Cardiovascular, Pulmonary and Renal Pathology

Liver-Type Fatty Acid-Binding Protein Attenuates Renal Injury Induced by Unilateral Ureteral Obstruction

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Liver-type fatty-acid-binding protein (L-FABP), which has high affinity for long-chain fatty acid oxidation products, may be an effective endogenous antioxidant. To examine the role of L-FABP in tubulointerstitial damage, we used a unilateral ureteral obstruction (UUO) model. We established human *L-FABP* (*hL-FABP*) gene transgenic (Tg) mice and compared the tubulointerstitial pathology of the Tg mice ($n = 23$) with that of the wild-type (WT) mice ($n = 23$). Mice were sacrificed on days 2, 4, 5, or 7 after UUO. Although mouse L-FABP was not expressed in WT mice, hL-FABP was expressed in the proximal tubules of the Tg mice with UUO (UUO-Tg) and in sham-operated Tg mice. The expression of renal hL-FABP was significantly increased in UUO-Tg compared with sham-operated Tg mice. The number of macrophages (F4/80) infiltrating the interstitium and the level of expression of MCP-1 and MCP-3 were significantly lower in UUO-Tg kidneys compared with UUO-WT kidneys. In UUO-Tg kidneys, the degree of the tubulointerstitial injury and the deposition of type I collagen were significantly lower than that of UUO-WT kidneys. On day 7, lipid peroxidation product accumulated in the UUO-WT kidneys but not in that of UUO-Tg kidneys. In conclusion, renal L-FABP may reduce the oxidative stress in the UUO model, ameliorating tubulointerstitial damage. (*Am J Pathol* 2006, 169:1107-1117; DOI: 10.2353/ajpath.2006.060131)

Deterioration of renal function is primarily determined by the extent of tubulointerstitial alterations in many forms of renal disease, regardless of the underlying pathogenesis.¹⁻³ Therefore, inhibition of tubulointerstitial damage leads to a reduction in progressive loss of renal function. Liver-type fatty acid-binding protein (L-FABP) is a 14-kD protein found in the cytoplasm of human proximal tubules.⁴ L-FABP binds fatty acids and transports them to mitochondria or peroxisomes, where fatty acids are β -oxidized, possibly playing a role in fatty acid homeostasis.⁵⁻⁷ Moreover, L-FABP has high affinity and capacity to bind long-chain fatty acid oxidation products and may be an effective endogenous antioxidant.⁸⁻¹⁰

The pathophysiological role of renal L-FABP in kidney disease has not yet been fully clarified. Because renal L-FABP is not expressed in the kidneys of rodents, we generated human *L-FABP* (*hL-FABP*) chromosomal transgenic (Tg) mice and determined the pathological significance of hL-FABP in an experimental model of fatty acid overload, in which oxidative stress might contribute to the progression of tubulointerstitial injury.¹¹ Under pathological condition, the expression of renal hL-FABP was up-regulated, and the tubulointerstitial inflammation of Tg mice was attenuated compared with that of wild-type (WT) mice.¹¹ However, this model provoked only mild to moderate tubulointerstitial damage. It is important to confirm the pathophysiological significance of hL-FABP in an experimental model leading to severe tubulointerstitial damage.

Unilateral ureteral obstruction (UUO) is a well-established model of severe renal interstitial injury.¹² A number of reports have indicated that the interstitium in the setting of UUO is under the continuous oxidant stress produced from tension, stress, or hypoxia induced by a marked decline in renal plasma flow.¹³⁻¹⁶ In this study,

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we used the UUO model to investigate the renoprotective function of renal hL-FABP.

Materials and Methods

Animals

Tg mice bearing the *hL-FABP* gene were generated (patent no. WO0073791).¹¹ In brief, the genomic DNA encoding the *hL-FABP* gene including its promoter region (13 kb) was microinjected into fertilized eggs obtained from BALB/c mice mated with CBA mice. ICR mice were used as the recipients for the transfected eggs. The resulting Tg mice were backcrossed for more than six generations onto C57/BL6 to obtain homozygous mutant mice on an inbred background. Only male mice were used. The integration of hL-FABP gene into the mouse genome was confirmed by polymerase chain reaction (PCR) of genomic DNA, and the expression of hL-FABP in the proximal tubules of the Tg mice was confirmed by Northern blot analysis, Western blot analysis, and immunohistochemistry.¹¹ The Tg mice did not show any obvious abnormalities in appearance or behavior. The distribution of hL-FABP expression was confirmed in the kidney, liver, and the intestine of the Tg mice using an enzyme-linked immunosorbent assay (ELISA) described below.¹¹ Mice were housed in the animal facilities of St. Marianna University School of Medicine with free access to food and water.

Twelve- to 16-week-old male Tg mice ($n = 23$; body weight, 31.6 ± 0.4 g; mean $\pm$ SE) and WT littermates on a C57/BL6 background ($n = 23$; body weight, 31.7 ± 0.5 g) were used for this study. The presence of the transgene was ascertained by visualizing the mice under UV light: the transgene was fused with the green fluorescent protein gene, and therefore, mice expressing the transgene were readily identified by green fluorescence signal. Both the Tg mice and the WT mice underwent either left UUO or sham surgery. UUO was performed using an established procedure.^{17,18} In brief, under intraperitoneal anesthesia the left ureter was ligated with 5-0 silk suture at two locations and cut between the ligatures to prevent retrograde urinary tract infection. Sham-operated mice, both WT ($n = 4$) and Tg mice ($n = 4$), had their ureters exposed and manipulated but not ligated. The incision was closed in two layers with 4-0 silk sutures and staples. Mice that were operated on were sacrificed under pentobarbital anesthesia 2 ($n = 5$), 4 ($n = 5$), 5 ($n = 4$), and 7 ($n = 5$) days after UUO. Sham-operated animals were killed to obtain control kidneys. After UUO or sham surgery, the left obstructed kidney and the right contralateral nonobstructed kidney of each mouse were harvested for various histological and biochemical analyses, as described below. The experimental protocol was approved by the ethics committees for animal experimentation of the participating institutions.

Serum Biochemistry

Total cholesterol was measured by an enzymatic method and serum lipid peroxidation was measured by hemoglobin-methylene blue at BCL Co. Research Service (Tokyo, Japan).^{19,20}

Renal Histological and Morphometric Analysis

For light microscopy analysis, the kidneys were sliced axially into 3-mm-thick sections, fixed in methyl Carnoy's solution, and embedded in paraffin. Paraffin sections (4 μ m thick) were stained with Azan-Mallory stain. The tubulointerstitial injury was categorized as proximal tubule dilation with epithelial atrophy and extracellular matrix accumulation that stained blue because of collagen deposition, excluding blood vessels. Under $\times 200$ magnification, five consecutive fields were randomly selected in the renal cortex,^{17,18} and the area with tubulointerstitial damage and the whole cortical area were measured by using an image analyzer (Leica Image Analyzer, Wetzlar, Germany). The degree of interstitial injury was defined as the ratio of the area of interstitial damage to the entire cortical area.

Immunohistological Analysis

Tissues were fixed in methyl Carnoy's solution and embedded in paraffin. An indirect immunoperoxidase method was used to identify the antigen. Macrophages were identified with rat monoclonal antibody F4/80 (Medical and Biological Laboratories Co., Ltd., Nagoya, Japan), and type I collagen was identified with a goat polyclonal antibody (Southern Biotechnology Associates, Birmingham, AL). The degree of macrophage infiltration in the cortical interstitium was measured as the average number of F4/80-positive cells per field at $\times 200$ magnification by using the image analyzer (Leica Image Analyzer).^{11,21} The positive area of type I collagen was evaluated as the ratio of the positive area of type I collagen to the entire cortical area under $\times 200$ magnification by using the image analyzer (Leica Image Analyzer). To perform the immunohistochemistry with monoclonal antibody against human L-FABP, tissues were fixed in 10% buffered formalin and embedded in paraffin. hL-FABP immunostaining in the kidneys of the mice was performed with mouse monoclonal antibody against human L-FABP, FABP-2, which was generated previously and reacted with the endogenous mouse L-FABP expressed in the liver.¹¹

TaqMan Real-Time PCR Assay

Total RNA of the kidney was extracted using an RNeasy mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Total RNA (0.5 μ g) was reverse-transcribed using ExScript RT reagent kit (Takara Shuzo, Kyoto, Japan). The TaqMan real-time PCR reactions were performed on a TaqMan ABI 7000 sequence detection system (Applied Biosystems, Foster City, CA)

Figure 1. UUO-induced expression of hL-FABP in Tg mice. **a:** The expression of hL-FABP transcripts was determined by TaqMan real-time PCR and was normalized to GAPDH. **b:** The expression of hL-FABP protein was determined by ELISA and was corrected for the total amount of protein. Values are mean $\pm$ SE. * $P < 0.05$ compared with the kidney of the sham-operated Tg mice; $\nabla P < 0.05$ compared with each contralateral kidney of UUO-Tg mice.

using TaqMan Universal PCR Master Mix (Applied Biosystems). hL-FABP, monocyte chemoattractant protein (MCP)-1, MCP-3, heme oxygenase-1 (HO-1), transforming growth factor- β (TGF- β), glutathione peroxidase-1 (Gpx1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were detected using TaqMan real-time PCR. Unlabeled specific primers and the TaqMan MGB probes (6-FAM dye-labeled) were purchased from Applied Biosystems. TaqMan conditions were as follows. After an initial hold of 2 minutes at 50°C and 10 minutes at 95°C, the samples were cycled 40 times at 95°C for 15 seconds and 60°C for 1 minute. Expression of hL-FABP, MCP-1, MCP-3, HO-1, and TGF- β mRNAs in each sample was evaluated after normalization with GAPDH expression.

Measurement of MCP-1 and hL-FABP by ELISA

To determine the quantity of MCP-1 and hL-FABP proteins in the kidney, the protein extracted by the method described above was measured with ELISA kits for MCP-1 (R&D Systems, Minneapolis, MN) and hL-FABP (CMIC Co., Ltd. Tokyo, Japan).^{11,22,23} The concentrations of MCP-1 and hL-FABP were corrected for the total amount of protein.

Western Blot Analysis

To determine the lipid peroxidation products produced by oxidative stress, ie, 4-hydroxy-2-nonenal (4-HNE)-modified proteins,^{15,24} protein extracts were prepared

from kidney tissues by homogenization in lysis buffer: 0.1 mol/L phosphate buffer, 1 μ g/ml chymotrypsin, 1 μ g/ml leupeptin, 1% Triton X-100, and 0.05 mmol/L phenylmethyl sulfonyl fluoride. In brief, kidney tissue was homogenized in the lysis buffer and was centrifuged for 30 minutes at 4°C at 15,000 rpm. The supernatant was transferred into a clean tube, and the protein concentration was measured using the Lowry protein assay (Bio-Rad Laboratories, Hercules, CA). A protein sample (40 μ g) was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated protein bands were analyzed by enhanced chemiluminescence (ECL system; Amersham Int., Buckinghamshire, UK). Monoclonal antibody against 4-HNE was used as primary antibody (JalCA, Shizuoka, Japan).

Statistical Analysis

All values are expressed as mean $\pm$ SE. The correlation between the two groups (nonparametric distribution) was analyzed by the Mann-Whitney *U*-test using unpaired data. Statistical significance was set at $P < 0.05$.

Results

Expression of hL-FABP

TaqMan real-time PCR assay showed that the *hL-FABP* gene was expressed in the kidney of Tg mice (Figure 1a) but not in WT mice (data not shown). hL-FABP protein

Figure 2. Photomicrographs of renal hL-FABP immunostaining. Representative photomicrographs showing immunostaining of hL-FABP in the kidneys of sham-operated Tg mice on day 7 (**a**), UUO-Tg mice on day 4 (**b**), sham-operated WT mice on day 7 (**c**), and UUO-WT mice on day 4 (**d**). Original magnifications, $\times 200$.

expression was confirmed with ELISA (Figure 1b). In the obstructed kidneys of the Tg mice with UUO (UUO-Tg), renal gene expression of hL-FABP was significantly higher (relative expression in arbitrary units) on day 2 (0.75 ± 0.24 , $P < 0.05$), day 4 (0.89 ± 0.35 , $P < 0.05$), and day 5 (0.82 ± 0.13 , $P < 0.05$) compared with the kidneys of sham-operated mice on day 7 (0.27 ± 0.03) and the contralateral kidneys of UUO-Tg. hL-FABP expression in kidneys of UUO-Tg decreased on day 7 to 0.35 ± 0.03 (arbitrary units), which was significantly higher than that in the contralateral kidneys of UUO-Tg (0.10 ± 0.07 , $P < 0.05$), but was not found in the kidneys of sham-operated mice on day 7 (Figure 1a).

In UUO-Tg kidneys, hL-FABP protein expression increased significantly on day 2 (4.8 ± 1.2 $\mu\text{g}/\text{mg}$ total protein), compared with sham (0.8 ± 0.1 $\mu\text{g}/\text{mg}$ total protein, $P < 0.05$) and the contralateral kidneys (day 2, 1.2 ± 0.1 $\mu\text{g}/\text{mg}$ total protein; $P < 0.05$), and decreased on day 7 to 3.5 ± 0.6 $\mu\text{g}/\text{mg}$ total protein (Figure 1b). The expression of hL-FABP in the contralateral kidneys on days 2, 4, 5, and 7 after UUO was similar to that in sham.

Immunohistochemical analysis showed that hL-FABP staining in the Tg mice was spread diffusely through the cytoplasm of the proximal tubules in the sham-operated Tg mice on day 7 (Figure 2a), in the contralateral kidneys of UUO-Tg (data not shown), and in the UUO-Tg kidneys on day 2 (data not shown), as well as in the cytoplasm and nuclei of the proximal tubules in the UUO-Tg kidneys on day 4 (Figure 2b), day 5 (data not shown), and day 7 (data not shown). Mouse L-FABP expression was not observed in the sham-operated WT mice (Figure 2c) or in the contralateral kidneys of UUO-WT (data not shown) and UUO-WT kidneys (Figure 2d).

Serum Biochemistry

Total cholesterol in the sham-operated Tg mice was similar to that in the sham-operated WT mice (Table 1). On

Table 1. Serum Lipids of UUO-Tg, UUO-WT, Sham-Operated Tg, and WT Mice

	Total cholesterol (mg/dl)	Serum lipid peroxidation (nmol/ml)
WT mice		
Sham operated	77.8 ± 4.0	1.35 ± 0.14
Day 2 after UUO	$104.4 \pm 3.5^*$	1.22 ± 0.12
Day 4 after UUO	$63.2 \pm 1.1^*$	2.18 ± 0.81
Day 5 after UUO	61.0 ± 6.4	4.00 ± 3.07
Day 7 after UUO	71.2 ± 4.9	1.52 ± 0.29
Tg mice		
Sham operated	74.0 ± 3.8	1.13 ± 0.23
Day 2 after UUO	$121.5 \pm 9.6^*$	1.48 ± 0.21
Day 4 after UUO	74.4 ± 4.1	1.36 ± 0.16
Day 5 after UUO	65.0 ± 11.4	1.05 ± 0.19
Day 7 after UUO	78.8 ± 8.8	1.13 ± 0.27

* $P < 0.05$ compared to the sham-operated Tg or WT mice.

day 2, in both UUO-Tg and UUO-WT, the concentration of total cholesterol significantly increased compared with sham-operated Tg or WT mice. There was no difference between the UUO-WT and the UUO-Tg. Serum lipid peroxidation in the sham-operated Tg mice was similar to that in the sham-operated WT mice (Table 1). The mean values of serum lipid peroxidation in UUO-WT on days 4, 5, and 7 or that in UUO-Tg on days 2 and 4 increased more than that in the sham-operated WT or Tg mice, but not significantly. There was no difference between the UUO-WT and the UUO-Tg mice (Table 1).

Expression of MCP-1

By TaqMan real-time PCR, the renal expression of MCP-1 was similar in the sham-operated Tg mice and the sham-operated WT mice (Figure 3a). Gene expression of MCP-1 significantly increased in the UUO-Tg kidneys on days 4, 5, and 7, or in the UUO-WT kidneys on days 2, 4, 5, and 7, compared with the kidneys of sham-operated Tg or WT mice and the contralateral kidneys of Tg or WT mice. The level of gene expression of MCP-1 in the UUO-Tg kidneys was lower than that in the UUO-WT kidneys on day 2, but the difference was not statistically significant (Figure 3a). Thereafter, the expression in the UUO-Tg kidneys was significantly lower than that in the UUO-WT kidneys on day 4 [0.20 ± 0.03 (arbitrary units) and 0.41 ± 0.02 (arbitrary units), respectively; $P < 0.01$], but on days 5 and 7, the expression was not significantly different from that in the UUO-WT kidneys [day 5, 0.33 ± 0.10 (arbitrary units) and 0.37 ± 0.10 (arbitrary units), respectively; day 7, 0.77 ± 0.13 (arbitrary units) and 0.65 ± 0.13 (arbitrary units), respectively; NS]. The level of gene expression was significantly higher in the contralateral kidneys of UUO-Tg on day 7 [0.04 ± 0.01 (arbitrary units)] and in that of UUO-WT on day 5 [0.05 ± 0.01 (arbitrary units)] than that in the sham-operated Tg or WT mice. There was no significant difference between the contralateral kidneys of UUO-Tg and UUO-WT mice.

The level of renal MCP-1 protein expression (measured by ELISA) of the sham-operated Tg mice was similar to that in the kidneys of the sham-operated WT mice (Figure

Figure 3. Expression of MCP-1 in mice with UUO. **a:** Expression of MCP-1 mRNA transcripts was determined by TaqMan real-time PCR and was normalized to that of GAPDH mRNA transcripts in the same sample. **b:** The expression of MCP-1 protein was determined by ELISA and was corrected for the total amount of protein. Values are the mean $\pm$ SE. ■, Tg mice; □, WT mice. $^{\S}P < 0.01$ and $^{*}P < 0.05$ compared with the UUO-WT kidneys. $^{*}P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; $^{\eta}P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice.

3b). In the UUO-Tg kidneys or the UUO-WT kidneys, MCP-1 protein expression on days 2, 4, 5, and 7 increased significantly ($P < 0.05$) compared with sham-operated Tg or WT mice and the contralateral kidneys of Tg or WT mice. On days 4, 5, and 7, MCP-1 protein expression in the UUO-Tg kidneys was significantly lower than in the UUO-WT kidneys (day 4, 39.6 ± 4.6 pg/mg protein and 67.0 ± 6.9 pg/mg protein, respectively; day 5, 45.5 ± 11.3 pg/mg protein and 103.8 ± 12.1 pg/mg protein, respectively; day 7, 92.1 ± 15.6 pg/mg protein and 139.0 ± 6.4 pg/mg protein, respectively; $P < 0.05$). In the contralateral kidneys of UUO-Tg on day 5 and day 7 or in that of UUO-WT on day 5 and day 7, the level of protein expression of MCP-1 was significantly higher than that in the sham-operated Tg or WT. There was no significant difference between the contralateral kidneys of UUO-Tg or UUO-WT mice.

Expression of MCP-3

By TaqMan real-time PCR, the renal expression of MCP-3 mRNA was similar in the sham-operated Tg mice and the sham-operated WT mice (Figure 4). In the UUO-Tg kidneys or in the UUO-WT kidneys on days 2, 4, 5, and 7, renal gene expression of MCP-3 significantly increased compared with the kidneys of sham-operated Tg or WT mice and the contralateral kidneys of Tg or WT mice. The level of gene expression of MCP-3 in the UUO-Tg kidneys was significantly lower than that in the UUO-WT kidneys on day 2 [day 2, 0.17 ± 0.04 (arbitrary units) and 0.41 ± 0.08 (arbitrary units), respectively; $P < 0.05$], day 4 [0.28 ± 0.09 (arbitrary units) and 1.03 ± 0.14 (arbitrary units), respectively; $P < 0.01$], and day 5 [1.51 ± 0.55 (arbitrary units) and 2.98 ± 1.33 (arbitrary units), respectively; $P < 0.05$] (Figure 4). On day 7, in the contralateral kidneys of UUO-Tg or in that of UUO-WT mice, the level of gene expression was significantly higher than that in

the sham of Tg or WT mice. There was no statistically significant difference between UUO-Tg contralateral kidneys and the UUO-WT contralateral kidneys.

Expression of TGF- β

Renal TGF- β gene expression measured by TaqMan real-time PCR was similar in the sham-operated Tg and the sham-operated WT mice (Figure 5). In the UUO-Tg kidneys, gene expression of TGF- β increased significantly ($P < 0.05$) on day 4 compared with the contralateral kidneys and on days 5 and 7 compared with the kidneys of sham-operated Tg mice and the contralateral kidneys of UUO-Tg. TGF- β expression increased significantly ($P < 0.05$) in the UUO-WT kidneys as early as on day 2

Figure 4. Expression of MCP-3 in mice with UUO. Expression of MCP-3 mRNA transcripts was determined by TaqMan real-time PCR and was normalized to that of GAPDH mRNA transcripts in the same sample. ■, Tg mice; □, WT mice. $^{\S}P < 0.01$ and $^{*}P < 0.05$ compared with the UUO-WT kidneys. $^{*}P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; $^{\eta}P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice.

Figure 5. Expression of TGF- β in mice with UUO. Expression of TGF- β mRNA transcripts was determined by TaqMan real-time PCR and was normalized to that of GAPDH mRNA transcripts in the same sample. ■, Tg mice; □, WT mice. * $P < 0.05$ compared with the UUO-WT kidneys. † $P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; # $P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice.

and continued to increase on days 4, 5, and 7; the expression level was significantly higher than in the kidneys of sham-operated WT mice and the contralateral kidneys of UUO-WT. On day 2, the level of TGF- β gene expression in the UUO-Tg kidneys was similar to that in the UUO-WT kidneys and was significantly lower than that in the UUO-WT kidneys on day 4 [0.83 ± 0.13 (arbitrary units) and 1.65 ± 0.14 (arbitrary units), respectively; $P < 0.05$]. However, there was no difference between the UUO-Tg kidneys and the UUO-WT kidneys on day 5 [1.45 ± 0.44 (arbitrary units) and 1.84 ± 0.74 (arbitrary units), respectively; NS] and day 7 [1.46 ± 0.28 (arbitrary units) and 2.53 ± 0.52 (arbitrary units), respectively; NS]. On days 2, 4, 5, and 7 after UUO, the expression of

TGF- β in the contralateral kidneys of UUO-Tg or UUO-WT was similar to that in sham, and there was no significant difference between the Tg and the WT.

Histological and Immunohistochemical Evaluation of Kidneys

In the UUO-Tg kidneys or the UUO-WT kidneys, the area of the tubulointerstitial damage was significantly greater than that in the kidneys of the sham-operated Tg or WT mice ($P < 0.05$; Figure 6, a–d) or that in the contralateral kidneys of UUO-Tg or UUO-WT mice. The area of damage in the UUO-Tg kidneys was similar to that in the UUO-WT kidneys on day 2 (0.04 ± 0.01 and 0.05 ± 0.01 , respectively; NS) and was significantly smaller than that in the UUO-WT kidneys on day 4 (0.17 ± 0.02 and 0.26 ± 0.02 , respectively; $P < 0.05$) and day 5 (0.18 ± 0.02 and 0.27 ± 0.03 , respectively; $P < 0.05$) (Figure 6e). Thereafter, the tubulointerstitial damage progressed in both groups, and the difference between the two groups was not statistically significant on day 7 (0.20 ± 0.06 and 0.19 ± 0.01 in the UUO-Tg and the UUO-WT, respectively; NS). In both the contralateral kidneys of Tg and WT mice and the kidneys of the sham-operated mice, tubulointerstitial damage was not observed.

Macrophage infiltrates were found in the interstitium in both UUO-Tg kidneys and UUO-WT kidneys on days 2, 4, 5, and 7 (Figure 7, a–d). In the UUO-Tg kidneys or the UUO-WT kidneys, the extent of infiltration was significantly greater than that in the kidneys of the sham-operated Tg or WT mice ($P < 0.05$). On day 2, there was significantly less infiltrate in the UUO-Tg kidneys than that

Figure 6. Histological evaluation of tubulointerstitial change after UUO in the Tg mice and the WT mice. Representative photomicrographs of Azan-Mallory-stained sections from the sham-operated Tg mice on day 7 (a), the UUO-Tg kidneys on day 4 (b), the sham-operated WT mice on day 7 (c), and the UUO-WT kidneys on day 4 (d). e: The tubulointerstitial injury was assessed semiquantitatively as described under Materials and Methods; values are mean $\pm$ SE. ■, Tg mice; □, WT mice. * $P < 0.05$ compared with the UUO-WT mice; † $P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; # $P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice. Original magnifications, $\times 200$.

Figure 7. Immunostaining of the tubulointerstitium with F4/80 after UUO in Tg mice and WT mice. F4/80 immunostaining in the kidney of sham-operated Tg mice on day 7 (**a**), UUO-Tg mice on day 4 (**b**), sham-operated WT mice on day 7 (**c**), and UUO-WT mice on day 4 (**d**). **e:** The number of F4/80-positive cells that had infiltrated the interstitium was assessed semiquantitatively as described under Materials and Methods; values are mean $\pm$ SE. ■, Tg mice; □, WT mice. $^{\S}P < 0.01$ and $^{*}P < 0.05$ compared with the UUO-WT kidneys; $^{*}P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; $^{\P}P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice. Original magnifications, $\times 200$.

in the UUO-WT kidneys (12.1 ± 2.6 and 30.2 ± 5.7 , respectively; $P < 0.05$). On day 4, the cellular infiltrate measurements were 57.5 ± 18.0 and 138.3 ± 35.1 in the UUO-Tg kidneys and the UUO-WT kidneys, respectively ($P < 0.05$). On day 5, the measurements were 59.9 ± 14.6 and 201.7 ± 18.7 ($P < 0.05$) in the UUO-Tg kidneys and the UUO-WT kidneys, respectively, and on day 7 the

measurements were 163.1 ± 11.3 and 266.6 ± 22.8 ($P < 0.01$) in the UUO-Tg kidneys and the UUO-WT kidneys, respectively (Figure 7e). There was no statistically significant difference between the contralateral kidneys of Tg and WT mice and the kidneys of the sham.

On days 4, 5, and 7, the deposition of interstitial type I collagen in the UUO-Tg kidneys or in the UUO-WT kid-

Figure 8. Type I collagen immunostaining after UUO in Tg mice and WT mice. Type I collagen immunostaining in the kidney of sham-operated Tg mice on day 7 (**a**), UUO-Tg mice on day 4 (**b**), sham-operated WT mice on day 7 (**c**), and UUO-WT mice on day 4 (**d**). **e:** The positive area of type I collagen was assessed semiquantitatively as described under Materials and Methods; values are the mean $\pm$ SE. ■, Tg mice; □, WT mice. $^{*}P < 0.05$ compared with the UUO-WT kidneys; $^{*}P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; $^{\P}P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice. Original magnifications, $\times 200$.

Figure 9. Expression of HO-1 in mice with UUO. The expression of HO-1 mRNA transcripts was determined by TaqMan real-time PCR and was normalized to that of GAPDH in the same sample. ■, Tg mice; □, WT mice. * $P < 0.05$ compared with the UUO-WT kidneys; † $P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; ‡ $P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice.

neys was significantly higher than that in the kidneys of the sham-operated Tg or WT mice or the contralateral kidneys of UUO-Tg or UUO-WT (Figure 8, a–d). On day 2, the extent of deposition was similar in the UUO-Tg kidneys and the UUO-WT kidneys and was significantly less in the UUO-Tg kidneys than in the UUO-WT kidneys on day 4 (0.02 ± 0.00 and 0.09 ± 0.02 , respectively; $P < 0.01$) and day 5 (0.05 ± 0.01 and 0.10 ± 0.01 , respectively; $P < 0.05$) (Figure 8e). The difference was not statistically significant between the sham-operated Tg and the sham-operated WT mice (0.02 ± 0.00 and 0.00 ± 0.00 , respectively; NS) or between the contralateral kidneys of UUO-Tg and the contralateral kidneys of UUO-WT. In the Tg or WT mice, there was no statistically significant difference between the contralateral kidneys and the kidneys of the sham.

Evaluation of Oxidative Stress

Because HO-1 is considered to be one of the most sensitive indicators of cellular oxidative stress,²⁵ the gene expression of HO-1 in the kidney was investigated. The level of renal gene expression of HO-1 measured by TaqMan real-time PCR in the sham-operated Tg mice was similar to that in the kidneys of the sham-operated WT mice (Figure 9). In the UUO-Tg mice, renal gene expression of HO-1 increased significantly on day 2 [1.03 ± 0.14 (arbitrary units)] compared with the kidneys of sham-operated Tg mice [0.25 ± 0.06 (arbitrary units)] and the contralateral kidneys of UUO-Tg, thereafter decreasing to a level that was similar to that of the kidneys of sham-operated Tg mice on days 4, 5, and 7, or that of the contralateral kidneys of UUO-Tg on days 5 and 7. In the UUO-WT kidneys, the expression of HO-1 increased significantly on day 2 [1.52 ± 0.09 (arbitrary units)] compared with the kidneys of sham-operated WT mice [0.17 ± 0.07 (arbitrary units)] and the contralateral kidneys; thereafter, the expression decreased on days 4, 5, and 7. However, the level on days 4, 5, and 7 was

Figure 10. Expression of Gpx1 in mice with UUO. The expression of Gpx1 mRNA transcripts was determined by TaqMan real-time PCR and was normalized to that of GAPDH in the same sample. ■, Tg mice; □, WT mice. * $P < 0.05$ compared with the UUO-WT kidneys; † $P < 0.05$ compared with the kidney of the sham-operated Tg or WT mice; ‡ $P < 0.05$ compared with each contralateral kidney of UUO-Tg or UUO-WT mice; † $P < 0.05$ compared with each UUO kidney.

significantly higher than that in the kidneys of the sham-operated WT mice or that of the contralateral kidneys of UUO-WT mice (Figure 9). The level of gene expression of HO-1 in the UUO-Tg kidneys was significantly lower than that in the UUO-WT kidneys on day 2 ($P < 0.05$) and was similar to that in the UUO-WT kidneys on day 4. In the contralateral kidneys of the UUO-Tg or the UUO-WT mice, the expression of HO-1 on days 2, 4, 5, and 7 after UUO was similar to that in sham-operated Tg or WT, and there was no statistically significant difference between the Tg and the WT.

Moreover, the expression of the anti-oxidative stress enzyme Gpx1^{26–28} was examined in the kidney. The level of renal gene expression of Gpx1 measured by TaqMan real-time PCR in the sham-operated Tg mice [1.51 ± 0.35 (arbitrary units)] was similar to that in the kidneys of the sham-operated WT mice [1.23 ± 0.16 (arbitrary units)] (Figure 10). In the UUO-Tg mice, renal gene expression of Gpx1 did not significantly increase compared with the kidneys of sham-operated Tg mice and the contralateral kidneys of UUO-Tg. In the UUO-WT kidneys, the expression of Gpx1 increased significantly on days 4 [2.03 ± 0.18 (arbitrary units)], 5 [2.13 ± 0.13 (arbitrary units)], and 7 [2.33 ± 0.30 (arbitrary units)] compared with the kidneys of sham-operated WT mice and the contralateral kidneys. The level of gene expression of Gpx1 in the UUO-Tg kidneys [1.55 ± 0.17 (arbitrary units)] was significantly lower than that in the UUO-WT kidneys [2.13 ± 0.13 (arbitrary units)] on day 5 ($P < 0.05$). On day 2, in the contralateral kidneys of UUO-Tg or in that of UUO-WT mice, the level of gene expression was significantly higher than that in the sham of Tg or WT mice or that in the UUO-Tg mice, and there was no statistically significant difference between the Tg and the WT mice. One of the lipid peroxidation products, a 4-HNE-modified protein band, was not observed in Western blot anal-

Figure 11. Presence of ~51.6-kd 4-HNE-modified protein in mice with UUO. Protein extracts of the kidneys (day 7) from the UUO-Tg and the UUO-WT mice were electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gels and subjected to Western blot analysis using monoclonal antibody against 4-HNE. A major band of ~51.6 kd was found in the UUO-WT.

ysis of the sham-operated Tg or WT mice or the contralateral kidneys of UUO-Tg or UUO-WT mice (Figure 11). Although this 4-HNE-modified protein was not detected in the duration of these experiments in the UUO-Tg kidneys, a protein band of ~51.6 kd was very weakly expressed on day 7 in the UUO-WT kidneys.

Discussion

In the present study, we demonstrated that the expression of renal hL-FABP was up-regulated in the UUO-Tg mice and that it suppressed the production of HO-1, Gpx1, MCP-1, MCP-3, TGF- β , and 4-HNE-modified proteins. The number of infiltrating macrophages and the deposition of type I collagen were significantly lower in the UUO-Tg kidneys than in the UUO-WT kidneys, and the tubulointerstitial damage was significantly attenuated in the UUO-Tg kidneys compared with the UUO-WT kidneys on days 4 and 5. These results suggested the possibility that renal hL-FABP reduced the oxidative stress, inhibited the production of inflammatory cytokine and lipid peroxidation product, and attenuated the tubulointerstitial damage in the UUO model.

Although we examined the influence of hL-FABP in the nonobstructed contralateral kidneys of UUO-Tg on UUO-Tg kidneys, the expression of hL-FABP in the contralateral kidneys of UUO-Tg was similar to that in the kidneys of the sham-operated Tg mice. Therefore, the observed effect is unlikely to be attributable to systemic influences of hL-FABP derived from the nonobstructed contralateral kidneys of UUO-Tg on UUO-Tg kidneys. Conceptually, a systemic effect would involve the release into circulation of hL-FABP, which may be elevated in the contralateral kidneys of UUO-Tg, leading to amelioration of the tubulointerstitial damage in the UUO-kidneys.

Oxidative stress derived from shear stress, or hypoxia attributable to reduced blood flow,^{16,29} is known to play a significant role in the progression of tubulointerstitial damage in the kidney of UUO mice.^{13–16} Recently it was reported that the L-FABP-expressing cell line has a reduced concentration of intracellular reactive oxygen species, and L-FABP has a cytoprotective function against oxidative stress.⁹ We found that the expression of HO-1, which is considered to be a sensitive and reliable marker of the onset of oxidant stress,²⁵ was transiently induced at an early stage after UUO in both the UUO-Tg kidneys and the UUO-WT kidneys, and its expression was significantly lower in the UUO-Tg. At the same time, hL-FABP expression in the proximal tubules of UUO-Tg kidneys was up-regulated by UUO on day 2. To reconfirm anti-oxidative potential of L-FABP, the expression of Gpx1,

which was reported to be induced by oxidative stress,^{26–28} was investigated. Its expression in the UUO-WT kidneys significantly increased compared with that in the UUO-Tg kidneys on day 5. Furthermore, the products of lipid peroxidation, 4-HNE-modified proteins, were suppressed on day 7 in the UUO-Tg kidneys. These results suggested the onset of oxidative stress in the kidney of UUO and the possibility that L-FABP may function as an antioxidant.

Regarding the role of renal L-FABP in kidney disease, an *in vitro* study suggested that L-FABP does not protect against cytotoxicity induced by fatty acids in the distal tubules.³⁰ However, we previously reported that L-FABP expression in the proximal tubules reduced inflammation in the interstitium and mildly inhibited the development of tubulointerstitial damage in a protein overload model.¹¹ In this study, the expression of MCP-1 and MCP-3, which are principal cytokines in chemotaxis and activation of macrophages,^{31–34} and TGF- β , which is thought to be a key molecule in fibrosis, were significantly suppressed.^{12,35} In addition, macrophage infiltration, deposition of type I collagen, and the progression of tubulointerstitial damage were significantly attenuated in the UUO-Tg kidneys compared with the UUO-WT kidneys. These results suggested that L-FABP expression in the proximal tubules ameliorated the development of tubulointerstitial damage.

On day 7, the late stage of the UUO model, the degree of tubulointerstitial damage was not significantly different between the UUO-Tg kidneys and the UUO-WT kidneys. Because many factors contribute to the pathogenesis of tubulointerstitial damage in the UUO model, it is likely that the presence of L-FABP alone had limited efficacy for prevention of tubulointerstitial damage after persistent injury.

In the UUO-Tg kidneys, immunohistochemical staining of hL-FABP was found not only in the cytoplasm but also in the nuclei on days 4, 5, and 7, and L-FABP was present in the nuclei. L-FABP was reported to transport bound ligands such as fatty acids to the nucleus and to interact with the nuclear protein peroxisome proliferator-activated receptor (PPAR),^{36–38} which initiates the gene expression of enzymes involved in lipid metabolism.^{39–43} hL-FABP might play an important role in PPAR-regulated gene expression in the UUO model. In the previous study of the protein overload model, we did not find positive staining of hL-FABP in the nuclei. We speculated that there were other factors involved in the transport of L-FABP from the cytoplasm to the nucleus in the UUO model. Because we did not examine the precise significance of the presence of L-FABP in the nucleus, further *in vitro* experiments are needed to clarify this point.

Likewise, serum total cholesterol increased on day 2 in both the UUO-Tg and the UUO-WT mice, thereafter decreasing to a level approximating that of the sham-operated Tg or WT mice. On day 2, the expression of Gpx1 significantly increased in the contralateral kidneys of the UUO-Tg or the UUO-WT mice compared with that in sham-operated Tg or WT mice. These results suggested that the surgical procedure performed on the UUO mice temporarily exerted oxidative stress on the mice and

suppressed the food intake of these mice, causing undernourishment and increasing the synthesis of total cholesterol in the liver. Hypercholesterolemia has been reported to cause interstitial inflammation and fibrosis.⁴⁴ Although we induced hypercholesterolemia (total cholesterol, 300 to 320 mg/dl) in mice with a high-fat diet, tubulointerstitial damage was not observed (data not shown). This result suggested that total cholesterol level in this study did not influence the progression of tubulointerstitial injury.

In the protein extracted from the UO-WT kidneys on day 7, a 4-HNE-modified protein of ~51.6 kd was detected very weakly by Western blot analysis. However, we did not find any lipid peroxidation products in the kidney by immunohistochemistry (data not shown). It may be that the level of the lipid peroxidation products in the UO kidneys are lower than can be detected by Western blot analysis.

Although in the contralateral kidneys of UO-Tg or UO-WT the expressions of MCP-1 and MCP-3 were significantly lower than those in UO-Tg kidneys or UO-WT kidneys and tubulointerstitial damage was not observed, the expression of MCP-1 and MCP-3 was significantly higher than that in the sham-operated Tg or WT kidneys at a later stage of UO such as day 5 or day 7. There is the possibility that any cytokines induced in the UO kidneys acted on the contralateral kidneys of UO in the late phase of UO.

In conclusion, we found that hL-FABP expressed in the proximal tubules was up-regulated in the UO model and that it suppressed the development of tubulointerstitial damage. Renal L-FABP is likely to be an effective endogenous antioxidant. This study provides a new insight that the agents up-regulating the expression of renal L-FABP in the proximal tubules might lead to prevention of tubulointerstitial damage and may offer a new strategy for inhibiting the progression of kidney disease.

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References

- Risdon RA, Sloper JC, De Wardener HE: Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968, 2:363-366
- Remuzzi G, Ruggenti P, Benigni A: Understanding the nature of renal disease progression. *Kidney Int* 1997, 51:2-15
- Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. *N Engl J Med* 1998, 339:1448-1456
- Maatman RG, van de Westerlo EM, van Kuppevelt TH, Veerkamp JH: Molecular identification of the liver- and the heart-type fatty acid-binding proteins in human and rat kidney. Use of the reverse transcriptase polymerase chain reaction. *Biochem J* 1992, 288:285-290
- Sweetser DA, Heuckeroth RO, Gordon JI: The metabolic significance of mammalian fatty-acid-binding proteins: abundant proteins in search of a function. *Annu Rev Nutr* 1987, 7:337-359
- Veerkamp JH, Peeters RA, Maatman RG: Structural and functional features of different types of cytoplasmic fatty acid-binding proteins. *Biochim Biophys Acta* 1991, 1081:1-24
- Veerkamp JH, van Kuppevelt TH, Maatman RG, Prinsen CF: Structural and functional aspects of cytosolic fatty acid-binding proteins. Prostaglandins Leukot Essent Fatty Acids 1993, 49:887-906
- Ek-Von Mentzer BA, Zhang F, Hamilton JA: Binding of 13-HODE and 15-HETE to phospholipid bilayers, albumin, and intracellular fatty acid binding proteins. Implications for transmembrane and intracellular transport and for protection from lipid peroxidation. *J Biol Chem* 2001, 276:15575-15580
- Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski FJ: Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells. *Hepatology* 2005, 42:871-879
- Raza H, Pongubala JR, Sorof S: Specific high affinity binding of lipoxygenase metabolites of arachidonic acid by liver fatty acid binding protein. *Biochem Biophys Res Commun* 1989, 161:448-455
- Kamijo A, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M, Honda A, Okabe M, Fujino T, Hirata Y, Omata M, Kaneko R, Fujii H, Fukamizu A, Kimura K: Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. *Am J Pathol* 2004, 165:1243-1255
- Klahr S, Morrissey J: Obstructive nephropathy and renal fibrosis. *Am J Physiol* 2002, 283:F861-F875
- Pat B, Yang T, Kong C, Watters D, Johnson DW, Gobe G: Activation of ERK in renal fibrosis after unilateral ureteral obstruction: modulation by antioxidants. *Kidney Int* 2005, 67:931-943
- Ricardo SD, Ding G, Eufemio M, Diamond JR: Antioxidant expression in experimental hydronephrosis: role of mechanical stretch and growth factors. *Am J Physiol* 1997, 272:F789-F798
- Sunami R, Sugiyama H, Wang DH, Kobayashi M, Maeshima Y, Yamasaki Y, Masuoka N, Ogawa N, Kira S, Makino H: Acatalasemia sensitizes renal tubular epithelial cells to apoptosis and exacerbates renal fibrosis after unilateral ureteral obstruction. *Am J Physiol* 2004, 286:F1030-F1038
- Kawada N, Moriyama T, Ando A, Fukunaga M, Miyata T, Kurokawa K, Imai E, Hori M: Increased oxidative stress in mouse kidneys with unilateral ureteral obstruction. *Kidney Int* 1999, 56:1004-1013
- Yang J, Dai C, Liu Y: Hepatocyte growth factor gene therapy and angiotensin II blockade synergistically attenuate renal interstitial fibrosis in mice. *J Am Soc Nephrol* 2002, 13:2464-2477
- Sato M, Kashiwara N, Yamasaki Y, Maruyama K, Okamoto K, Maeshima Y, Sugiyama H, Sugaya T, Murakami K, Makino H: Renal interstitial fibrosis is reduced in angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2001, 12:317-325
- Yagi K: A simple assay of serum lipid hydroperoxides. *Jpn J Clin Chem* 1997, 26:89-94
- Yagi K: A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 1976, 15:212-216
- Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hase H, Kaneko T, Hirata Y, Goto A, Fujita T, Omata M: Urinary free fatty acids bound to albumin aggravate tubulointerstitial damage. *Kidney Int* 2002, 62:1628-1637
- Kamijo A, Sugaya T, Hikawa A, Yamanouchi M, Hirata Y, Ishimitsu T, Numabe A, Takagi M, Hayakawa H, Tabei F, Sugimoto T, Mise N, Kimura K: Clinical evaluation of urinary excretion of liver-type fatty acid binding protein as a marker for monitoring chronic kidney disease: a multi-center trial. *J Lab Clin Med* 2005, 145:125-133
- Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hikawa A, Hirano N, Hirata Y, Goto A, Omata M: Urinary fatty acid binding protein as a new clinical marker for the progression of chronic renal disease. *J Lab Clin Med* 2004, 143:23-30
- Toyokuni S, Uchida K, Okamoto K, Hattori-Nakakuki Y, Hiai H, Stadtman ER: Formation of 4-hydroxy-2-nonenal-modified proteins in the renal proximal tubules of rats treated with a renal carcinogen, ferric nitrilotriacetate. *Proc Natl Acad Sci USA* 1994, 91:2616-2620
- Applegate LA, Luscher P, Tyrrell RM: Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. *Cancer Res* 1991, 51:974-978
- de Haan JB, Bladier C, Griffiths P, Kelner M, O'Shea RD, Cheung NS, Bronson RT, Silvestro MJ, Wild S, Zheng SS, Beart PM, Hertzog PJ,

- Kola I: Mice with a homozygous null mutation for the most abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. *J Biol Chem* 1998, 273:22528–22536
27. Muse KE, Oberley TD, Sempf JM, Oberley LW: Immunolocalization of antioxidant enzymes in adult hamster kidney. *Histochem J* 1994, 26:734–753
28. De Haan JB, Crack PJ, Flentjar N, Iannello RC, Hertzog PJ, Kola I: An imbalance in antioxidant defense affects cellular function: the pathophysiological consequences of a reduction in antioxidant defense in the glutathione peroxidase-1 (Gpx1) knockout mouse. *Redox Rep* 2003, 8:69–79
29. Ohashi R, Shimizu A, Masuda Y, Kitamura H, Ishizaki M, Sugisaki Y, Yamanaka N: Peritubular capillary regression during the progression of experimental obstructive nephropathy. *J Am Soc Nephrol* 2002, 13:1795–1805
30. Zimmerman AW, Veerkamp JH: Fatty-acid-binding proteins do not protect against induced cytotoxicity in a kidney cell model. *Biochem J* 2001, 360:159–165
31. Shimizu H, Maruyama S, Yuzawa Y, Kato T, Miki Y, Suzuki S, Sato W, Morita Y, Maruyama H, Egashira K, Matsuo S: Anti-monocyte chemoattractant protein-1 gene therapy attenuates renal injury induced by protein-overload proteinuria. *J Am Soc Nephrol* 2003, 14:1496–1505
32. Wada T, Furuichi K, Sakai N, Iwata Y, Kitagawa K, Ishida Y, Kondo T, Hashimoto H, Ishiwata Y, Mukaida N, Tomosugi N, Matsushima K, Egashira K, Yokoyama H: Gene therapy via blockade of monocyte chemoattractant protein-1 for renal fibrosis. *J Am Soc Nephrol* 2004, 15:940–948
33. Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Hashimoto H, Ishiwata Y, Tomosugi N, Mukaida N, Matsushima K, Egashira K, Yokoyama H: Gene therapy expressing amino-terminal truncated monocyte chemoattractant protein-1 prevents renal ischemia-reperfusion injury. *J Am Soc Nephrol* 2003, 14:1066–1071
34. Ou ZL, Natori Y: Gene expression of CC chemokines in experimental acute tubulointerstitial nephritis. *J Lab Clin Med* 1999, 133:41–47
35. Bascands JL, Schanstra JP: Obstructive nephropathy: insights from genetically engineered animals. *Kidney Int* 2005, 68:925–937
36. Huang H, Starodub O, McIntosh A, Atshaves BP, Woldegiorgis G, Kier AB, Schroeder F: Liver fatty acid-binding protein colocalizes with peroxisome proliferator activated receptor alpha and enhances ligand distribution to nuclei of living cells. *Biochemistry* 2004, 43:2484–2500
37. Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F: Liver fatty acid-binding protein targets fatty acids to the nucleus. Real time confocal and multiphoton fluorescence imaging in living cells. *J Biol Chem* 2002, 277:29139–29151
38. Wolfrum C, Borrmann CM, Borchers T, Spener F: Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha- and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. *Proc Natl Acad Sci USA* 2001, 98:2323–2328
39. Schoonjans K, Staels B, Auwerx J: Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996, 37:907–925
40. Lemberger T, Desvergne B, Wahli W: Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu Rev Cell Dev Biol* 1996, 12:335–363
41. Wolfrum C, Ellinghaus P, Fobker M, Seedorf U, Assmann G, Borchers T, Spener F: Phytanic acid is ligand and transcriptional activator of murine liver fatty acid binding protein. *J Lipid Res* 1999, 40:708–714
42. Martin G, Poirier H, Hennuyer N, Crombie D, Fruchart JC, Heyman RA, Besnard P, Auwerx J: Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. *J Biol Chem* 2000, 275:12612–12618
43. Issemann I, Green S: Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990, 347:645–650
44. Eddy AA: Interstitial fibrosis in hypercholesterolemic rats: role of oxidation, matrix synthesis, and proteolytic cascades. *Kidney Int* 1998, 53:1182–1189