Enhanced interstitial expression of caldesmon in IgA nephropathy and its suppression by glucocorticoid–heparin therapy

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Abstract

Background. With progressive renal disease, structural derangement increasingly encompasses the tubulointerstitial compartment. Tubulointerstitial injury is a critical determinant of renal functional reserve and prognosis in renal disease. Interstitial cells acquiring characteristic of myofibroblasts are an important contributor to interstitial fibrosis. Caldesmon, a calmodulin or actin binding protein, is a molecular marker of differentiation in smooth muscle cells and has recently been shown by us to be a good marker of mesangial cell activation in IgA nephropathy patients.

Methods. We studied whether the expression of caldesmon in interstitium of the kidney was enhanced in the process of glomerular disease and whether it would be a marker of interstitial activation in specific disease states. We performed immunohistochemical staining with anti-caldesmon antibodies in 38 biopsy specimens from IgA nephropathy patients and analysed them quantitatively with a computer-aided manipulator. Interstitial caldesmon expression was compared with histological changes and clinical parameters.

Results. Caldesmon expression was enhanced where renal function has been shown to correlate better with interstitial cell infiltration. Follow-up of these patients (average 24 months) revealed a significant suppression of urinary protein excretion and significant improvement of creatinine clearance.

Conclusion. These results suggest that the interstitial caldesmon expression is associated with the progression of IgA nephropathy, and glucocorticoid–heparin therapy may reverse the phenotypic change of interstitial cells during the disease process of glomerulonephritis.

Key words: caldesmon; glucocorticoid–heparin therapy; IgA nephropathy; interstitial cell; myofibroblast; phenotypic change

Introduction

In the progression of renal disease, interstitial fibrosis is found regardless of whether the primary injury is of glomerular or tubulointerstitial origin. The decline in renal function has been shown to correlate better with tubulointerstitial changes than with glomerular damage per se. [1,2]. Thus, tubulointerstitial changes are thought to be critical determinant of renal functional reserve and prognosis in glomerular disease. There is increasing evidence that interstitial cells are activated during initial injury and undergo a variety of phenotypic changes as characterized by alteration of cell morphology, acquisition of proliferative phenotype, an increased synthesis of extracellular matrix and an enhanced secretion of growth factors such as platelet-derived growth factor and transforming growth factor-β [3]. Recent findings demonstrated that the enhanced expression of α-smooth muscle actin is a marker of interstitial phenotypic change in various forms of glomerular disease and these activated interstitial cells are called ‘myofibroblasts’ [4]. The myofibroblast is a cell...
phenotype with features of both fibroblasts and smooth muscle cells. A pivotal role for myofibroblasts has been identified in fibrogenesis in non-renal tissues, including skin wound healing, hepatic fibrosis and pulmonary fibrosis [5]. Appearance of myofibroblasts may also play a key role in the progression of renal fibrosis in both glomerular and interstitial lesions.

Caldesmon is a major calmodulin- and actin-binding protein found in smooth muscle and non-muscle cells [6]. It plays a vital role in the Ca^{2+}-dependent regulation of smooth muscle and non-muscle contraction [7]. The two caldesmon isoforms (h-caldesmon and l-caldesmon) resulting from an alternative splicing have been identified. While h-caldesmon is dominantly expressed in differentiated smooth muscle cells, l-caldesmon is widely distributed in non-muscle cells. In particular, the isoformal interconversion of caldesmon is tightly associated with the phenotypic modulation of smooth muscle cells, in which the caldesmon isoform converts from the l- to the h-form during differentiation and vice versa [8,9]. Caldesmon is therefore a favourable molecular marker for studying the phenotypic modulation of smooth muscle cells. Our recent study in human IgA nephropathy has demonstrated that the glomerular expression of caldesmon is enhanced in mesangial lesion, and that the quantification of the caldesmon immunostaining revealed an association between caldesmon expression and mesangial proliferation in IgA nephropathy [10]. These results demonstrate that the enhanced expression of caldesmon is a feature of the phenotypic change in glomerular mesangial cells during the progression of proliferative glomerulonephritis.

IgA nephropathy, the most common glomerular disease in Japan, occurs in 40–50% of primary glomerulonephritis, and 40% of IgA nephropathy patients progress to end-stage renal failure. In the present study, to determine if caldesmon expression is associated with the phenotypic changes of interstitial cells in the progressive renal tubulointerstitial fibrosis, we investigated the interstitial expression of caldesmon in human IgA nephropathy kidneys by immunohistochemical studies, using monoclonal antibodies. We found a positive association between the interstitial expression of caldesmon and the pathological changes of the interstitium. Furthermore, caldesmon expression was decreased in concomitant with decreased interstitial cell infiltration after glucocorticoid and heparin therapy. Thus, caldesmon is a reliable marker of the phenotypic changes of interstitial cells in the process of the disease progression of glomerulonephritis.

Subjects and methods

Patients and tissue samples

Tissue samples were obtained by renal biopsy of 38 patients with IgA nephropathy over a 2-year period (April 1995 to October 1996 at Osaka University Hospital). Normal human kidney tissues (n = 5) were obtained from kidneys that were surgically excised because of the presence of a localized neoplasm. The peripheral normal tissues were used for this study as the controls.

Therapy and re-biopsy

To investigate the effects of glucocorticoid (40 mg/day) and continuous heparin infusion (10 000–25 000 units/day) therapy, re-biopsy was performed after the therapy (4–8 weeks). The target dose of heparin administration was set as activated partial thromboplastin time (APTT) prolonged to twice those before the therapy. This protocol of the therapy and re-biopsy was evaluated and approved by the committee of Department of Internal Medicine and Therapeutics, Osaka University School of Medicine. We recommended this protocol to every patient except those who had minor histological changes or advanced glomerular changes. Among the 38 cases studied, therapy and re-biopsies were performed in 15 cases who gave consent to this protocol. Written informed consents were obtained from all patients who entered this therapy and re-biopsy protocol. Follow-up information (mean 24 months) was available from 14 patients among these 15 patients.

Fixation

All tissues except for those of immunoelectron microscopy were fixed in formalin and processed and embedded in paraffin, utilizing conventional techniques. Tissue blocks were stored at 4°C until use. Four-micrometre-thick serial sections were cut for the immunohistochemical studies as well as for the conventional periodic acid–Schiff (PAS) staining and Masson’s trichrome staining.

Immunohistochemistry

Paraffin-embedded sections were deparaffinized with xylene and graded ethanol, then only the sections that would be reacted with anti-caldesmon antibody were heated in an 800-W microwave oven for 15 min. Next they were exposed to 3% hydrogen peroxide in methanol for 5 min to inhibit the endogenous peroxidase activity, and then treated with horse serum diluted 1:20 with saline for 20 min to block the non-specific staining. The sections were then incubated with one of the primary mouse monoclonal antibodies for 60 min at room temperature and subsequently processed using an avidin–biotinylated peroxidase complex method (Vectastain ABC kit, Vector Laboratories, Inc., Burlingame, CA, USA) with diaminobenzidine as the chromogen. Sections were counterstained with methyl green or haematoxylin.

Antibodies

Three mouse monoclonal antibodies were used for immunohistochemical evaluation. Mouse IgG3 anti-caldesmon monoclonal antibody, which recognizes both low- and high-molecular-weight isoforms of caldesmon, was obtained from YLEM (Roma, Italy) and was used in 1:100 dilution. Mouse IgG3 anti-α-SMA monoclonal antibodies were from Immunotech SA, France. Mouse IgG3, CD68 monoclonal antibodies which recognized human macrophages were from Dako (Glostrup, Denmark).
Interstial immunoperoxidase staining for caldesmon and \( \alpha \)-SMA in each section was quantified by a computer-aided manipulator (Quantimet 500 Leica K.K Tokyo Japan). The results were expressed as a fractional volume (Fv) according to the following formula. These analyses were performed by one of the authors, in a blind fashion.

Fractional volume (Fv \( \% \)) = \( \frac{\text{area of positive staining}}{\text{area of whole field}} \times 100 \)

Each area was measured quantitatively by a computer-aided manipulator at a final magnification of \( \times 400 \). In each section non-overlapping 10 fields were selected at random and then evaluated. The individual Fv was obtained as the average Fv of these 10 fields in each section.

Glomerular caldesmon expression was also examined, and the caldesmon score in each glomerulus selected at random was calculated by the following method.

Glomerular caldesmon score \( (\% ) = \frac{\text{caldesmon area}}{\text{whole glomerular area}} \times 100 \)

The whole glomerular area was measured by tracking out the glomerular tuft. The caldesmon area which reacted to anti-caldesmon antibody was measured in the glomerular area. In each section, eight glomeruli longer than 160 \( \mu \text{m} \) in diameter were selected at random and then evaluated. The individual score was obtained as the average score of these glomeruli in each section.

As to evaluation of CD68 staining, all patients were divided into two groups, low CD68 positive cells infiltrated group and intense CD68-infiltrated group. The low CD68 group was defined as less than 5 CD68-positive cells/100 cells in the interstitium. The intense CD68 group was defined as five or more CD68-positive cells/100 cells in the interstitium.

**Analysis of histology by light microscopy**

Interstitial mononuclear cell infiltration and interstitial fibrosis were evaluated semiquantitatively as follows. Interstitial mononuclear cell infiltration was scored as \( 0-3 \) ( \( 0\% = 0, \ 0-20\% = 1, \ 20-50\% = 2, \ > 50\% = 3 \)). Interstitial fibrosis was evaluated by Masson’s trichrome-stained sections, and was scored as \( 0-3 \) ( \( 0\% = 0, \ 0-20\% = 1, \ 20-50\% = 2, \ > 50\% = 3 \)).

**Immunoelectron microscopy**

Blocks were post-fixed in Zamboni’s solution containing 0.05% glutaraldehyde and immersed in a 30% sucrose solution in 0.1 M phosphate buffer (pH 7.40) (PB) overnight until they sank. Then the blocks were frozen in liquid nitrogen for 5–10 s, thawed in PB, and 40-\( \mu \text{m} \) thick sections were cut on a vibratome. These sections were processed for immunoelectron microscopy by pre-embedding method described in a previous paper [11]. An avidin—biotinylated peroxidase complex method was used on the labelling technique.

**Evaluation of clinical data**

Urinary protein excretion (g/day), serum creatinine (mg/dl), creatinine clearance (ml/min), systolic blood pressure (mmHg), serum IgA (mg/dl), and age at the renal biopsy were analysed. Urinary protein excretion and creatinine clearance were also evaluated after 2-year follow-up.

**Statistical analysis**

Values are expressed as the mean ± standard deviation. A comparison of the caldesmon score between the normal control and IgA nephropathy was performed using the Mann–Whitney U test. A comparison of caldesmon expression with histological change (interstitial mononuclear cell infiltration and fibrosis) was performed using ANOVA. A comparison of the Fv of caldesmon between the low-CD68 group and the intense-CD68 group was performed using the Mann–Whitney U test. The correlation of the Fv of caldesmon and the Fv of \( \alpha \)-SMA was performed using Spearman’s rank correlation test. The correlation of the glomerular caldesmon score and the interstitial Fv of caldesmon was performed using Spearman’s rank correlation test. The comparison of the clinical data between the high-intensity and low-intensity groups was performed using the Mann–Whitney U test. A comparison of each score between before and after glucocorticoid-heparin therapy was performed using the Wilcoxon signed-ranks test. A comparison of the changes in Fv of caldesmon between non-responder (patients with unchanged interstitial cell infiltration) and responder (patients with decreased interstitial cell infiltration) was performed using the Mann–Whitney U test.

**Results**

**Expression of caldesmon in normal and IgA nephropathy kidneys**

The distribution of Fv of caldesmon in individual patients for caldesmon immunoreactivity in all cases is given in Figure 1. In normal controls the interstitial caldesmon expression was weak. None of the normal kidneys showed prominent interstitial caldesmon expression. On the other hand, the extent of interstitial

![Graph](https://via.placeholder.com/150)

**Fig. 1.** A comparison of the Fv of caldesmon between the normal control group \( (n = 5) \) and the IgA nephropathy group \( (n = 38) \). Each dot shows the individual caldesmon score. There is a statistically significant difference between the two groups \( (P<0.01) \).
caldesmon expression in IgA nephropathy kidneys were distributed in a wide range as shown in Figures 1 and 2. IgA nephropathy patients had significantly higher Fv of caldesmon than normal controls. Enhanced interstitial caldesmon expression was virtually limited to the area where interstitial pathological changes were observed. In Figure 2, the representative appearance of the interstitial caldesmon expression in IgA nephropathy with a different degree of interstitial damage is shown. As the severity of the interstitial damage increases, the staining of the caldesmon in the interstitium seems to increase accordingly.

**Immunoelectron microscopy**

The morphology of tissue from IgA nephropathy for immunoelectron microscopy with caldesmon was adequate to identify individual cells in tubulointerstitial compartment. Interstitial immunolabelling was confined to the cytoplasm of fibroblast-like cells and fibroblast-like processes (Figure 3). Glomerular mesangial cells and vascular smooth-muscle cells also

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**Fig. 2.** Immunohistochemical interstitial staining for caldesmon in IgA nephropathy kidneys. (A) almost normal interstitial lesion; little interstitial staining is shown. It has 9.2% of the Fv of caldesmon. (B) interstitial lesion with mild interstitial change; enhanced interstitial staining is shown. It has 18.3% of the Fv of caldesmon. (C) severe interstitial lesion; enhanced interstitial staining is shown. It has 32.4% of the Fv of caldesmon. × 400.

**Fig. 3 (A)** Immunoelectron microscopy for caldesmon, demonstrating fibroblast-like interstitial cell cytoplasmic labeling (double arrow) adjacent to a tubular basement membrane (arrow). Nu = nucleus; CP = cytoplasmic process; Ed = endothelial cell; CL = capillary lumen. The bar represents 1 μm. (B) High-power magnification of the part of Figure 3 (A) clearly showing interstitial cell cytoplasmic caldesmon labelling.
A comparison of caldesmon and CD68 expression in the same interstitial lesion using consecutive sections. (A) caldesmon immunostaining; (B) CD68 immunostaining. ×200.

A comparison of caldesmon immunostaining and Masson’s trichrome stain in the same interstitial lesion using consecutive sections. (A) caldesmon immunostaining; (B) Masson’s trichrome stain. ×200.

demonstrated intracellular cytoplasmic labelling for caldesmon (data not shown). There was no specific immunolabelling in tubular epithelial cells.

A comparison of the leukocyte infiltration and histological changes with the caldesmon expression

We compared the caldesmon expression with the leukocyte infiltration and histological changes using consecutive specimens. Representative illustrations of caldesmon and CD68 immunoreactivity in the interstitium with a prominent mononuclear cell infiltration are shown in Figure 4. Caldesmon immunoreactivity and Masson’s trichrome stain of the consecutive specimen with a moderate interstitial fibrosis are shown in Figure 5. The caldesmon expression was faint in the interstitium of the specimen showing no apparent mononuclear cell infiltration and fibrosis. But the enhanced expression of caldesmon and α-SMA was detected in the interstitium with prominent mononuclear cell infiltration or with mild to moderate fibrosis.

To determine if the caldesmon expression was associated with the histological changes, we compared the Fv of caldesmon with mononuclear cell infiltration and fibrosis score. Fv of caldesmon was higher as mononuclear cell infiltration score increased (Figure 6). With regard to the fibrotic changes, the Fv of caldesmon was significantly increased in group 1 compared with group 0. But in case of advanced fibrosis, in
fractional volume of caldesmon (%)  

\begin{figure}
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\includegraphics[width=0.8\textwidth]{fig7.png}
\caption{A comparison of the caldesmon immunostaining with interstitial fibrosis. (d) $P<0.05$ vs group 0; (e) NS vs group 0; (f) NS vs group 1.}
\end{figure}

\begin{figure}
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\includegraphics[width=0.8\textwidth]{fig8.png}
\caption{A comparison of the clinical data with the caldesmon expression}
\end{figure}

There is clinical evidence that excretion of more than 1 g of protein per day is a bad prognostic feature in IgA nephropathy. Thus patients were divided into two groups: one that excreted 1 g or more protein per day (high urinary protein group) and one that excreted less than 1 g of protein excreted per day (low urinary protein group). To seek the possible association of the caldesmon expression with the progression of IgA nephropathy, histological scores (fractional volume of caldesmon and $\alpha$-SMA, interstitial mononuclear cell infiltration and fibrosis score) and clinical parameters at the beginning of the study (serum creatinine, creatinine clearance, blood pressure, serum IgA and age) were compared between the two groups. As shown in Table 1, patients in the high urinary protein group had a significantly greater fractional volume of caldesmon and $\alpha$-SMA than those in the low urinary protein group. With regard to the other clinical parameters, no statistically significant difference were noted between the two groups (Table 1). In addition, interstitial mononuclear cell infiltration and fibrosis score were significantly greater in the high urinary protein group than in the low urinary protein group.

**The correlation between $\alpha$-SMA expression and caldesmon expression**

Interstitial renal fibrosis is associated with the interstitial cells expressing $\alpha$-SMA. Phenotypic modulation of interstitial cells to express $\alpha$-SMA appears to be an early feature of progressive renal scarring. In this study we have also investigated the interstitial $\alpha$-SMA expression in the IgA nephropathy kidneys. Using consecutive specimens, co-localization of caldesmon and $\alpha$-SMA expressions in the interstitium is shown in Figure 9. The relationship between the Fv of caldesmon and $\alpha$-SMA is shown (Figure 10). There was a statistically significant correlation between caldesmon and $\alpha$-SMA expression.

**The correlation between caldesmon expression in glomeruli and interstitium**

Since IgA nephropathy is a primary glomerular disease, changes in the interstitium are considered to be a consequence of glomerular damage. We compared the glomerular and interstitial expressions of caldesmon in individual patients. As shown in Figure 11, there was a significant correlation between glomerular caldesmon scores and interstitial Fv of caldesmon in overall analysis. However, some cases with low glomerular caldesmon scores showed high interstitial Fv of caldesmon.

**The effects of glucocorticoid and heparin therapy**

We investigated the effects of glucocorticoid and continuous heparin infusion therapy on the expression of caldesmon and $\alpha$-SMA, morphological scores and other clinical parameters. Fifteen patients underwent re-biopsies after the therapy (4–8 weeks). The immunostaining, morphological scores, and the clinical parameters were compared before and after the therapy. Creatinine clearance (Ccr) of those 15 patients before the therapy was $104 \pm 38$ ml/min. The clinical parameters were not changed by the therapy, except that daily urinary protein excretion significantly decreased ($1.30 \pm 1.33$ vs $0.435 \pm 0.687$ g/day). With regard to the morphological score, Fv of caldesmon and $\alpha$-SMA and fibrosis score were not significantly decreased after the therapy, but only the mononuclear cell infiltration score was significantly decreased after the therapy.
Table 1. Comparison of clinical data with caldesmon expression

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<th>u-prot &gt; 1.0 g/day (n=13)</th>
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Fig. 9. A comparison of the caldesmon immunostaining and a-SMA in the same interstitial lesion using consecutive sections. (A) caldesmon immunostaining; (B) a-SMA stain. ×200.

Fig. 10. The correlation between a-SMA and caldesmon expression. Each dot shows the a-SMA and caldesmon expression in each IgA nephropathy patient. The correlation is statistically significant (P<0.0001).

(1.27 ± 0.594 vs 0.867 ± 0.743). So those 15 patients were divided into two groups: non-responder (patients with unchanged interstitial cell infiltration) and responder (patients with decreased interstitial cell infiltration). Then between these two groups, the difference in the changes of caldesmon expression by the therapy was evaluated. The changes of caldesmon was calculated as follows.

The changes of caldesmon expression by the therapy = [(Fv of caldesmon (post)−Fv of caldesmon (pre))/Fv of caldesmon(pre)] × 100

Fig. 11. The correlation between the glomerular caldesmon score and interstitial Fv of caldesmon. Each dot shows the glomerular caldesmon score and interstitial Fv of caldesmon in each IgA nephropathy patient. The correlation is statistically significant (P<0.001).
The change in Fv of caldesmon was $-26.6\%$ in the responder, and was significantly different compared to the non-responder ($7.35\%$) (Figure 12), indicating that the Fv of caldesmon decreased in patients with decreased interstitial mononuclear cell infiltration.

In addition we investigated the effects of the therapy after a 2-year follow up. The doses of glucocorticoid tapered off during these 2 years. Fourteen of 15 patients were analysed as to urinary protein excretion (g/day) and creatinine clearance (ml/min) after a 2-year follow up. Creatinine clearance was significantly increased and urinary protein excretion was decreased compared to before the therapy (Figure 13).

**Discussion**

In the present study, we observed that changes in the amount and distribution of caldesmon positive cells within the interstitium of the kidneys from IgA nephropathy patients, and that the quantification of the caldesmon immunostaining revealed an association between caldesmon expression and pathological changes of tubulointerstitial compartment ($\alpha$-SMA expression, mononuclear cell infiltration, macrophage infiltration and fibrosis) in IgA nephropathy.

Tubulointerstitial injury is an invariant finding in the chronically diseased kidney, irrespective of the type of disease or the compartment in which the disease originates. The decline in renal function has been suggested to correlate better with tubulointerstitial changes than with glomerular damage. The pathways in various compartments of the kidney that culminate in tubulointerstitial injury were: (i) vascular effect; (ii) glomerular injury (proteinuria, haematuria, cytokines, autacoids); (iii) interstitial processes (lymphocytes, macrophages, fibroblasts, extracellular matrix); (iv) tubular epithelial processes (antigenicity, metabolism, growth factors, cytokines, structural alterations, collagen synthesis); (v) other processes (nephron obstruction, crystalline deposits) [1]. Since IgA nephropathy is a primary glomerular disease, the tubulointerstitial changes in IgA nephropathy patients may first develop from the glomerular injury. We found that in overall patients, glomerular and interstitial caldesmon expressions showed significant correlation. However, we also found that in a few patients the degree of interstitial changes did not correlate with that of glomerular changes. Thus, interstitial processes may partly play roles as an independent factor in the development of tubulointerstitial lesions in IgA nephropathy. In the present study, our observations were focused on the interstitial processes, especially on the role of interstitial...
myofibroblasts. Previous work demonstrated that interstitial α-SMA expression is enhanced in IgA nephropathy and α-SMA-positive cells are considered to be myofibroblasts which are important mediators of tubulointerstitial scarring and have useful prognostic implications [12]. α-SMA is currently considered to be a sensitive marker of the phenotypic change in the interstitial cells in vivo. Our present study demonstrated an enhanced expression of caldesmon was localized in interstitial fibroblast of IgA nephropathy. We have also shown co-localization of caldesmon and α-SMA expressions in the interstitium to further characterize the phenotype of interstitial myofibroblasts. Thus, caldesmon is a reliable molecular marker of the phenotypic change in the interstitial cells.

The concept of the myofibroblast’s playing a pivotal role in renal interstitial fibrosis has attracted increasing attention. The presence of myofibroblasts within the interstitium of patients with IgA nephropathy suggests a role for these cells in the renal scarring. Phenotypic changes of renal interstitial fibroblasts leading to their expression of α-SMA and the acquisition of contractile properties would happen during the progression of IgA nephropathy. However, we cannot exclude that myofibroblasts appeared to be derived from the cortical arterial and arteriolar walls and diffuse into the interstitium and accumulate in the periglomerular and peritubular space. Immunoelectron microscopy revealed that activated mesangial cells as well as interstitial fibroblasts expressed caldesmon but tubular cells did not, suggesting that the epithelial-mesenchymal transformation may not occur in IgA nephropathy. Moreover, the prominent periglomerular and peritubular distribution of α-SMA is consistent with the pattern of collagen mRNA expressing cells seen in experimental interstitial fibrosis [13]. In in vitro cell culture study, renal fibroblasts have been shown to produce collagen in response to a variety of stimuli, including cytokines and growth factors [14,15]. Fibroblasts cultured from diseased kidneys exhibit significant abnormal hyperproliferative growth and collagen biosynthesis when compared to their normal counterparts.

Our present study demonstrates that the presence of myofibroblasts in the renal interstitium correlates well with interstitial leukocyte infiltrates, including macrophages. Leukocytes have a role in activation and recruitment of fibroblast-like cells. Interestingly, results of a cell culture study suggest that leukocyte-derived transforming growth factor β may act as a stimulus for acquisition of myofibroblast features [16]. Investigators using several model systems have also shown that macrophage-derived TGF-β1 may be pivotal in the process of myofibroblast modulation [17].

Another major finding in this study is that glucocorticoid–heparin therapy (4–8 weeks) seemed to suppress the interstitial expression of caldesmon and α-SMA in the patients with decreased interstitial cell infiltration. It supports the idea that leukocyte-derived growth factors and/or cytokines may act as a stimulus for acquisition of myofibroblast features. In the present study, we also found that after 2-year follow up urinary protein excretion was not increased and renal function was improved and that even short term glucocorticoid therapy could improve clinical states of the disease over 2 years in comparable with the reduction of caldesmon expression in tubulointerstitial fibroblasts as well as in glomeruli observed after 4–8 weeks duration of the therapy. Although we have not evaluated the histological change after 2-year follow up, we speculate that the therapy was shown to have a favourable effect on the progression of the disease at least for 2 years. These results are comparable with the recent report by Kobayashi and colleges [18]. Future long-term follow up is required to evaluate the efficacy of this therapy. How glucocorticoids beneficially influence the interstitial lesions and reverse the phenotype of interstitial cells in IgA nephropathy still remains unclear. Glucocorticoids may have an anti-inflammatory effect on the interstitial lesions in IgA nephropathy, resulting in decreased interstitial cell infiltration and the reduction of the phenotypic change of interstitial cells. Alternatively, a direct suppression of caldesmon expression by glucocorticoids could be the mechanism; however, so far, there has been no report on the direct action of glucocorticoids on caldesmon in smooth-muscle cells or interstitial cells in culture.

In the present study, we used heparin in addition to glucocorticoids. Heparin was demonstrated to suppress mesangial cell proliferation and matrix expansion in anti-Thy 1.1 glomerulonephritis [19]. Thus, heparin may also have beneficial effects in vivo in IgA nephropathy patients. However, there was a report that heparin induced α-SMA expression in cultured fibroblasts [20]. Thus it is unlikely that heparin directly suppressed the interstitial α-SMA expression in IgA nephropathy patients.

Of note is the fact that the distribution pattern of caldesmon in the interstitium was enhanced particularly in the perivascular, periglomerular and peritubular lesion and was similar to that of α-SMA in IgA nephropathy. The degree of response to the therapy was also similar between caldesmon and α-SMA. Our previous study demonstrated that the similar up-regulation of caldesmon and α-SMA were observed in the mesangial lesion in IgA nephropathy. Taken together, we speculate that to a large extent, the gene regulation of caldesmon and α-SMA is mediated by the same molecular mechanism in the phenotypic change of interstitial and mesangial cells in the process of glomerulonephritis.

In fact, there are several common sequence elements within the promoter of caldesmon and α-SMA. For example, CArG elements exist in the 5′ regulatory regions of both genes and they appear to be the targets of growth-factor-induced signals that produce a rapid transcriptional activation of the caldesmon and α-SMA gene [21,22]. However, the involvement of CArG element in the transcriptional regulation of α-SMA and caldesmon in the process of myofibroblast formation has not been elucidated. We are investigating the molecular mechanism of myofibroblast formation...
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during the process of glomerular or interstitial fibrosis. We made transgenic mouse harbouring z-SMA gene regulatory region-chloramphenicol acetyltransferase (CAT) fusion genes to analyse the z-SMA promoter activity in vivo [23]. We found that the gene region from −891bp to +3812 bp (containing intron 1) is important in the gene expression of z-SMA in cultured mesangial cells, and in mesangial proliferative glomerulonephritis in vivo. Further analysis of z-SMA promoter transgenic mice will give us an insight into the molecular mechanism of myofibroblast formation in vivo.

In conclusion, the study presented here demonstrates that the enhanced interstitial expression of caldesmon is an additional sensitive marker of interstitial injury in IgA nephropathy patients. The responsive decrease of caldesmon expression after the glucocorticoid—heparin therapy may suggest diagnostic and/or prognostic significance of caldesmon in the assessment of IgA nephropathy. Naturally, future long-term follow-up of the patients involved in the present study is required to establish the prognostic significance of caldesmon in IgA nephropathy.

References


Received for publication: 8.7.98
Accepted in revised form: 18.1.99