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

Shengwen Li, Koichiro Nomata, Tomayoshi Hayashi, Daiyu Aoki, Shigeru Kanda, Hiroshi Kaneta

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.

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. One of the selectin family, L-selectin, is thought to play an important role in the pathogenesis of this disorder.

A carbohydrate antigen (CA19-9) is a high molecular weight glycoprotein, which is a ligand for L-selectin, and is detected by a monoclonal antibody that is produced by immunizing mice with human colon cancer cells. The antigenic determinant is a sialylated derivative of the Lewis (Le) blood group antigen. CA19-9 is known to be elevated in the serum of patients with intraabdominal malignancies, particularly in those who suffer from pancreatic cancer. 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.

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.

Unilateral ureteral obstruction (UUO) is a well-established experimental model of renal hydronephrosis leading to interstitial fibrosis. Previous studies have investigated the molecular and cellular mechanisms of interstitial fibrosis in UUO, and the accumulation of leucocytes within the interstitium of the kidney is well recognized in the course of hydronephrosis.

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 anesthetized by an intraperitoneal injection of pentobarbital (5.0 mg/kg body wt) and right
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. 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% H2O2 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 two hours at room temperature, and streptavidin-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).

**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.

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)

Immunohistological study of CA19-9

It has been reported that serum CA19-9 is elevated in the patients with hydrenephrosis. 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 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 CA 19-9. (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. (X200)

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 the selectin family and is involved in site-specific lymphocyte adhesion. Selectins bind to sialyl Le (α2-3 sLeα), sialyl Le (α2-3 Leβ) and related carbohydrates. 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. 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. 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 possibilities 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 sLeα and sLeβ positive. Firstly, they could synthesize sialyl Lewis epitopes directly, which would require the presence of functional α1,3 fucosyltransferase (α1,3 Fuc-Ts) or α1,4 fucosid-
transferase (α1,4 Fuc-Ts) and α2,3 sialyltransferase activities in endothelial cells. Secondly, these epitopes could be absorbed passively from the blood. The genes encoding α1,3 Fuc-Ts form a family and 5 human and 3 mouse α1,3 Fuc-Ts genes have been cloned. The mouse α1,3 Fuc-Ts can synthesize the Leα epitope and mouse α1,3 Fuc-Ts transcripts were abundant in the mouse kidney. However, whether mouse α1,3 Fuc-Ts has the ability of α1,4 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.

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