidney Int.* **52**: 103-10, 1997
3. Berg EL, Magnani J, Warnock RA, Robinson MK, Butcher EC. Comparison of L-selectin ligand specificities: The L-selectin can bind the E-selectin ligands sialyl Le^x and sialyl Le^A. *Biochem Biophys Res Comm.* **184**: 1048-55, 1992
4. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridomaantibodies *Somatic Cell Genet.* 5957-71, 1979
5. Magnani JL, Nilsson B, Brockhaus M et al. A monoclonal antibody-defined antigen associeted with gastrointestinal cancer is a ganglioside containing silylated lacto-N-fucopentaose. *J Biol Chem.* **257**: 14365-69, 1982
6. Magnani JL, Steplewski Z, Koplowski H, Ginsburg V. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19-9 in the sera of patient as a mucin. *Cancer Res.* **43**: 5489-92, 1983
7. Fujita J, Obayasi Y, Yamadori I et al. Marked elevation of CA19-9 in a patient with idiopathic pulmonary fibrosisCA19-9 as a bad prognostic factor. *Respirology.* **3**: 211-14, 1998
8. Deugnier YM, Rabot AF, Guyader D et al. Serum increase and liver overexpression of carbohydrate 19-9 antigen in-patients with genetic haemochromatosis. **Gut. 35:** 1107-11, 1994
9. Takase K, Inayama Y, Misaki H, Kitamura H, Nakatani Y, Hosaka M. Hydronephrosis and hydroureter with extremely high levels of serum carbohydrate antigens 19-9 and Span-1a case report. *J Urol.* **155**: 1386, 1996
10. Inayama Y, Uesato M, Takase K, Motono N, Nakatani Y, Kitamura H. Immunohistochemical study of CA19-9 and Span-1 in cases of hydronephrosis. *Urol Int.* **57**: 93-8, 1996
11. Shudo R, Saito T, Takahasi K et al. Giant hydronephrosis due to a ureteral stone, and elevated serum levels of CA19-9. *Intern Med.* **38**: 887-91, 1999
12. Aoki D, Nomata K, Kanda S et al. A case of pyonephrosis caused by ureteral stones with elevated serum levels of CA19-9. *Acta Urol Jpn.* **45:** 629-32, 1999
13. Klahr S: New insights into the consequences and mechanisms of renal impairment in obstructive nephropathy. *Am J Kidney Dis.* **18** : 689-99, 1991
14. Kuncio GS, Neilson EG, Haverty T et al. Mechanisms of tubulointerstitial fibrosis. *Kidney Int.* **39**: 550-6, 1991
15. Schreiner GF, Harris KPG, Purkerson ML, Klahr S. Immnological aspects of acute ureteral obstruction: immune cell infiltrate in the kidney. *Kidney Int.* **34**: 487-93, 1988
16. Wen JG, Ringgaard S, Frokiaer J et al. Contralateral compensatory kidney growth in rats with partial unilateral ureteral obstruction monitored by magnetic resonance imaging. *J Urol.* **162**: 1084-9, 1999
17. Gingrich JR, Barros RJ, Morton RA et al. Metastatic prostate cancer in a transgenic mouse. *Cancer Res.* **56**: 4096-102, 1996
18. Isshiki S, Togayachi A, Kudo T et al. Cloning, expression, and characterization of a novel UDP-galactose: beta-N-acetylglucosamine betal, 3-galactosyltransferase (beta3Gal-T5) responsible for synthesis of type 1 chain in colorectal and pancreatic epithelia and tumor cells derived thereform. *J Biol Chem.* **274**: 12499-507, 1999
19. Watanabe J, Nomata K, Noguchi H et al. All-trans retinoic acid enhances gap junctional intercellular communication among renal epithelial cells in vitro treated with renal carcinogens. *Eur J Cancer.* **35:** 1003-8, 1999
20. Bevilacqua M, Butcher E, Furie B et al. Selectines: A family of adhesion receptors. *Cell.* **67**: 18, 1991
21. Turunen JP, Paavonen T, Majuri ML et al. Sialyl lewisx and L-selectin dependent site specific lymphocyte extravasation into renal transplants during acute rejection. *Eur J Immunol.* **24**: 1130-6, 1994
22. Moriyama T, Kawada N, Ando A et al. Up-regulation of HSP47 in the mouse kidneys with unilateral ureteral obstruction. *Kidney Int.* **54**: 110-9, 1998
23. Hansson GC, Zopf D. Biosynthesis of the cancer-associated sialyl-L e^a antigen. *J Biol Chem.* **260**: 9388-92, 1985
24. Kudo T, Ikehara Y, Togayachi A et al. Expression cloning and characterization of a novel murine alpha1, 3-Fucosyltransferase, mFuc-TIX, that synthesizes the Lewis x (CD15) epitope in brain and kidney. *J Biol Chem.* **273**: 26729-38, 1998