CRBP-I in the renal tubulointerstitial compartment of healthy rats and rats with renal fibrosis

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Abstract

Background. Cellular retinol-binding protein I (CRBP-I), a member of the intracellular lipid-binding protein (iLBP) superfamily, is a specific marker of quiescent stellate cells in the healthy human liver. In the diseased fibrotic/cirrhotic liver, portal and septal myofibroblasts acquire CRBP-I expression, while activated hepatic stellate cells maintain their CRBP-I expression. Here, we investigate the distribution of CRBP-I in the renal cortex of healthy rats and rats with renal fibrosis.

Methods. Kidneys of healthy and adriamycin-treated rats were studied by immunohistochemistry, using antibodies against CRBP-I, desmin, vimentin and α-smooth muscle actin (α-SMA). Double stainings were done with immunofluorescence. Western blotting was performed to semi-quantify the expression levels of vimentin, desmin, α-SMA and CRBP-I.

Results. In the normal rat kidney, the convoluted proximal tubular epithelial cells express CRBP-I; no expression is found in the interstitium, nor in the glomeruli. In the adriamycin-induced fibrotic rat kidney, CRBP-I expression diminishes in the convoluted proximal tubular epithelial cells, whereas peritubular myofibroblasts in the interstitium acquire CRBP-I expression.

Conclusions. In the tubulointerstitial compartment of the adriamycin-induced fibrotic rat kidney, CRBP-I is expressed in a different pattern than in the healthy rat kidney. As the convoluted proximal tubular epithelial cells dedifferentiate during fibrosis, CRBP-I expression decreases. Furthermore, de novo expression of CRBP-I is found in activated myofibroblast-like cells in the interstitium of adriamycin-treated rats. CRBP-I is therefore a useful marker to identify a subpopulation of activated/myodifferentiated fibroblasts in the rat kidney.

Keywords: CRBP-I; dedifferentiation; myofibroblast; renal fibrosis; tubulointerstitium

Introduction

Vitamin A (retinol) is stored in the liver and transported in the bloodstream to peripheral tissues, such as the kidney, bound to the plasma retinol-binding protein (RBP). Retinol bound to RBP forms a macromolecular complex with another carrier protein, the tetrameric carrier of thyroxine, transthyretin (TTR, formerly known as pre-albumin) [1]. Once RBP and retinol are released from TTR, retinol can enter into a target cell. Recently, the STRA6 receptor has been identified as a specific RBP receptor with robust retinol uptake activity [2]. When retinol is internalized, it forms a complex with a cellular retinol-binding protein (CRBP) [3]. Retinol can then be metabolized and converted to retinoic acid, which can act as a signalling molecule in the cell in which it is produced, or can diffuse through the plasma membrane to influence the development of neighbouring cells. Retinoic acid can also be modified enzymatically to alter its signalling specificity [4].

The CRBP-I is a good marker to identify hepatic stellate cells in the normal human and rat liver.
In fibrotic or cirrhotic livers CRBP-I is co-expressed with α-SMA in portal and septal myofibroblasts, and in activated stellate cells [10,11]. Hepatic stellate cells are well-defined cells that are part of a diffuse stellate cell system [12,13]. Peritubular fibroblasts in the kidney cortex have been identified as being part of this system [14–17].

The aim of the present study is to investigate whether CRBP-I might also be a marker for stellate-like cells or myofibroblasts in the healthy and fibrotic rat kidney. To induce fibrosis in the rat kidney we used adriamycin (doxorubicin). Adriamycin is a broad-spectrum chemotherapeutic drug, clinically used to treat a variety of tumours. Its clinical use, however, is severely restricted by a dose-limiting cardiotoxicity, due to the fact that adriamycin has been linked to the formation of free radicals within the heart muscle. In the kidney of rodents, the initial damage of adriamycin occurs in the glomerulus; the filtration barrier becomes defected leading to glomerulosclerosis and eventually to tubulointerstitial (TI) fibrosis [18,19].

We report the down- and up-regulation of CRBP-I in renal fibrosis in rats during, respectively, the differentiation of proximal tubular epithelial cells and the myodifferentiation of fibroblastic cell types in the renal interstitium.

**Subjects and methods**

**Animals**

All animal studies were conducted under a protocol approved by the committee for the care and use of laboratory animals of the ‘Vrije Universiteit Brussel’. Adult male Wistar rats (Ifca Credo, Brussels, Belgium), weighing 200–220 g, were divided at random into two groups. To one group adriamycin was administered (AD group, n = 8). Adriamycin (doxorubicin, Pharmacia, Brussels, Belgium) administration consisted of two consecutive intravenous tail injections (2 mg/kg, with an interval of 20 days) after anaesthesia with pentobarbital (Nembutal 60 mg/kg i.p.). The second group of rats (control group, n = 6) was injected with the same volume of saline solution. Animals in the control and adriamycin groups were allowed ad libitum tap water and standard chow (A04, UAR, Epinay, France). All animals were killed 20 weeks after the second injection. Blood pressure and body weight were determined weekly. Systolic arterial blood pressure was measured in conscious restrained rats using the tail-cuff method (IITC Life Science Instruments, Woodland Hills, CA, USA) at room temperature. One day before killing, rats were individually housed in metabolic cages for the collection of 24-h urine samples. The rats were anaesthetized again with pentobarbital before killing and blood was collected by cardiac puncture. The kidneys were harvested, weighed and processed for either (immuno)histological evaluation or western blot analysis. Serum and urine urea, creatinine and total protein were analysed by the Kodak Ektachem method (Kodak Eastman, Rochester, NY, USA). Total serum cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides were determined by enzymatic methods or calculated.

**Histological examination**

Transversal slices of kidneys were fixed in 4% buffered formaldehyde at room temperature for 24 h and embedded in paraffin. Sections of 5 µm were cut and stained with haematoxylin-eosin-saffron, periodic acid Schiff (PAS), Masson trichrome and Jones methenamine silver. The sections were examined by a pathologist in a blind fashion by light microscopy. Glomerulosclerosis was scored in 100 glomeruli per rat, counting the number of quadrants showing sclerosis. TI lesions were also described [20].

**Isolation of the tubulointerstitial compartment**

Kidneys were removed from adult male Wistar rats, and the cortex was isolated by dissection. The cortex was minced and gently forced through a 200-µm cell strainer keeping the tissue hydrated with PBS; the filtrate was consecutively passed through a 180-µm cell strainer. The TI compartment was taken from the 180-µm mesh. The purity of the TI suspension was confirmed through microscopic evaluation and found to be >98%. The TI suspension was subjected to centrifugation at 4°C for 5 min at 1000 g. Pellets were resuspended in lysis buffer, and subjected to a second centrifugation at 4°C for 13 min at 20 000 g [21]. The resulting supernatant was used for immunoblot analysis. For long-term storage, samples were kept at −20°C.

**Western blot analysis**

Protein concentrations were determined with a Pierce Micro BCA protein assay kit (Pierce BioScience, Aalst, Belgium). Aliquots of 100 µg protein were treated with 5% β-mercaptoethanol and 0.01% bromophenol blue, then heated at 96°C for 5 min and fractionated (15 µl/lane) in a 10–20% Tris–HCl gradient gel (Bio-Rad Laboratories, Nazareth, Belgium). Proteins were electrotransferred to nitrocellulose membranes (100 V for 1 h) in Tris-glycine buffer. The membrane was incubated in blocking buffer (PBS, 0.05% Tween 20 and 5% non-fat milk) for 1 h at room temperature and then incubated for 1 h at room temperature in blocking buffer with the indicated primary antibody, anti-desmin (mouse monoclonal, DE-U-10, Sigma-Aldrich, Bornem, Belgium, 1/1000), anti-vimentin (mouse monoclonal, V9, DAKO, Heverlee, Belgium, 1/1000), anti-α-SMA (mouse monoclonal, 1A4 Sigma-Aldrich, Bornem, Belgium, 1/5000), anti-β-actin (mouse monoclonal, AC-74 Sigma-Aldrich, Bornem, Belgium, 1/5000) and anti-CRBP-I (rabbit polyclonal, FL-135, Tebu Bio, Boechout, Belgium, 1/500). The membrane was washed once for 15 min and twice for 5 min in PBS with 0.05% Tween 20 and incubated in blocking buffer with horseradish peroxidase (HRP)-linked sheep anti-mouse IgG (GE Healthcare Europe-Amersham, Diegem, Belgium) or HRP-linked goat anti-rabbit IgG (Abcam, Cambridge, UK) at 1/5000 dilution. The washes were repeated and finally the membrane was developed with a chemiluminescent agent ECL on Hyperfilm MP (GE Healthcare Europe-Amersham, Diegem, Belgium).

**Immunohistochemistry**

Fresh kidney-tissue slices of 1 mm thickness were fixed in 4% formaldehyde for 24 h and embedded in paraffin.
Five-μm sections were cut and stained according to the following protocol: dewaxed sections were subjected to heat antigen retrieval in citrate buffer (10 mM, pH 6) in a 98°C waterbath. After blocking, sections were automatically stained using the iView detection kit with the NexEs immunostainer (Ventana Medical Systems, Tucson, AZ, USA) or manually for anti-desmin (mouse monoclonal, DE-U-10, Sigma-Aldrich, Bornem, Belgium, 1/1000), antivimentin (mouse monoclonal, V9, DAKO, Heverlee, Belgium, 1/1000), α-SMA (rabbit polyclonal, Abcam, Cambridge, UK, 1/500) and anti-CRBP-I (mouse monoclonal, 1/20). The CRBP-I monoclonal antibody was raised against a synthetic peptide specific for the CRBP-I protein sequence according to the previous work [20]. We demonstrated the specificity of the monoclonal antibody against CRBP-I by the enzyme-linked immunosorbent assay, by western blot and by immunohistochemical staining using rat and human liver. Streptavidin-HRP biotinylated goat anti-mouse IgG/IgM (Ventana Medical Systems, Tucson, AZ, USA) or donkey anti-rabbit IgG (GE Healthcare, Diegem, Belgium) were used as the secondary antibody for immunohistochemistry. The staining was visualized with a hydrogen peroxide substrate and 3,3′-diaminobenzidine tetrahydrochloride (DAB) chromogen. Tissues were counterstained with Harris haematoxylin (1/8) for 30 s and mounted with Faramount. For each stain, positive and negative controls were used.

Expression vectors

Total RNA was isolated out of the rat liver by the TriZol method (Invitrogen, Merelbeke, Belgium) and purified by spin technology (RNeasy Mini kit, Qiagen, Venlo, The Netherlands). By reverse transcription, we generated cDNA using the high-capacity cDNA Archive kit (Applied Biosystems, Lennik, Belgium). A typical PCR-reaction mixture containing 10 pmol of each primer (CRBP-I forward primer: CACCATGCCGTGGAGACTTCAAC; CRBP-I reverse primer: TCAGTGACTTTCTTGAA-CAC), 2 mM dNTP (Fermentas, St Leon-Rot, Germany), reaction buffer with 50 mM MgCl₂ and 25 mM MgSO₄ (Bioline, Melsbroek, Belgium), 1.1 g/ml DMSO (Sigma-Aldrich, Bornem, Belgium), 5 μ/l Taq DNA polymerase (Invitrogen, Merelbeke, Belgium), 2.5 μ/l Pfu DNA polymerase (Fermentas, St Leon-Rot, Germany) and 100 ng of template DNA. PCR products were run on a 2% agarose gel in TAE buffer, extracted by the QIAEX II agarose gel extraction protocol (Qiagen, Venlo, The Netherlands) and polished using Pfu. For expression in mammalian cells, a GFP-tagged CRBP-I protein was generated using a pENTR directional TOPO cloning reaction subsequently followed by a LR recombination into a pEGFP vector. The sequences were confirmed by Big Dye Sequencing (Applied Biosystems, Lennik, Belgium).

Double immunofluorescence

For double immunofluorescence, dewaxed paraffin sections were subjected to heat antigen retrieval in the citrate buffer (10 mM, pH 6) in a microwave oven. After blocking of the unspecific binding with 2% BSA/PBS, the sections were incubated with a primary antibody mix-

### Table 1. General and functional renal parameters in control and adriamycin-treated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AD</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>469 ± 22</td>
<td>379 ± 17*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 5</td>
<td>182 ± 4*</td>
</tr>
<tr>
<td>Serum urea (mg/100 ml)</td>
<td>34 ± 3</td>
<td>106 ± 36*</td>
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<tr>
<td>Serum creatinine (mg/100 ml)</td>
<td>0.55 ± 0.004</td>
<td>1.46 ± 0.41*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/100 g body weight)</td>
<td>0.37 ± 0.04</td>
<td>0.19 ± 0.05**</td>
</tr>
<tr>
<td>Total urinary protein (mg/24 h)</td>
<td>10 ± 2</td>
<td>518 ± 29**</td>
</tr>
<tr>
<td>Serum triglycerides (mg/100 ml)</td>
<td>90 ± 16</td>
<td>360 ± 58*</td>
</tr>
<tr>
<td>Serum cholesterol (mg/100 ml)</td>
<td>72 ± 2</td>
<td>289 ± 32*</td>
</tr>
<tr>
<td>Serum high-density lipoprotein cholesterol (mg/100 ml)</td>
<td>33 ± 1</td>
<td>110 ± 9*</td>
</tr>
<tr>
<td>Serum low-density lipoprotein cholesterol (mg/100 ml)</td>
<td>23 ± 3</td>
<td>108 ± 16*</td>
</tr>
</tbody>
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Statistical significance [AD (adriamycin) group versus control group]: *P < 0.01; **P < 0.05.

### Results

**General observations and clinical parameters**

All the animals, which were injected twice with adriamycin (2 mg/kg) to induce renal fibrosis and those in the matched control (saline) group, survived and remained in good shape during the course of the experiment. The proteinuria in the adriamycin-treated rats shows a 50-fold increase (Table 1). Adriamycin induces higher serum levels of total cholesterol, high- and low-density lipoprotein cholesterol, triglycerides, total protein and a higher systolic blood pressure compared to the matched control group (Table 1).
Histological evaluation

Adriamycin-treated animals show glomerular and TI changes. Glomerulosclerosis is seen in 21.5% ± 2.6% of the glomeruli (13.8% ± 1.9% of the quadrants) versus 1% ± 1% in control animals (P < 0.01). A moderate interstitial lymphocytic infiltration and dilated tubules containing large proteinaceous casts are present only in adriamycin-treated animals. The injured tubules show the loss of brush border and cell height with sparse cellular debris into the lumen.

Altered expression of vimentin, desmin, α-SMA and CRBP-I

CRBP-I is located in the cytoplasm of the convoluted proximal tubular epithelial cells in the healthy rat kidney [5,6]; therefore we specifically isolated the TI compartment to determine the protein expression of CRBP-I and typical markers of fibrogenesis, such as desmin, vimentin and α-SMA. TI lysates were obtained by a sieving method first described by Burlington et al. [21] and analysed by western blotting.

Vimentin increases 5- to 10-fold in the adriamycin-treated group; fold induction compared to the control group was obtained by semi-quantification analysis using Scion Image software (Scion, Frederick, MD, USA) (data not shown). The elevated expression of vimentin in the renal tubulointerstitium of adriamycin-treated rats is due to the increased number of mesenchymal (-derived) cells (Figure 1A). The expression of desmin (a marker of cardiac, skeletal and smooth muscle) is 3- to 5-fold higher in the TI compartment of the adriamycin group compared to the control group. The activation of fibroblasts in the TI lysates of adriamycin-treated rats contributes to the elevated levels of desmin found (Figure 1A). Figure 1A also shows a 5- to 10-fold increase in α-SMA expression (a smooth muscle and a myofibroblastic cell marker) in the fibrotic rat kidney. The higher α-SMA expression confirms the renal TI fibrogenesis in the adriamycin-treated rats, characterized by the abundant myodifferentiation of fibroblastic cell types.

In 1984, studies on the healthy rat kidney showed the presence of CRBP-I in the cytoplasm of the convoluted proximal tubular epithelial cells [5,6]. Here, we observe a decrease in CRBP-I expression in the TI lysates of the adriamycin-induced fibrotic rat kidney compared to the healthy rat kidney (Figure 1A). The specificity of the polyclonal CRBP-I antibody is shown by western blot detection of a GFP-CRBP-I fusion protein expressed in HEK293T cells (Figure 1B).

Localization of CRBP-I, desmin, vimentin and α-SMA

To study in more detail the altered expression of CRBP-I and the above-mentioned fibrotic markers in the adriamycin-treated rats, we performed immunohistochemical stainings for CRBP-I, desmin, vimentin and α-SMA on the kidneys of the control and adriamycin group. Immunohistochemical staining for CRBP-I in the healthy rat kidney (Figure 2A) shows positive epithelial cells in the proximal convoluted tubules. The apical membrane of proximal tubular epithelial cells shows strong reactivity for CRBP-I, associated with a moderate cytoplasmic positivity. We find no or undetectable levels of CRBP-I expression in the glomeruli, nor in the interstitium of the healthy rat kidney. Whereas in the normal kidney CRBP-I stains solely the proximal tubular epithelial cells, in the adriamycin-induced fibrotic rat kidney we find CRBP-I-positive cells in the interstitium with a morphology similar to peritubular fibroblasts or myofibroblasts. In contrast, the CRBP-I expression in the proximal tubular epithelial cells decreases in the adriamycin-induced fibrotic rat kidney. CRBP-I expression is not found in the glomeruli of the adriamycin-induced fibrotic rat kidney. Some background staining, probably due to the serum presence, is observed in the capillaries. Myofibroblast-like cells around Bowman’s capsule are also positive for CRBP-I (Figure 2B and C). The specificity of the monoclonal CRBP-I antibody is shown by western blot detection of a GFP-CRBP-I fusion protein expressed in HEK293T cells (Figure 1B).

Mesangial cells and some podocytes in the glomeruli of healthy animals express desmin. In addition, interstitial fibroblasts and pericytes stain positive for desmin, whereas the epithelium of the tubules is negative (Figure 2D). The adriamycin-induced fibrotic rat kidney stains for desmin in the same pattern as in the healthy kidney while, due to the activation of myofibroblastic cell types, the number...
Fig. 2. Immunohistochemistry for CRBP-I (A–C): in the normal rat kidney strong expression of CRBP-I is observed in convoluted proximal tubular epithelial cells (A). In the fibrotic rat kidney diminished expression of CRBP-I is found in the convoluted proximal tubular epithelial cells, in contrast to the de novo expression of CRBP-I in (myo)fibroblast-like cells (arrowhead) located in the interstitium (B and C). Immunohistochemistry for desmin (D–F): in the normal rat kidney expression of desmin is observed in mesangial cells and in few podocytes (arrowhead) of the glomerulus; in the interstitium possible fibroblasts/pericytes are also stained (D). In the fibrotic rat kidney desmin is observed in the same cell types as in the normal kidney; the number of desmin-positive cells in the interstitium, however, is elevated due to the activation of fibroblastic cell types to myofibroblasts (E and F). Immunohistochemistry for vimentin (G–I): in the normal rat kidney vimentin expression is observed in podocytes and parietal epithelial cells (Bowman’s capsule) of the glomerulus; epithelial cells of the tubules do not show expression of vimentin; in the interstitium endothelial cells and fibroblasts both stained positive for vimentin (G). In the fibrotic rat kidney vimentin is observed in the same cell types as in the normal kidney; however, diseased epithelium of the tubules, atrophic, dedifferentiated tubular epithelial cells de novo express vimentin; in the interstitium endothelial cells, fibroblasts and myofibroblasts all are positive for vimentin (H and I). Immunohistochemistry for α-SMA (J–L): in the normal rat kidney expression of α-SMA is observed in arteries and arterioles (J). In the fibrotic rat kidney interstitial myofibroblasts are positive for α-SMA (K and L). Abbreviations: PTEC, proximal tubular epithelial cell; Int, interstitium; Glom, glomerulus; P, podocyte; BC, Bowman’s capsule.
of positive cells in the interstitium increases (Figure 2E and F).

Immunohistochemistry for vimentin shows positive staining in the mesangial cells, the podocytes and the parietal epithelial cells of Bowman's capsule in the glomeruli of the healthy rat kidney. The epithelial cells of the tubules are negative, while in the healthy interstitium endothelial cells and fibroblasts are immunoreactive for vimentin (Figure 2G). In the adriamycin-induced fibrotic rat kidney the glomeruli stain in the same pattern as in the healthy kidney; furthermore, dedifferentiated tubular epithelial cells are immunoreactive for vimentin. Endothelial cells, fibroblasts and myofibroblasts, in the renal interstitium of the adriamycin-treated rats, are all positive for vimentin (Figure 2H and I).

Smooth muscle cells of arteries and arterioles are immunoreactive for α-SMA in the healthy rat kidney (Figure 2J). The blood vessels remain positive for α-SMA in the adriamycin-induced fibrotic rat kidney. In addition, interstitial and periglomerular myofibroblasts acquire α-SMA expression in the adriamycin-treated rats (Figure 2K and L).

CRBP-I, a marker for myofibroblastic cells in renal fibrosis

To identify the interstitial cell type, which acquires CRBP-I expression during renal fibrogenesis, we performed double stainings. Immunofluorescence for CRBP-I in the adriamycin-induced fibrotic rat kidney shows that some proximal convoluted epithelial cells stay immunoreactive (Figure 3A and D). Around dilated proximal tubules we find the interstitial cell type, which acquires CRBP-I expression. In kidneys of adriamycin-treated rats, desmin stains myofibroblast-like cells in the interstitium (Figure 3B). Double immunofluorescent staining with desmin and CRBP-I (Figure 3C) reveals that 90% ± 4% of the CRBP-I-stained interstitial population is double positive for desmin. Of the desmin-stained interstitial population 75% ± 3% is double positive for CRBP-I. In adriamycin-treated rats, α-SMA stains myofibroblastic cells in the renal interstitium (Figure 3E). Double immunofluorescent staining with α-SMA and CRBP-I shows that 77% ± 4% of the CRBP-I-stained interstitial population is double positive for α-SMA. Of the α-SMA-stained interstitial population 55% ± 3% is double positive for CRBP-I.

This shows that in the renal interstitium of adriamycin-treated rats CRBP-I is co-expressed with desmin and α-SMA in a distinct subpopulation of myofibroblastic cells, suggesting that CRBP-I is a good marker for renal fibrosis.

Discussion

The development of renal fibrosis is a complex process depending on a range of cellular and molecular mediators. Cytokines, growth factors, signalling pathways and the renin–angiotensin system have all been implicated to play an important role in the progression of TI fibrosis leading to a functional decline of the kidney tissue. TI fibrosis, the common pathological pathway after renal injury, is characterized by the excessive accumulation of the extracellular matrix (ECM) and the activation of renal fibroblasts. Activated fibroblasts undergo a phenotypical change, which has been named myodifferentiation. The adriamycin nephropathy is an efficient model to induce chronic renal failure with glomerulosclerosis and TI fibrosis. Typical for this model are large protein casts in the tubules, proteinuria and arterial hypertension. Primarily the damage occurs at the level of the slit diaphragms of podocytes in the glomerular compartment. This defect in the filtration barrier explains the higher level of proteins in the tubules which in turn causes secondary injuries in the TI compartment. At
the molecular level, the p38 MAPK and TGF-β1/Smad2 signalling pathways are two reported profibrogenic pathways that have been implicated in the adriamycin-induced nephropathy [22]. Other inflammatory and profibrotic signalling pathways might play an additive role in the process of renal fibrosis. CRBP-I immunostaining allows for the identification of quiescent stellate cells present in the human liver along the sinusoids. The cellular body of the hepatic stellate cell stains positive, outlining unstained lipid droplets inside the cytoplasm [10]. Through UV-autofluorescence these perinuclear retinoid (vitamin A) droplets can be visualized. However, we have seen, as did Nagy et al. [15] and Kida et al. [17], that the UV-autofluorescence detection method does not give a positive result in the kidney of rats and mice fed a normal diet. This is probably due to the low sensitivity of this method. Similarly, in the fibrotic rat kidney no UV-autofluorescence is found. During human liver fibrogenesis, CRBP-I expression has been demonstrated in the matrix-producing cells (quiescent/activated stellate cells and myofibroblasts) of the liver [10,11]. Activation/myodifferentiation of hepatic stellate cells during liver fibrosis is accompanied by the loss of the characteristic vitamin A-containing lipid droplets [23]. This suggests that CRBP-I in these cells is not exclusively devoted to intracellular transport and esterification of retinol. Here, we investigated the expression of CRBP-I in a rat model of renal fibrosis. Our study confirms CRBP-I expression in the convoluted proximal tubular epithelial cells of the normal rat kidney [5,6]. No, or undetectable, levels of CRBP-I are observed in the healthy renal interstitium of rats. Nakatani et al. [16] and Kida et al. [17] showed by Cygh/STAP (cytoglobin/stellate cell activation-associated protein), which is expressed in murine and rat quiescent hepatic stellate cells, the presence of splanchnic fibroblast-like cells in the renal interstitium. In contrast, we do not find renal interstitial cell types, such as pericytes/stellate cells or fibroblasts, immunoreactive for CRBP-I in the healthy renal interstitium. This discrepancy might be due to the different embryological origin of the liver and kidney. Both tissues originate from mesenchyme; however, cells of the liver are derived from the intestinal mesenchyme, whereas the kidney cells are derived from the mesenchyme surrounding the ureteric bud. During adriamycin-induced renal fibrosis we could demonstrate the presence of activated interstitial fibroblasts (resident or haematopoetic derived), due to the co-localization of CRBP-I with desmin and α-SMA. We conclude that these cells are a subpopulation of myofibroblasts, as seen in the liver [10,11]. Earlier studies have also reported this up-regulation of CRBP-I in skin granulation myofibroblasts [24], in arterial smooth muscle cell activation [25] and TGF-β- and RA-stimulated cultured fibroblasts [26]. To our knowledge, this study demonstrates for the first time the same phenomenon during renal fibrogenesis. The intricate relation between CRBP-I expression and myodifferentiation might indicate a possible role for CRBP-I, and consequently retinoids, in tissue repair.

Furthermore, the appearance of vimentin and the reduced expression of CRBP-I in the convoluted proximal tubular epithelial cells during adriamycin-induced renal fibrosis might reflect the undifferentiated state of these cells. Previous in vivo and in vitro studies have shown that vimentin, an intermediate filament protein expressed by mesenchymal (derived) cells, is detectable in renal proximal tubular epithelial cells after sustained injuries such as unilateral ureteral obstruction [27], ischaemia [28], oxidative stress [29] and toxicological stress [30]. This phenotypical conversion of epithelial to mesenchymal cells during fibrogenesis and carcinogenesis has been defined as EMT (epithelial to mesenchymal transition) [31–34]. The disappearance of CRBP-I has been documented for certain epithelial cancers, where the differentiated attributes such as cell–cell and cell–matrix junctions of these epithelial cells are lost [35–37]. CRBP-I has been shown to act as a potent inhibitor, in a retinoic acid receptor–dependent manner, of the PI3K/Akt survival pathway [38]. It has been suggested that the down-regulation of CRBP-I, in many cancers and therefore maybe also in renal fibrosis, might be due to the hypermethylation of the CRBP-I gene promoter [39]. A correlation between human carcinogenesis and vitamin A/retinoic acid bioactivity has been proposed [35–37]. Further studies should be done to establish a similar correlation between vitamin A/retinoic acid bioactivity [40] and human fibrogenesis.

In conclusion, this study shows that during renal fibrosis there is a concomitant down-regulation of CRBP-I in the convoluted proximal tubular epithelial cells and appearance of CRBP-I expression in a subpopulation of interstitial matrix-producing myofibroblastic cells.

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References


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