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Relationship between vimentin expressing renal tubules and interstitial fibrosis in chronic progressive nephropathy in aged rats

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Abstract We investigated the relationship between regenerating renal tubular epithelial cells and myofibroblast development in chronic progressive nephropathy (CPN) of aged male F344 rats. We used established criteria to classify disease in rats with CPN as grade 1 (n=9), grade 2 (n=10), grade 3 (n=7) and grade 4 (n=4). Five young rats served as controls (grade 0). The ratio of fibrotic tissues per unit area, assessed in collagen type III-immunostained sections by morphometric analysis, increased significantly with advancing grade of CPN. Vimentin-expressing, regenerating renal tubules were found from grade 1 and continued to increase in number up to grade 3, decreasing slightly, however, in grade 4. Similar kinetics were seen for the number of α-smooth muscle actin-positive myofibroblasts, and there was a significant correlation between the number of regenerating renal tubules and myofibroblast development (correlation coefficient=0.83, P<0.01). The myofibroblasts developed in close association with the fibrotic areas seen in grades 1–4; the cells also reacted to desmin or vimentin, indicating the activated state. Immunohistochemistry for platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β revealed that vimentin-positive renal tubules were positive for PDGF-BB, but negative for TGF-β, and that interstitial reactive cells showed no positive reactions for both factors. The present studies on rat CPN showed that regenerating renal tubules may be a major source of a fibrogenic growth factor, PDGF-BB, and that the PDGF-BB might induce the development of fibrogenic cells, myofibroblasts, culminating in progressive interstitial fibrosis.

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Introduction

Renal interstitial fibrosis is a hallmark of progressive renal disease following renal injury and has been observed in kidneys of aged animals and humans [31, 36, 39, 59]. The pathogenesis of the fibrosis is complex and uncertain. Recent studies have shown that infiltrating macrophages and fibrogenic cells, myofibroblasts, are key cells in the progressive renal fibrosis and that there is a positive correlation between macrophage infiltration and the appearance of myofibroblast in experimental renal fibrogenesis [4, 14, 16, 27, 51]. Furthermore, some studies have suggested that degenerative or regenerative renal tubules might contribute to the development of renal interstitial fibrosis [13, 18, 28, 36]; this idea is based on findings showing that proximal renal tubular epithelial cells are capable of synthesizing collagen types I and III [10, 22] and that fibrogenic growth factors such as platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β might be secreted by injured renal tubular epithelial cells, resulting in proliferation of myofibroblasts producing extracellular matrix (ECM) [13, 22, 31]. However, the roles of renal tubules in association with interstitial fibrosis have not been fully elucidated.

Vimentin is an intermediate filament expressed mainly in cells of mesenchymal origin. In normal kidneys vimentin is detected in the epithelial cells and mesangial cells in the glomeruli, vessels and interstitial cells, but not in renal tubular epithelial cells [5]. However, vimentin expression has been observed in developing renal tubular epithelial cells [23], in neoplastic cells of renal tubular epithelial cell-derived tumours [53, 55], and in regenerating renal epithelial cells after injury [20, 40, 54, 56]. Thus, vimentin expression in renal tubules has been interpreted as a marker of cellular proliferation and dedifferentiation [49].

Chronic progressive nephropathy (CPN) is a well-known spontaneous lesion observed in various strains of rats [6, 8, 19, 30]. The CPN is characterized histologically by glomerular lesions, protein casts, degenerative or regenerative renal tubular lesions and interstitial fibrosis with mononuclear cell infiltration; such changes gradually progress with aging, and rats older than 24 months may develop the end-stage kidney leading to markedly impaired renal function. Rat CPN is a useful model for the investigation of chronic progressive fibrosis in humans.

To shed some light on the mechanisms behind renal fibrosis, in the present study we analysed rat CPN by immunohistochemistry, with a particular focus on the correlation between vimentin-positive, regenerating renal tubules and the appearance of myofibroblasts; in addition, immunohistochemical localizations for PDGF-BB and TGF-β were investigated with reference to vimentin-positive renal tubules and renal fibrogenesis.

Methods

Thirty male F344/DuCrj rats obtained at the age of 6 weeks from Charles River Japan were examined at the age of 24 months. Five rats aged 8 weeks served as controls. All animals were housed in a barrier-maintained room at a temperature of 24±1°C, 60±10% relative humidity, and a 12-h light–dark cycle and given a standard commercial laboratory diet for rats and tap water ad libitum.

Rats were killed with ether, and the kidneys were fixed in Methacarn solution (60% methanol, 30% chloroform and 10% acetic acid) for 3–4 h. Midsagittal sections were embedded in paraffin. Sections were cut at 4 μm thick and stained with haematoxylin and eosin (HE) and by Masson's trichrome method. According to criteria described previously [8, 30], we classed rat CPN as follows: grade 0, no lesions or minimal scattered tubular lesions; grade 1, focal glomerular basement membrane thickening and slight mesangial thickening in some glomeruli, and a few tubular basement membranes thickened; grade 2, multifocal areas of scattered dilated and atrophic tubules with thickened basement membranes; grade 3, glomerular and tubular lesions more pronounced, with atrophy and sclerosis, thickening of the capsule, cellular infiltration and mild interstitial fibrosis; grade 4, adhesions of the glomerular tufts to the wall, marked tubular dilatation with proteinaceous casts, more pronounced cellular infiltration and interstitial fibrosis. Rats examined in the present study were distributed by grade as follows: grade 0, 5 animals (controls); grade 1, 9 animals; grade 2, 10 animals; grade 3, 7 animals; grade 4, 4 animals.

The following primary antibodies were used for immunohistochemistry: anti-swine vimentin at a dilution of 1:400 (clone V9; Dako, Kyoto, Japan), anti-human α-smooth muscle actin (α-SMA) at a dilution of 1:400 (clone 1A4; Dako), anti-chicken desmin at a dilution of 1:800 (polyclonal rabbit; Dako), anti-rat collagen type III at a dilution of 1:100 (polyclonal rabbit; Chemicon International, Temecula, CA, USA), anti-proliferating cell nuclear antigen (PCNA) at a dilution of 1:200 (clone PC10; Dako), anti-human PDGF-BB at a dilution of 1:10 (polyclonal rabbit; Genzyme, Cambridge, MA, USA) and anti-bovine TGF-β at a dilution of 1:10 (monoclonal mouse IgG_1; Genzyme).

The immunohistochemical methods have been described in detail elsewhere [37]. Briefly, deparaffinized, Methacarn-fixed sections were incubated with 1% hydrogen peroxide in methanol for 15 min at room temperature (RT) to inactivate endogenous peroxidase and were then washed with phosphate-buffered saline (PBS). After incubation with 10% goat serum in PBS for 20 min at RT, sections were reacted with the aforementioned primary antibodies for 14 h at 4°C followed by incubation with secondary antibodies for 2 h at 37°C, and were subsequently reacted with avidin–biotin complex reagents (Vector Laboratories, Burlingame, CA, USA) for 30 min at RT. The secondary antibodies used were biotinylated goat anti-mouse IgG antibody (Vector) for monoclonal mouse antibodies against for vimentin, α-SMA, PCNA, TGF-β, and biotinylated goat anti-rabbit IgG antibody (Vector) for polyclonal rabbit antibodies against collagen type III, desmin, and PDGF-BB. After visualization with 3.3′-diaminobenzidine tetrahydrochloride as a substrate, sections were counterstained with haematoxylin. Non-immunized mouse and rabbit sera, which were processed instead of the primary antibody, served as negative controls. To confirm the specificity of immunostaining with the polyclonal PDGF-BB antibody described above, we used an additional PDGF-BB antibody (monoclonal mouse IgG_2b; Upstate Biotechnology, Lake Placid, NY, USA), and both antibodies to PDGF-BB showed identical immunostaining patterns. Rat fetal lung tissues, which are known to express PDGF-BB immunopositive reaction [21], served as positive controls.

Vimentin-positive renal tubules were determined by counting the number of luminal cross sections that showed vimentin labelling of more than half the epithelial cells; the counts were conducted in the cortex and the outer stripe of the medulla at a magnification of ×100. α-SMA-positive interstitial cells were counted at a magnification of ×400 throughout the cortex and the outer stripe of the medulla. To evaluate renal interstitial fibrosis, the collagen type III-immunolabelled area was quantitated by computer-aided morphometry using a colour image analyser (MacSCOPE; Mitani, Tokyo, Japan) in ten randomly selected areas (0.5 mm^2 each) of the cortex and outer stripe of the medulla [37, 58]. The fibrotic rate was presented as the percentage of collagen immunolabelling area per unit area. Glomeruli and vascular structures were excluded from these analyses.

Small blocks of the cortical area in CPN were fixed in 2.5% glutaraldehyde in cacodylate buffer, post-fixed in 1% osmium tetroxide, and then embedded in epoxy resin (Nisshin EM, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Hitachi H-500 transmission electron microscopy.

The numbers of vimentin-positive renal tubules and α-SMA-positive cells, and the fibrotic rate were expressed as the group mean±standard deviation for each grade of CPN. Student's *t*-test was used to compare the control (grade 0) with the other grades. A *P*-value smaller than 0.05 was considered significant. The relationship between the number of vimentin-positive renal tubules and the number of α-SMA-positive cells was evaluated using the correlation coefficient.

Results

As described previously [5], in all rats examined glomerular epithelia and vascular endothelia were reactive for vimentin. In grade 0 CPN, a few renal tubules lined with basophilic epithelial cells reacted faintly to vimentin [6, 7], but the majority of renal tubules were negative for vimentin (Fig. 1). In contrast, vimentin-positive renal epithelial cells were frequently seen in grades 1–4 CPN; the positive reactions were localized mainly in epithelial cells lining dilated or atrophic proximal tubules and collecting ducts with thickened basement membrane (Fig. 1). The vimentin staining developed in the cytoplasm, often in the basal portion of the epithelial cells. In the vimentin-positive renal tubules, many epithelial cells gave a positive reaction to PCNA, indicating proliferating, regenerating cells (Fig. 2). As shown in Fig. 3, the number of vimentin-positive renal tubules increased with advancing grade of CPN, reaching a peak in grade 3 CPN; however, in grade 4 CPN (the most advanced

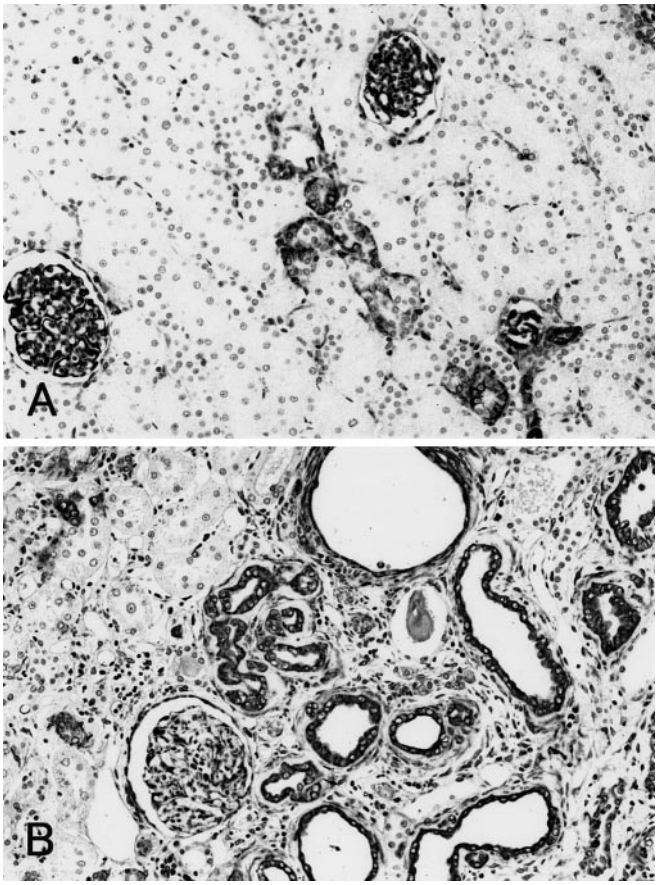

Fig. 1A, B Vimentin expression of renal tubules in control (8-week-old) and aged rats. **A** In control kidney [progressive chronic nephropathy (CPN), grade 0], glomerular epithelia, interstitial cells and vessels and occasional renal tubules are positive for vimentin. **B** In contrast, most of regenerating renal tubular epithelial cells react to vimentin in grade 3 CPN of an aged rat. ×120

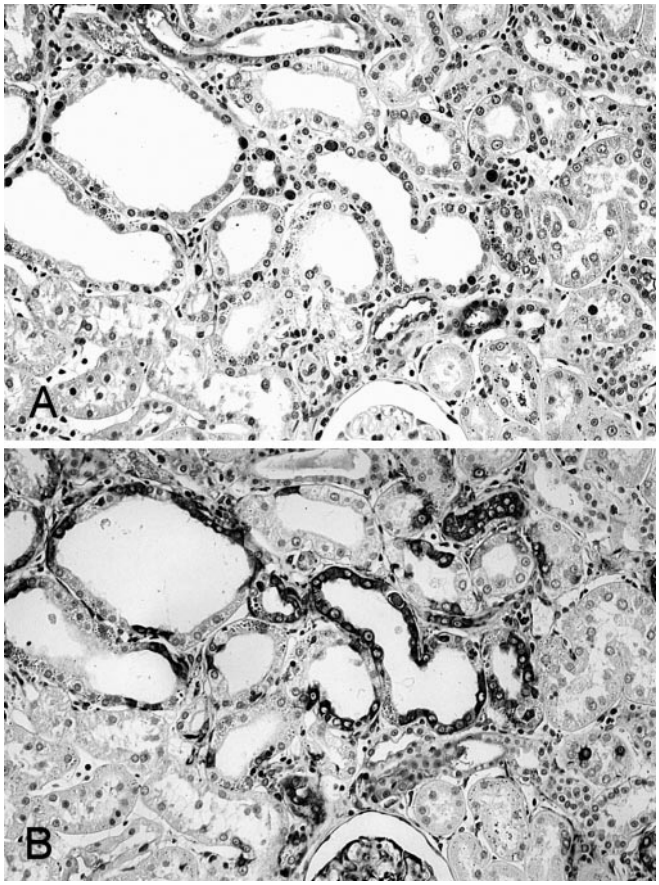

Fig. 2A, B Immunoreactivities for proliferating cell nuclear antigen (PCNA) and vimentin on serial sections of grade 3 CPN. **A** Many epithelial cells lining dilated or atrophic renal tubules give a positive reaction to PCNA, indicating active proliferation (dark nuclei). **B** These cells correspond to vimentin-positive, regenerating renal epithelial cells. ×140

stage), the number was slightly lower than in grade 3 CPN.

Smooth muscle cells constituting blood vessels were positive for α-SMA in CPN of all grades, indicating the specificity of this staining. An antibody against α-SMA was used for identification of myofibroblasts [12, 42, 44]. In grades 1–4 CPN, α-SMA-positive cells were found in the tubulointerstitial fibrotic areas as well in the surroundings of the sclerotic glomeruli. The positive cells in the fibrotic areas were located mainly around the vimentin-positive renal tubules, surrounding the basement membrane of the tubules; the cells often showed filamentous reactions in their cytoplasm. These α-SMA-positive cells also reacted to desmin or vimentin, indicating activated myofibroblasts (Fig. 4). Electron microscopy showed that the myofibroblastic cells had characteristically long myofilament bundles arranged parallel to the plasma membrane(Fig. 5) and possessed well-developed rough endoplasmic reticulum and some mitochondria. Many collagen fibres were seen adjacent to the cells.

The number of α-SMA-positive cells increased with advancing degree of CPN, reaching a peak in grade 3.

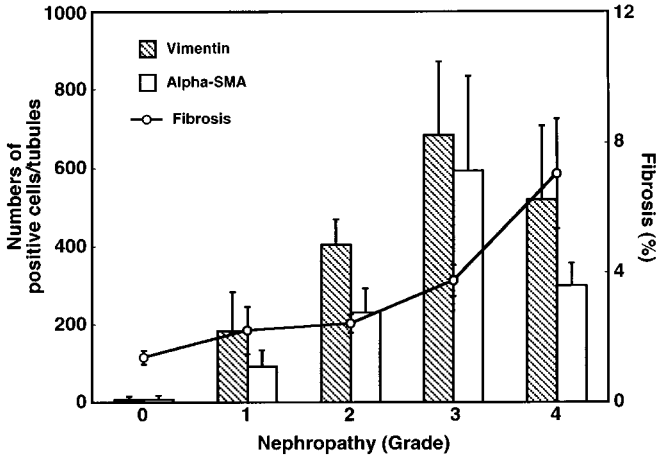

Fig. 3 Numbers of vimentin-positive renal tubules and α-smooth muscle actin (α-SMA)-positive myofibroblasts, and the percentage fibrosis in CPN of various grades. Control rats represented as grade 0 CPN. Both vimentin-positive tubules and α-SMA-positive cells were counted in the defined area (see 'Methods'). Mean ±SD

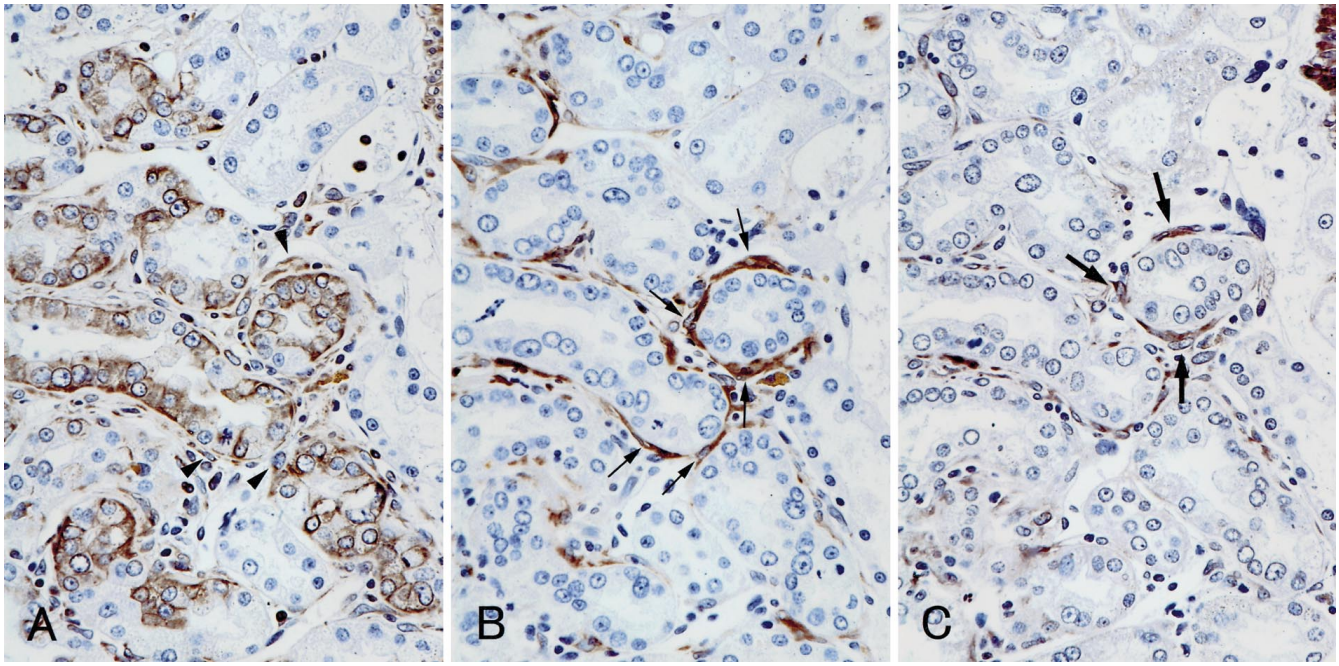

Fig. 4 Immunoreactivities for **A** vimentin, **B** α-SMA, and **C** desmin on serial sections of grade 2 CPN. α-SMA-positive myofibroblasts (*thin arrows*) are seen surrounding vimentin-positive renal tubules. These cells also react to desmin (*thick arrows*) or vimentin (*arrowheads*). ×240

However, in grade 4 CPN, the number was slightly lower than in grade 3 CPN (Fig. 3).

Interstitial fibrosis in CPN was assessed by collagen type III immunostaining. The fibrotic areas seen in grades 1–4 CPN were stained blue by Masson's trichrome method, indicating deposition of collagen fibres. In the initial stages of collagen deposition and fibril formation, collagen type III appears in greater amounts than collagen type I [31, 59]. Thus, we assessed development of the fibrotic areas in collagen type III-immunostained sections by morphometric analysis. As shown in Fig. 3, compared with grade 0 CPN, the percentage of fibrotic tissue per unit area increased significantly with increasing grade of CPN; grade 4 CPN had the greatest fibrotic area. In grades 1–4 CPN, the immunoreactivity for collagen type III was seen in the tubulointerstitia and periglomerular areas (Fig. 6). In the tubulointerstitia, the intense reactivity was present exclusively adjacent to the dilated or atrophic renal tubules expressing vimentin. In

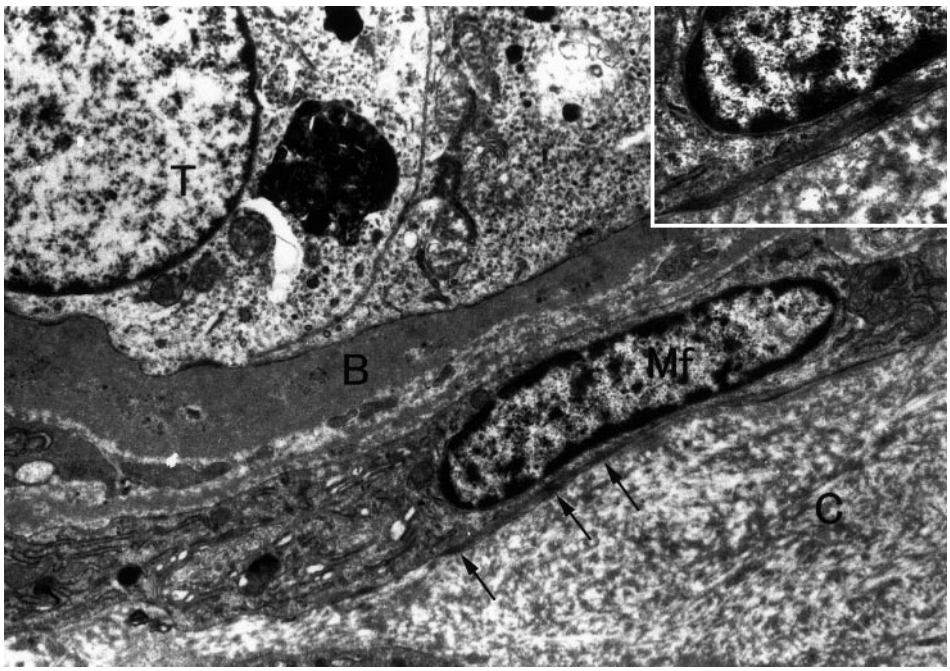

Fig. 5 Electron micrograph of a myofibroblastic cell adjacent to a renal tubular epithelial cell in grade 3 CPN. Myofilament bundles (*arrows*) are characteristically seen in the cytoplasm. (*T* regenerating tubular cell, *B* thickened tubular basement membrane, *Mf* myofibroblastic cell, *C* collagen fibres). ×8, 800 **Inset** higher power view of myofilament bundles

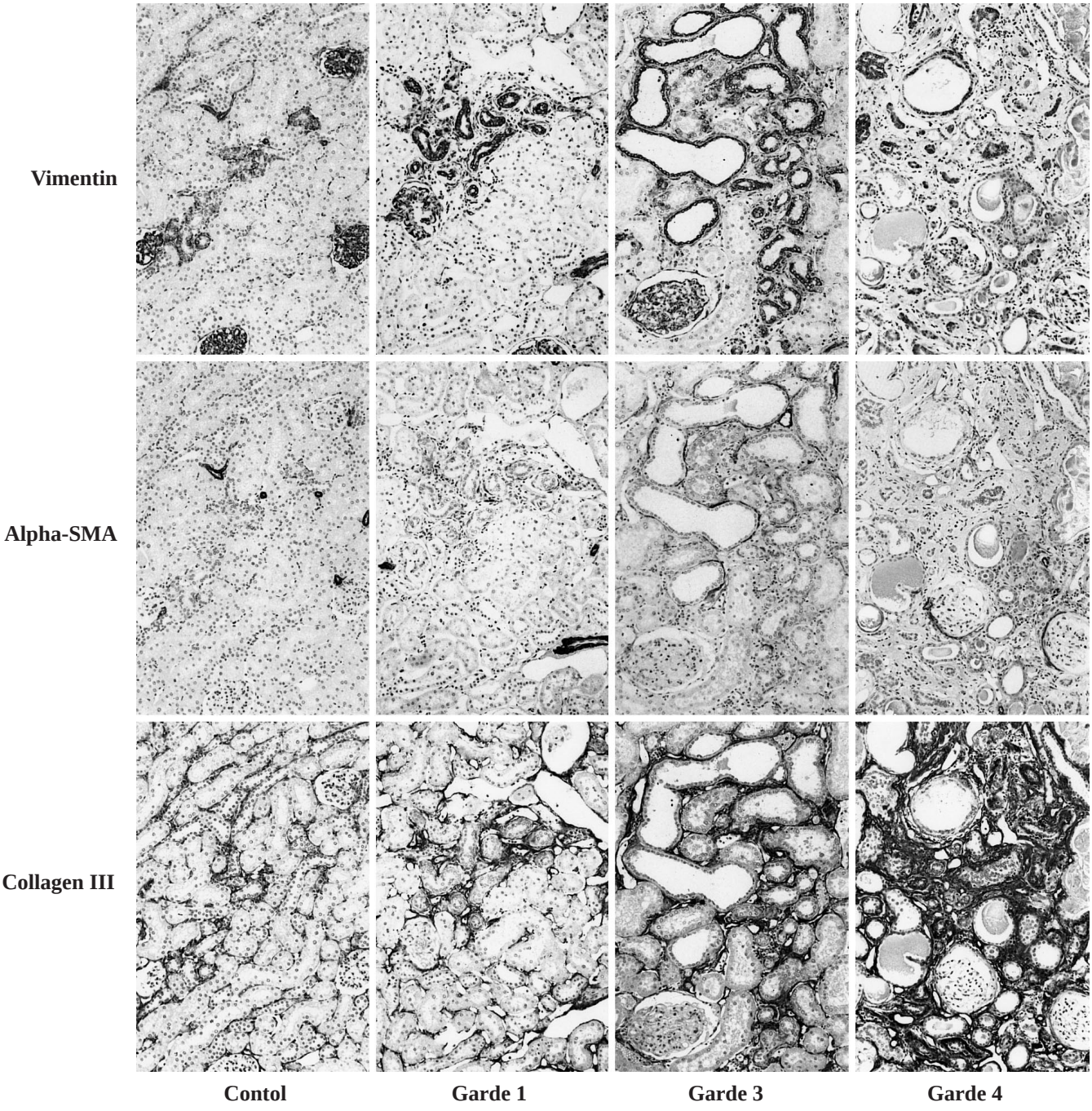

Fig. 6 Immunoreactivities for vimentin, α-SMA, and collagen type III on serial sections of grades 0, 1, 3 and 4 CPN. Immunoreactivities for vimentin, α-SMA, and collagen type III are markedly increased in grades 1, 3 and 4, as compared with grade 0. However, in grade 4, regardless of the decreased expressions of vimentin and α-SMA, staining intensity for collagen type III appears to be increased. ×60

grade 0 CPN there was simply a small number of type III collagen fibres in the interstitia.

As described above and shown in Fig. 3, in grades 1–3 CPN the increased number of α-SMA-positive cells was closely related to increased number of vimentin-positive renal tubules, and in grade 4 CPN the numbers of both declined. There was, then, a highly significant correlation between the number of α-SMA-positive cells and the number of vimentin-positive renal tubules (correlation coefficient=0.83, $P<0.01$; Fig. 7). In conformity with the increased number of α-SMA-positive cells the collagen type III accumulation was increased in grades 1–3 CPN, but in grade 4 CPN, regardless of the decreased number of α-SMA-positive cells the collagen accumulation was still increased. Grade 4 CPN appears to be the end-stage of contracted kidneys developed through scar formation, consisting in increased ECM and decreased number of cellular components [30].

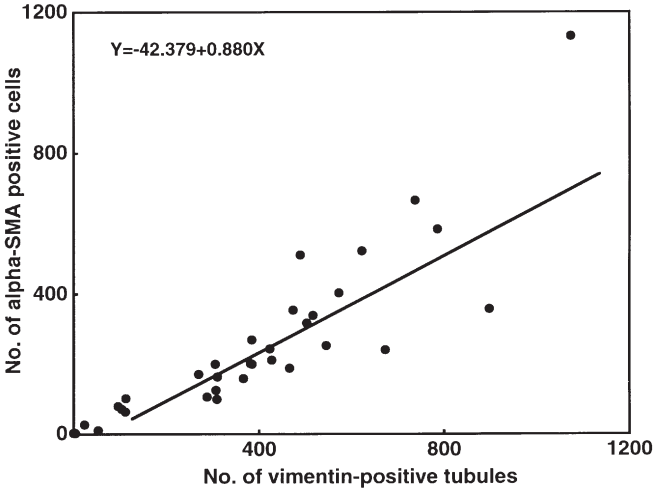

Fig. 7 Correlation between α-SMA-positive myofibroblasts and vimentin-positive renal tubules in rat CPN. Both vimentin-positive tubules and α-SMA-positive cells were counted in the defined area (see 'Methods'). Each *dot* represents an individual animal. Correlation coefficient=0.83 ($P<0.01$)

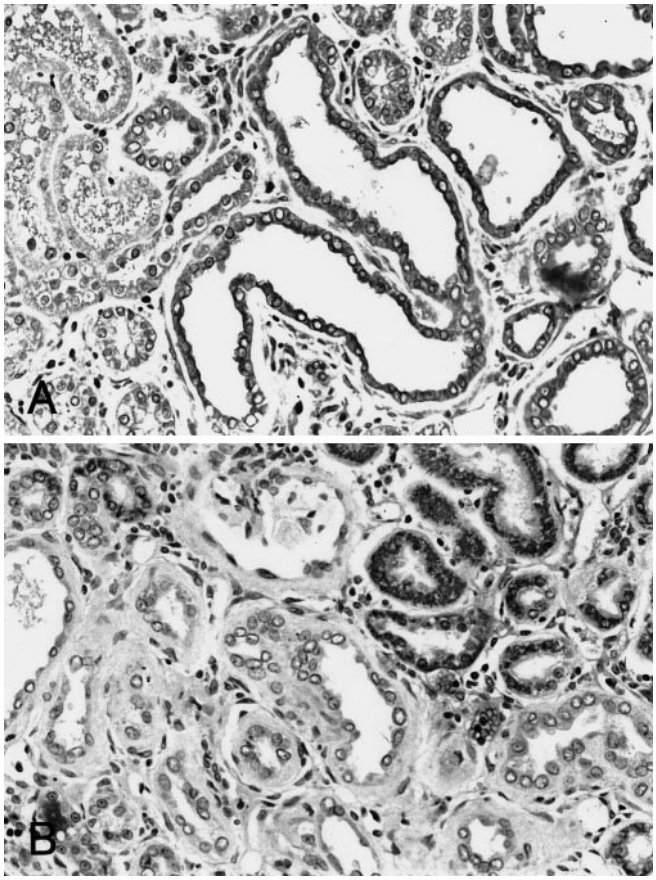

Fig. 8A, B Immunoreactivities for platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β in grade 3 CPN. **A** PDGF-BB expression is seen intensely in dilated or atrophic regenerating renal tubules, whereas **B** no reactivity for TGF-β is observed in regenerating renal tubules. However, occasional cortical renal tubules with normal structures are reactive for TGF-β. ×180

In grades 1–4 CPN, intense immunostaining for PDGF-BB was localized in the epithelial cells lining dilated or atrophic renal tubules with thickened basement membrane (Fig. 8). Intact proximal tubules and apparently normal tubules with slightly thickened basement membranes were weakly or occasionally positive for PDGF-BB. These PDGF-BB-positive renal tubules were corresponding to vimentin-positive renal tubules. TGF-β expression was found in normal cortical tubular cells of all grades, including control (grade 0), whereas no reaction for TGF-β was detected in dilated or atrophic renal tubules in cortical areas of grades 1–4 CPN (Fig. 8). Interstitial reactive cells in CPN of all grades were negative for PDGF-BB and TGF-β.

Discussion

The pathogenesis of rat CPN appears to be related to protein leakage caused by increased porosity of the basement membrane [6, 19]. In the renal tubules, degenerative epithelial cells are sloughed from the basement membrane and fall into the lumina; thereafter, the tubules are lined with regenerating epithelial cells with basophilic cytoplasm. The regenerating epithelial cells in proximal tubules and collecting ducts are immunopositive for vimentin [20, 35, 40, 54, 56], and show proliferating activity by bromodeoxyuridine [30, 32] and PCNA immunostaining. In progressive tubular injury and following fibrosis, tubuloglomerular feedback has been assumed to play a central role in the transition from acute to chronic renal injury [7, 28, 59]; in addition to continuing exposure to an aetiologic agent, presumably dietary proteins, tubular damage can lead to loss of glomerular functions and subsequent structural changes, and conversely glomerular changes can accelerate renal tubular damage. The increased number of vimentin-positive renal tubules with increasing degrees of CPN suggest that degenerative or regenerative changes occur repeatedly in nephrons throughout life. As a result, glomerulosclerosis and interstitial fibrosis appear to develop progressively in rat CPN. Previous studies on rat CPN have concentrated mainly on glomerulosclerosis [8, 9, 19]. However, the mechanisms of the interstitial fibrosis are unclear. Our morphometric analysis showed that with advancing grade of CPN, collagen type III accumulated abnormally in the fibrotic areas. Furthermore, we have confirmed abnormal increases in collagen type I, collagen type IV and fibronectin in advanced CPN [38]. Similar increases in such ECM components have been reported in hydronephrosis and interstitial fibrosis induced by nephrotoxic chemicals [26, 27, 46, 57]. Rat CPN may be a useful model for chronically developed renal fibrosis.

Studies on hydronephrosis models caused by ureteral obstruction and chemical-induced renal fibrosis have demonstrated that α-SMA-positive myofibroblasts have a crucial role in renal interstitial fibrosis [13, 14, 24, 58]. In the present study, with an increase in the fibrotic tissues in CPN the number of α-SMA-positive myofibro-

blasts was significantly increased, indicating that myofibroblasts are also related to the progression of fibrosis in rat CPN. On electron microscopy, the cells were characterized by some myofilament bundles and well-developed rough endoplasmic reticulum. Similar cells have been reported in hepatic [25], pulmonary [2] and cardiac fibrosis [37, 52] and dermal wound healing [12, 44]. It has been well established that myofibroblasts may show an heterogeneous cytoskeletal repertoire in pathological settings [42, 44]. In the fibrotic areas of CPN, myofibroblasts also expressed vimentin and desmin; this indicates an activated state capable of producing ECM [42, 44].

Some studies have suggested that renal tubules may participate in interstitial fibrosis in response to injury [13, 18, 28, 36]. Therefore, we focused on the relationship of regenerating renal tubules to myofibroblasts. Interestingly, we found a significant correlation between vimentin-positive renal tubules and α-SMA-positive myofibroblast development (Fig. 7). In addition, we found that myofibroblasts appeared exclusively around vimentin-positive renal tubules. It has been suggested that fibrogenic factors released by infiltrating macrophages in injured areas might induce the modulation of pre-existing fibroblasts to myofibroblasts, leading to fibrosis [41–43, 45]. We investigated a possible production of TGF-β and PDGF-BB by regenerating renal tubular epithelial cells by immunohistochemistry and found TGF-β in the cytoplasm of normal cortical renal tubular epithelial cells, but not in vimentin-positive, regenerating renal tubules. In contrast, intense immunoreactivity of PDGF-BB were detected in the vimentin-positive, dilated and atrophic renal tubules, while renal tubules of a normal appearance though with slightly thickened basement membrane showed weakly or occasionally positive reaction for PDGF-BB. These PDGF-BB-positive renal tubules also demonstrated positive reactions to PCNA, indicating active tubular proliferation. PDGF-BB has been reported to be produced by renal tubular epithelial cells as well as infiltrating macrophages and platelets in diseased kidneys [3, 24, 29]. Tang et al. demonstrated in rat kidneys that exogenous administration of PDGF-BB induces the proliferation of renal tubulointerstitial myofibroblasts [48]. In angiotensin II-mediated renal injury in rats, tubulointerstitial fibrosis, collagen accumulation and tubular cell proliferation have been associated with increased PDGF-BB expression [24]. PDGF-BB is a potent mitogen and chemoattractant of interstitial fibroblasts and may be an important mediator of renal fibrosis [1, 24, 48]. Moreover, it has been reported that renal interstitial cells have PDGF receptors [3], and that the number of the receptors was increased in pathological conditions [17]. In tubulointerstitial fibrosis in a 5/6-nephrectomy model, Kliem et al. demonstrated that PDGF-B chain mRNA and both protein expression and its receptor was markedly increased in renal tubules, which associated with increasing tubular proliferative activity [29]. On the basis of these findings, we assumed that PDGF-BB expression was initiated at an early stage of tubular proliferation, that the expression was then increased in association with tubular proliferation/differentiation, and that vimentin-positive renal tubular epithelial cells might be a major source of PDGF-BB capable of inducing myofibroblast development. To specify the source of PDGF-BB, further investigations should be conducted with in situ hybridization studies.

In experimental rat hydronephrosis, there was a close relationship between the appearance of interstitial macrophages and TGF-β gene expression, and immunolocalization for TGF-β was found exclusively on peritubular mononuclear cells, apparently macrophages [15]. We failed to demonstrate TGF-β immunoreactivity on infiltrating macrophages in grades 1–4 CPN, although the number of macrophages demonstrable with ED1 (rat macrophage-specific antibody) immunostaining was very small [38]. Analogous negative staining for TGF-β in interstitial infiltrates has been shown in chronic ureteral obstruction [57]. There might be some differences in the functions of infiltrating macrophages between the acute phase of experimental renal fibrosis and chronic fibrosis in CPN. The possible contribution of TGF-β to interstitial fibrosis in rat CPN remain to be determined. As described above, further, TGF-β was not detected in the vimentin-positive, regenerating renal tubular epithelial cells. This cytokine has been shown to inhibit cell proliferation, especially by preventing resting-stage cells from entering the cell cycle [11, 34]. These findings imply that, with regard to tubular regeneration, the disappearance of TGF-β in the vimentin-positive tubular cells relieves the inhibition of cell proliferation, while production of PDGF-BB promotes cell proliferation via an autocrine pathway [50].

In grade 4 (end-stage kidney), the numbers of vimentin-positive tubules and α-SMA-positive cells were decreased, whereas the fibrotic rate demonstrable on morphometric analysis was increased. In the end-stage kidney, because almost all areas of the kidney were damaged owing to the advanced glomerulosclerosis, collapse and increased interstitial fibrosis, the regenerative capacity of renal tubules appeared to be reduced. Konishi et al. demonstrated that DNA synthesis in the tubular epithelium increased with the degree of CPN, whereas the synthesis decreased in the end-stage with progressive tubular injury and interstitial fibrosis [30]. They also indicated that interstitial cells (fibroblasts and endothelial cells) reduced DNA synthesis in the end-stage kidney [30]. This appears to support our findings that the regenerative tubules and the myofibroblasts were reduced in the end-stage of the rat CPN. Recent studies have demonstrated that the process of fibrosis represents an imbalance between ECM protein generation and degradation [15, 27, 47]. In addition to overproduction of ECM by interstitial cells, decreased ECM degradation by suppression of metalloproteinase activities or activation of tissue inhibitors of metalloproteinases or both contribute to excessive ECM accumulation leading to fibrosis. Therefore, severe fibrosis in grade 4 rat CPN may be associated partly with an imbalance in ECM metabolism.

In conclusion, α-SMA-positive myofibroblasts participate in interstitial fibrosis in rat CPN and there is a sig-

nificant correlation between the vimentin-positive, regenerating renal tubules and the appearance of myofibroblasts in fibrogenesis. The regenerating renal epithelial cells are immunopositive for PDGF-BB but not for TGF-β, indicating a possible participation of PDGF-BB in proliferation of myofibroblasts. Our findings suggest that repeated pathological change (degeneration and regeneration) in nephrons is the most important event for development of interstitial fibrosis in rat CPN.

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