Immunohistochemical Analysis of Molecular Events in Tubulo-Interstitial Fibrosis in a Mouse Model of Diffuse Mesangial Sclerosis (ICGN Strain)

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ABSTRACT. Diffuse mesangial sclerosis (DMS) is one of the hereditary glomerular diseases and histologically characterized by severe glomerulosclerosis and subsequent tubulo-interstitial fibrosis (TIF). In DMS patients, renal dysfunction correlates well with TIF, rather than with glomerular lesions. Thus, molecular mechanisms whereby TIF in DMS progresses should be addressed. Previously, we found that nephrotic ICGN mice manifest DMS-like lesions and develop renal dysfunction in accordance with onset of TIF. In the present study, we investigated fibrogenic events involved in the progression of TIF after DMS manifestation, using the DMS mouse model. Immunohistochemistry revealed that expression of transforming growth factor-beta (TGF-β) was rare in the interstitial cells of the nephrotic mice at the early-stage of DMS, while the TGF-β expression became evident in the late-stage DMS mice. Platelet-derived growth factor (PDGF) was mildly expressed in the distal tubules of the early-stage DMS mice, whereas the PDGF expression markedly increased at the late-stage of DMS. As a result, α-actin-positive myofibroblastic cells were found dominant in the interstitial spaces of the late-stage DMS mice. Finally, TIF became severe in accordance with the overexpressions of these molecules. Our results suggest that in our murine model: 1) persistent proteinuria leads to over-expression of TGF-β and PDGF in non-glomerular areas; 2) these cytokines provoke interstitial myofibroblast accumulation; and 3) the myofibroblasts produce fibrotic matrix proteins in the interstitial spaces. This process may possibly contribute to the development of TIF in DMS patients.

KEY WORDS: diffuse mesangial sclerosis, growth factor, ICGN mouse, myofibroblast, tubulo-interstitial fibrosis.

Renal dysfunction has been, in numerous chronic renal diseases, characterized by a progressive loss in glomerular filtration ratio. In some cases, glomerular injury could be a crucial determinant for progression of a loss in renal function [7]. During the past several years, much interest has been focused on investigating molecular basis of glomerulosclerosis [16, 35, 41]. In contrast, decline in renal function correlates, in most patients with chronic kidney disease, more closely with extents of tubular and interstitial lesions than with that of the glomerular injury [4, 29, 38]. Therefore, recent attention is now being paid to the molecular basis of tubulo-interstitial lesions [9, 17, 33, 43].

In intractable nephrotic syndrome (NS), patients exhibit glomerular sclerotic injury and massive proteinuria, leading to renal failure [19]. Indeed, the glomerular damage may be responsible for clinical manifestation of proteinuria, but tubular and tubulo-interstitial lesions rather than the glomerular injury seem to be a reliable histological marker for predicting prognosis of the patients [3, 42]. Until now, molecular basis of tubulo-interstitial fibrosis (TIF) has been investigated in some NS models with or without glomerular injury [9].

Glomerulosclerosis is often observed in patients with hereditary NS [11, 34]. In particular, diffuse mesangial sclerosis (DMS) is one of the NS-related disorders and histopathologically characterized by mesangial expansion in more than 80% of glomeruli. Habib et al. described that tubular dilatation and interstitial expansion was impressive in cases with renal failure [13, 14]. This observation raised a possibility that tubulo-interstitial lesions could be a key predictor for diagnosis of DMS patients, although it may be difficult to determine whether the non-glomerular lesions cause or reflect renal dysfunction. Thus, molecular and cellular events of tubulo-interstitial lesions should be addressed. Nevertheless, no information is available about molecular pathogenesis of TIF following DMS manifestation, probably due to a lack of animal models of DMS in NS.

The nephrotic ICGN mice manifest severe albuminuria and hypoalbuminemia and therefore are considered to be a suitable model for intractable nephrotic syndrome (NS) [20–26, 31, 32]. Recently, we found that the NS mice develop DMS-like lesions by 6 weeks of age, and therefore, we postulated that the mice would provide a new tool for investigating pathogenesis of DMS in intractable NS [25]. Furthermore, we found that manifestation of the renal dysfunction in the NS model depends on the extent of tubular atrophy with interstitial expansions rather than that of DMS [21–24]. In this study, we immunohistochemically examined the molecular and cellular events involved in the progression of TIF following DMS manifestation in the NS mice in order to obtain clues as to how renal dysfunction occurs in DMS patients. From the present results, we discuss a possible event(s) involved in pathogenesis of TIF in intractable NS with DMS.

MATERIALS AND METHODS

Animals: Sixteen female nephrotic mice (ICGN strain),
prepared by mating between homozygous (nep/nep) males and heterozygous (nep/-) females [20–26], were used. Of all the offspring, homozygous mice were confirmed to be nephrotic by the SDS-PAGE of the urine samples [20]. They were kept under a specific-pathogen free condition in our institute and were fed a commercial diet (MF; Oriental Yeast, Tokyo, Japan). The clinical signs shown by the mice were monitored daily. The mice aging 14 weeks (n=8) were used as the early-stage DMS mice, and those aging 26 weeks (n=8) were used as the late-stage DMS mice, based on renal dysfunction [26]. The urine and serum samples were collected at the time of sacrifice and then frozen at –80°C until used.

Biochemical analyses: The urine and serum samples were subjected to the following analyses [20–26]: Urinary albumin (uAlb) and serum albumin (sAlb) concentrations were examined by a bromo-cresol green method with a commercial kit (A/G B-test, Wako, Osaka, Japan) to evaluate the severity of albuminuria and hypoalbuminemia [21]. To assess the loss of renal functions, BUN and serum creatinine (sCr) concentrations were determined by a urease indophenol method with a kit (urea nitrogen-B test, Wako) and by Jeffe’s method with a kit (Creatinine test, Wako), respectively [21, 22, 26].

Renal histopathology: At the scheduled necropsy, renal organs were fixed in cold neutral buffered formalin (pH 7.4) and were routinely embedded in paraffin. Each section was cut into 4 μm slices, de-waxed, and stained with hematoxylin and eosin (H.E.) solution. The renal specimens were observed under a light microscope, and abnormal findings were recorded. Tubular injury, characterized by the tubular dilatation and epithelial cellular atrophy with the tubulo-interstitial expansion was recorded, and scored according to the extent of cortical involvement on a scale of 0 to 4: 0=normal; 0.5=small focal area; 1=5–25% of the cortex; 2=25–50% of the cortex; 3=50–75% of the cortex; and 4=>75% of the cortex [21, 22, 26]. To evaluate the extent of the glomerulosclerosis, the glomerular extra-cellular matrix (ECM) index was determined by an established method, based on mesangial expansions [37]. In addition, the mononuclear cell (MNC) index and glomerular cell counts were determined to estimate the interstitial inflammation and glomerular atrophy, respectively [21].

Immunohistochemistry: The remaining renal tissues were fixed in 70% ethanol and were subjected to a routine process for the paraffin embedding [21]. To visualize the expressions of fibrogenic growth factors, rabbit IgG against human transforming growth factor-beta (TGF-β) (1:30) (R&D Systems, Minneapolis, U.S.A.) and goat IgG against human platelet-derived growth factor (PDGF) (1:100) (Becton Dickinson, Bedford, U.S.A.) were used for the primary reactions, and an avidin-biotin coupling (ABC) peroxidase technique was performed on the sections, using a commercial kit (Vectastain Elite ABC, Vector Lab., Burlingame, U.S.A.), according to the manufacturer’s instructions. To identify the PDGF receptor (PDGF-Rc), rabbit IgG against murine PDGF-Rc (1:200) (Oncogene, New York, U.S.A.) was applied on the dewaxed sections, followed by the ABC immuno-peroxidase technique. To identify myofibroblasts, a direct technique using a kit of mouse monoclonal IgG against human alpha-smooth muscle actin (α-SMA) (EPOS system, DAKO, Glostrup, Denmark) was used. To examine the extent of fibrotic areas, rabbit IgG against rat type I collagen [col (I)] (1:200) (Cosmo-Bio, Tokyo, Japan) and that against mouse fibronectin (Chemicon, Temecula, CA, U.S.A.) were used for the primary reactions, followed by the ABC peroxidase technique, as described above.

Semi-quantification for the immunolabelings: For the immunostaining for TGF-β, at least 20 randomly chosen non-overlapping high power fields (hpf) (×400 magnification) were examined, and the mean number of the TGF-β-positive interstitial cells per hpf was used as the staining score for interstitial TGF-β. The degrees of the PDGF-positive tubules, PDGF-Rc-positive interstitial cells and α-SMA-positive cells were evaluated in the at least 20 randomly chosen non-overlapping hpf and their extents were semiquantitatively by means of a scale of 0 to 3: 0=negative; 1=mild; 2=moderate; and 3=severe [15, 27]. The immunostaining scores for PDGF and its receptor, and α-SMA were determined by the mean values of these scales. Furthermore, glomerular staining score for α-SMA and col (1) was expressed as the mean scale of the positive areas in at least 20 glomeruli [10].

Statistical analyses: All data were expressed as mean ± standard error (S.E.) (n=8). A Student’s t-test was used to compare group means, with p<0.05 accepted as significant. Multiple regression analyses were employed to evaluate significance of the relationship between variables.

RESULTS

Clinical and histological findings: Almost all of the nephrotic ICGN mice assigned to the early-stage DMS group appeared to be healthy, while the nephrotic mice, divided into the late-stage DMS group, manifested clinical signs such as the exercise intolerance, paled ears, weight loss and edema [21]. The clinical and histopathological findings are shown in Table 1 and Fig. 1. In the early-stage DMS mice, albuminuria (uAlb level: ≥0.5 g/dl) and hypoalbuminemia (sAlb level: <2.5 g/dl) were found to be evident, but renal dysfunction, as determined by the increases in the BUN and sCr levels, was shown to be mild (Table 1). The BUN and sCr levels in the late-stage DMS showed a 2.5-fold and an 1.5-fold increases of those of the early-stage DMS mice, respectively.

The early-stage DMS mice exhibited little lesions in the tubular and interstitial areas (Fig. 1A), whereas the late-stage DMS mice manifested severe lesions such as the tubular dilatation, tubular epithelial atrophy and interstitial expansions with the MNC infiltration (Fig. 1B). The glomerular sclerotic lesions, characterized by mesangial expansion and capillary collapsing, were found to be severe in both the early-stage and late-stage DMS mice (Figs. 1C & D). The tubular injury index in the late-stage DMS mice
was 5.0-fold higher than that in the early-stage DMS mice, while no significant differences in the glomerular ECM index was seen between the early-stage and late-stage of DMS (Table 1). In addition, the MNC index in the late-stage mice was 4.3-fold higher than that in the early-stage DMS mice, while the glomerular cell count was significantly lower in the late-stage DMS mice than in the early-stage DMS mice.

**Renal expressions of TGF-β, PDGF and PDGF-Rc:** So far, TGF-β and PDGF have been implicated to be a key molecule for eliciting renal fibrosis [5, 40]. Thus, we first examined whether these fibrogenic molecules could be expressed in our DMS model. Figure 2 represents the extraglomerular expressions of TGF-β, PDGF and PDGF-Rc. The immunolabeling for TGF-β was scarcely noted in the tubulo-interstitial cells in the early-stage DMS mice (Fig. 2A), while the TGF-β expression was frequently seen in the interstitial cells (Fig. 2B) as well as in the proximal tubules (data not illustrated) of the late-stage DMS mice. In the early-stage DMS mice, the immunostaining for PDGF was weakly seen in the proximal tubules (Fig. 2C), whereas the PDGF expression was obviously detected on the distal tubules in the late-stage mice (Fig. 2D). The positive staining for PDGF-Rc was scatteringly detected in the tubulo-interstitial cells of the early-stage DMS mice (Fig. 2E). In contrast, the PDGF-Rc staining was frequently detected in the interstitial cells (Fig. 2F) as well as in the same sites as the PDGF expression (data not illustrated). The immuno-

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**Table 1. Clinical and histological findings in the nephrotic mice (ICGN strain)**

| Parameters                  | Early-stage | Late-stage | Differences  
<table>
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<tr>
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<tr>
<td>Age</td>
<td>14 weeks</td>
<td>26 weeks</td>
<td></td>
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<tr>
<td>uAlb level (g/dl)</td>
<td>1.24 ± 0.26</td>
<td>0.63 ± 0.13</td>
<td>n.s.</td>
</tr>
<tr>
<td>sAlb level (g/dl)</td>
<td>2.15 ± 0.08</td>
<td>2.02 ± 0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>BUN level (mg/dl)</td>
<td>32.4 ± 2.04</td>
<td>81.3 ± 10.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>sCr level (mg/dl)</td>
<td>0.66 ± 0.04</td>
<td>1.02 ± 0.08</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Tubular injury index</td>
<td>0.63 ± 0.08</td>
<td>3.13 ± 0.30</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Glomerular ECM index</td>
<td>3.39 ± 0.09</td>
<td>3.36 ± 0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mononuclear cell index</td>
<td>0.19 ± 0.13</td>
<td>0.81 ± 0.09</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Glomerular cell count</td>
<td>31.1 ± 0.35</td>
<td>25.7 ± 0.73</td>
<td>p&lt;0.001</td>
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a) Evaluated with a Student t-test, b) Mean ± S.E. (n=8), and c) Not significant. Abbreviations: BUN, blood nitrogen urea; sCr, serum creatinine; sAlb, serum albumin; and uAlb, urinary albumin.

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**Fig. 1.** Representative microphotographs of the renal lesions in the nephrotic ICGN mice, used as a model of DMS (H.E. stain). (A & B) Photographs of the tubular dilatations in an early-stage DMS mouse (A) and a late-stage DMS mouse (B) (× 110). (C & D) Photographs of the glomerular sclerotic lesions in the early-stage DMS mouse (C) and the late-stage DMS mouse (D) (× 260).
staining scores for TGF-β, PDGF and PDGF-Rc in the late-stage DMS mice were significantly higher than those in the early-stage DMS mice (Fig. 2G-I). With regard to the intra-glomerular expressions, TGF-β-positive cells were detected in the glomeruli of the late-stage mice, with a rare frequency, but no immunolabelings for PDGF and for PDGF-Rc were detected in the intra-glomerular components of all the mice used in the present study (data not illustrated).

**Interstitial myofibroblast formation and TIF:** During a process of developing tissue fibrosis, myofibroblasts play an important role for depositing fibrotic materials (such as collagen or fibronectin), probably as ECM-producing cells [1, 28]. Therefore, we investigated a possible involvement of myofibroblasts in TIF after DMS progression in our model. Figure 3 shows the interstitial expressions of α-SMA, col (1) and fibronectin. Myofibroblasts, identified as the α-SMA-positive cells, were mild in the peri-tubular and periglomerular spaces of the early-stage DMS mice (Fig. 3A), whereas the interstitial myofibroblasts were found to be severe in the late-stage DMS mice (Fig. 3B). Interestingly,
there were no significant differences in glomerular α-SMA stainings between the early- and late-stage of DMS. The interstitial deposition of col (1), a marker for TIF, was mild in the early-stage DMS mice (Fig. 3C), while the interstitial deposition of col (1) was found severe in the late-stage DMS mice (Fig. 3D). With regard to glomerulosclerosis, the glomerular col (1) score in the late-stage DMS mice was not different from that in the early-stage DMS mice (3.10 ± 0.40 vs. 3.21 ± 0.38). Finally, the expression of fibronectin was examined on the DMS mice. The interstitial deposition of fibronectin was found to be mild in the early-stage DMS mice (Fig. 3E), whereas the late-stage NS mice showed severe deposition of the ECM protein (Fig. 3F). The staining scores for interstitial α-SMA, col (1) and fibronectin were significantly higher in the late-stage DMS mice than in the early-stage DMS mice (Fig. 3G-I).

**Correlations between fibrotic parameters and clinical or histological findings:** To further understand a significance of molecular and cellular events noted in the DMS mice, we finally prepared scattergraphs showing the relationships between fibrotic parameters and clinical or histological findings: The BUN level closely correlated with not only the

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Fig. 3. Representative microphotographs and semi-quantification of the interstitial myofibroblast formation and deposition of ECM proteins. (A & B) Photographs of the renal expression of α-SMA in an early-stage (A) and a late-stage DMS mouse (B) (× 110). (C & D) Photographs of the interstitial deposition of col (1) in an early-stage (C) and a late-stage DMS mouse (D) (× 220). (E & F) Photographs of the interstitial accumulation of fibronectin in an early-stage (E) and a late-stage DMS mouse (F) (× 130). (G-I) Comparisons of the immunostaining scores for the α-SMA, col (1) and fibronectin proteins between these two DMS stages. Data are expressed as mean ± S.E. (n=8). Statistical analysis: ***, p<0.001 compared with the early-stage nephrotic mice. For abbreviations see text.
tubular injury index but also the staining score for tubulo-interstitial collagen (1) (Fig. 4A). No closed relationships were seen between the BUN level and the extent of the glomerulosclerosis, expressed as the glomerular collagen (1) score, while there was a good correlation between the BUN level and glomerular cell count. The frequency of the TGF-β-positive tubulo-interstitial cells showed a close correlation with the interstitial collagen (1) score (Fig. 4B). Furthermore, the staining score for PDGF expressed in the distal tubules and for PDGF-Rc expressed in the interstitial cells both were correlated closely with the extent of TIF. Consequently, the interstitial staining score for α-SMA was shown to be linked to the interstitial collagen (1) score. Next, we examined the correlation of the fibrogenic parameters, noted in the interstitial and tubular areas, with the glomerular collagen (1) score. Overall, no relationships were seen between any expressions of these expressions and the glomerular staining score for collagen (1) (Fig. 4C). In contrast, these fibrotic molecules were all negatively correlated with the glomerular cell count (Fig. 4D), suggesting a possible involvement of glomerular cellular atrophy (rather than glomerular ECM accumulation) to further progression of TIF after DMS establishment.

**DISCUSSION**

The nephrotic mice (ICGN strain) manifest DMS-like lesions in the glomeruli by 6 weeks of age [25]. Tubular injuries such as the dilated tubules and epithelial cellular

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**Fig. 4.** Analysis of the molecular and cellular events involved in the progression of renal dysfunction and fibrosis in the DMS mice. (A) Relationships between the BUN level and extents of tubulo-interstitial or glomerular fibrosis. (B) Relationships between the interstitial collagen (1) score and the scores for TGF-β, PDGF, PDGF-Rc and α-SMA. (C) Relationships between the glomerular collagen (1) score and the staining scores for TGF-β, PDGF, PDGF-Rc and α-SMA. (D) Relationships between the glomerular cell number and the scores for these fibrogenic molecules. Symbols: Open square, early-stage; and closed square, late-stage of DMS. Statistical analysis: n.s., not significant. For abbreviations see text.
atrophy occur from at least 14 weeks after birth, while in 26 weeks of age, most of the DMS mice manifest the depressive signs due to renal failure [21, 22, 26]. Therefore, 14-week-old and 26-week-old ICGN mice were available as an animal model manifesting pathophysiological conditions of early-stage and late-stage DMS, respectively. Using the mice being at different stages of DMS, we examined the molecular and cellular changes during the progression of TIF in order to provide a clue of therapeutic strategies for DMS patients.

Until now, TGF-β has been suggested to be a key mediator for ECM production [5]. In our model, immunolabelings for TGF-β were predominantly detected in the interstitial cells of the late-stage DMS mice. The TGF-β-positive interstitial cells were immunoreactive for Mac-1, a specific marker for macrophages (data not shown). Furthermore, TGF-β-positive immunolabelings were also detected in the proximal tubular cells in the late-stage DMS mice. Considering a role of urinary protein(s) to elicit macrophage recruitment in interstitium [8], tubular epithelial cells exposed to albuminuria in the ICGN mice may provoke the interstitial MNC infiltration. With regard to this, it was shown that macrophages infiltrating into interstitium play a key role in onset of TIF as TGF-β-producing cells [8]. In addition, it is widely accepted that tubular epithelial cells could produce TGF-β [8, 36]. Therefore, we predict that both the macrophages and tubular epithelial cells could be a source of TGF-β during TIF progression in our DMS/NS model.

It is well documented that PDGF plays an etiological role(s) in development of glomerular sclerosis [16, 41]. Interestingly, this growth factor was over-expressed on the distal tubular cells, especially at the late-stage of DMS. Our present finding was similar to that in a 5/6-nephrectomized rat model [17], which of etiology was largely different from that of our NS model. The extent of the distal PDGF expression was correlated closely with the interstitial deposition of col (1). The lower tubule-derived PDGF may play a critical role in the ECM production in the interstitial cells. In fact, an in vivo study suggested that PDGF directly causes myofibroblast formation as well as ECM over-deposition in interstitial spaces [40]. Increased expression of PDGF-Rc in the interstitial cells during TIF progression in our DMS model may support a hypothesis that possible paracrine stimulation of interstitial fibroblasts by PDGF may provoke fibrogenic events [18].

As mentioned above, renal function was impaired in accordance with TGF-β and PDGF overexpressions in our DMS mice. Thus, it is of importance to discuss how interstitial and tubular expressions of TGF-β and PDGF could be induced under the nephrotic conditions. Long-lasting proteinuria can elicit macrophage infiltration, probably through upregulating expression of a molecule(s) attractant for monocyte/macrophages (i.e., monocyte chemoattractant protein-1 [8, 9]). Of note, interstitial macrophages can be an initial source of TGF-β and PDGF [27, 30]. Considering these cellular and molecular sequences, interstitial mononuclear cells noted in the nephrotic ICGN mice (see, Figs. 1B & 2B) may lead to increases in tubular TGF-β and PDGF expression. Another possibility is that urinary proteins per se might directly stimulate production of these fibrogenic cytokines in tubular epithelial cells, as suggested elsewhere [8, 9, 22, 43].

Overall, in parallel with the increased expressions of TGF-β and PDGF, myofibroblast formation (identified as the α-SMA expression) became evident in the tubulo-interstitial and peri-glomerular spaces of the late DMS mice. These findings suggested a phenotypic switch of the resident interstitial fibroblasts, as described elsewhere [28, 39]. Myofibroblasts may be generated by stimulation with TGF-β in a paracrine or autocrine manner [28, 45]. Furthermore, a recent work suggests an importance of PDGF in induction and maintenance of myofibroblast formation [40]. Indeed, immunolabeling not only for TGF-β but also for PDGF-Rc was noted in the myofibroblast-like cells. Therefore, the myofibroblasts seem to be induced and expanded through the autocrine stimulation by TGF-β as well as through paracrine stimulation by PDGF. Furthermore, the immunostaining score for α-SMA was correlated well with the BUN level. In patients with chronic renal failure, myofibroblast appearance could be, in fact, a reliable histological predictor for prognosis of patients with progressive nephropathy [6, 12, 39]. In our DMS/NS model, peri-glomerular myofibroblasts, noted in the late-stage mice, may accelerate progression of the glomerular obsolescence, because it was shown that myofibroblasts surrounding sclerotic glomerulus could infiltrate into the glomerular tufts, leading to further scarring [1, 28].

Consequently, interstitial fibrotic lesion, as identified by the depositions of col (1), was found evident in the late-stage of DMS/NS. In contrast, no significant fibrogenic events were seen in the age-matched non-nephrotic mice (data not shown). Scattergrams showed that staining scores for TGF-β, PDGF, PDGF-Rc and α-SMA in the tubular and interstitial areas were all positively correlated with the BUN levels as well as interstitial staining score for col (1) (Fig. 4A & B). Furthermore, we found that mRNAs of TGF-β and PDGF were markedly increased in the late-stage DMS mice (unpublished data). Taking together, we predict that tubular and interstitial lesions in our model may occur, largely through hyperplasia of tubulo-interstitial myofibroblasts, which would be induced by TGF-β and PDGF.

Glomerulosclerosis is histologically characterized by over-deposition of ECM as well as a loss in parenchymal cellular components. Of interest, there were no significant relationships between each extra-glomerular scores for TGF-β, PDGF, PDGF-Rc and α-SMA and the intra-glomerular col (1) score (Fig. 4C), while the glomerular cell count was negatively correlated with any staining scores for the extra-glomerular fibrogenic molecules (Fig. 4D). Therefore, it is highly possible that the glomerular cellular atrophy (rather than ECM over-deposition) may in part cause or promote the interstitial fibrogenic events in our DMS model. It has been implied that persistent and severe glomerulosclero-
sis causes post-glomerular capillary destruction, leading to acceleration of tubular atrophy [2, 4, 33]. In fact, a number in the interstitial vessels was much less in the late-stage DMS mice than in the early-stage DMS mice (data not shown). It is known that hypoxic condition promotes TGF-β (and possibly, PDGF) production in several organs [33, 44]. Thus, we predict that destruction of vascular architecture in DMS may cause renal tissue hypoxia and then upregulate extra-glomerular expressions of TGF-β (and PDGF), leading to further tubulo-interstitial fibrogenesis.

From the previous [20–26] and present observations obtained with the murine DMS model, possible molecular and cellular sequences of renal fibrosis noted in DMS patients are summarized as follows: 1) Mesangial ECM deposition occurs probably as a hereditary event [25]; 2) A number in glomerular parenchymal cells gradually decreases under persistent glomerulosclerotic and nephrotic conditions; 3) Depending on progression of glomerular destruction and severe proteinuria, TGF-β and PDGF expressions are upregulated in interstitial and tubular areas; 4) These fibrogenic cytokines cause a phenotypic switch of resident interstitial cells to tubulo-interstitial and peri-glomerular myofibroblasts; and 5) Tubulo-interstitial myofibroblasts produce and deposit ECM proteins in peri-tubular spaces, leading to TIF manifestation, while peri-glomerular myofibroblasts further aggravate pre-existing glomerulosclerosis. Considering that phenotypes of DMS could be inherited as an autosomal recessive trait in human [11] as well as in our murine model [20], not only mesangial sclerosis but also tubular lesion might be in part produced by a mutated gene(s), and the genomic studies are currently in progress.

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MOLECULAR EVENTS OF RENAL FIBROSIS IN DMS


