Significance of Intraglomerular Expression of α -Smooth Muscle Actin, Desmin, and PDGF Receptor on Glomerulosclerosis in the Rat Remnant Kidney Model

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Abstract: To elucidate the possible pathomechanism of glomerulosclerosis by partial nephrectomy, male SD rats received 3/4 nephrectomy and were maintained without further treatment for 8 weeks (3/4 nephrectomized group). Another group received a sham operation consisting of laparotomy and manipulation of the renal pedicles but without destruction of renal tissue and were maintained without treatment for 8 weeks (sham-operated group). The following findings were observed in the 3/4 nephrectomized group. The levels of urinary protein elevated after 4 weeks and thereafter reached a plateau. A high blood pressure was present at week 8. Histopathologically, glomerulosclerotic lesions were characterized by capillary obsolescence, proliferation of glomerular cells (including mesangial cells), mesangial matrix increase, and macrophage influx, and associated with expression of α -smooth muscle actin (α -SMA) and desmin in the glomerular tuft (mainly mesangial cells) and platelet-derived growth factor (PDGF) receptor in the glomerular tuft. The present results suggest that the key pathologic changes for glomerulosclerosis are impaired endothelial cells of glomerular capillaries by rapidly increased blood flow into the remaining nephrons and the resultant production of growth factors such as PDGF leading to mesangial cell proliferation. Furthermore, the present expression of α -SMA and desmin in mesangial cells suggests a phenotype switch of mesangial cells responsible for the induction of glomerulosclerosis. (J Toxicol Pathol 1999; 11: 229~234)

Key words: Pathomechanism, Glomerulosclerosis, Mesangial cells, α -SMA, Desmin, PDGF receptor, Rat, 3/4 Nephrectomy

Introduction

During the last twenty years, a great number of studies have focused on the process leading to progression of chronic renal diseases, and many renal diseases have been proven to progress to end-stage renal failure with glomerulosclerosis independent of the initial pathogenic mechanisms¹. The most widely investigated model of the pathological sequelae which occur after extensive destruction of renal parenchyma is the rat remnant kidney model²⁻⁵. One of the central pathological findings in this model has been documented to be a rapid postablation increase in glomerular size due to increased cell numbers, increased cell size, increased deposition of extracellular material, and capillary dilation⁶⁻⁹. Many pathomechanisms have been proposed to be responsible for the ultimate development of glomerulosclerosis, including glomerular hypertension and hyperfiltration¹⁰, glomerular hypertrophy¹¹, epithelial cell damage^{12,13}, intracapillary thrombosis^{6,14}, macrophage influx^{1,14}, macromolecule influx^{1,14-16}, and serum lipid abnormalities^{17,18}. In addition, factors such as platelet-derived growth factor (PDGF) or transforming growth factor β 1 (TGF- β 1) are

known to be induced as a consequence of intracapillary abnormal platelet-platelet activation within the glomerulus¹⁹. The *in vitro* experiments demonstrated that these growth factors are responsible for mesangial cell proliferation¹⁹ and increased production of mesangial matrix^{20,21}. However, there are few *in vivo* studies concerning histological or immunohistochemical features of mesangial cells involved in glomerulosclerosis. In contrast, a correlation between mesangial or glomerular cell proliferation and subsequent development of sclerosis has been found in minimal change diseases progressing to focal sclerosis in humans²². Taking together the above-mentioned findings, the mechanisms of glomerulosclerosis are complicated and have not been sufficiently clarified.

With this background, the present study was performed to investigate the pathomechanism of glomerulosclerosis in the rat remnant kidney model, particularly pathologic features of mesangial cells.

Materials and Methods

Male SD rats, four weeks old, were obtained from Charles River Japan Inc. (Shiga, Japan) and housed at a maximum of 3 to a polycarbonated cage with white chips as bedding in an air-conditioned animal room (room temperature, 24 $\pm$ 3°C; relative humidity, 60 $\pm$ 10%; lighting cycle, 12 light/12 dark). Animals without any abnormal findings after a two-week acclimatization period were selected for the present study. Pellet diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo, Japan) and distilled water were available *ad libitum* throughout the study.

A total of 14 rats were divided into 2 groups consisting

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Received: 6 July 1998, Accepted: 7 September 1998

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of 7. Seven rats were subjected to removal of three-fourths of kidney tissue, i.e. the left side was all dissected out and half of the right side was removed by ablation of subcapsular portions (3/4 nephrectomized group). These animals were then maintained without further treatment for 8 weeks. The remaining seven rats received a sham operation consisting of laparotomy and manipulation of the renal pedicles but without destruction of renal tissue and were maintained without treatment for 8 weeks (sham-operated group).

At weeks 2, 4, 6, and 8, the 24-hr urine was collected from the animals to determine the protein level. At week 8, the blood pressure was recorded by the tail cuff method. All the animals were then autopsied and the right kidney was dissected out. Kidney tissues were fixed in methanol Carnoy solution, embedded in paraffin, sectioned at 4–5 μ m, and stained with hematoxylin and eosin (H-E) for microscopic examination. Azan and PAS staining were also performed. In addition, immunohistochemical staining using a monoclonal antibody against proliferating cell nuclear antigen (PCNA), α -smooth muscle actin (α -SMA), and desmin, obtained from DAKO (Glostrup, Denmark), platelet-derived growth factor (PDGF) receptor obtained from Calbiochem (Cambridge, England) and ED-1 obtained from Biomedicals AG (Switzerland), was performed at a dilution of 1:100, 1:50, 1:100, 1:100 and 1:500, respectively. DAKO ABC kits were used for performance of ABC immunohistochemistry with 3, 3'-diaminobenzidine as the chromogen and hematoxylin for counterstaining. The number of glomerulosclerotic lesions was counted on all glomeruli per section of the right kidney. Glomerulosclerosis index was presented as the ratio of the number of glomeruli with sclerotic lesions to the number of glomeruli per renal section. The number of composing cells and PCNA-positive cells per glomerulus was counted and the labeling index was presented as the number of PCNA-positive cells per 100 glomerular cells. The staining score of α -SMA, desmin, and PDGF receptor was semiquantitatively graded as follows: 0, no or very weak staining of the glomerular tuft; 1, a weak staining with up to 10% of the glomerular tuft; 2, a focal staining with 10 to 25% of the glomerular tuft; 3, a focal staining with 25 to 50% of

the glomerular tuft; 4, a diffuse, strong staining with 50 to 75% of the glomerular tuft. The number of ED-1 positive cells per glomerulus was also counted.

Data for blood pressure, urinary protein, glomerulosclerosis index, the number and PCNA labeling index of glomerular cells, staining scores of α -SMA, desmin, and PDGF receptor and the number of ED-1 positive cells were used to generate the mean and standard deviation values (standard error for blood pressure and urinary protein), and intergroup differences were then analyzed with the Student's *t* test.

Results

The level of urinary protein in the 3/4 nephrectomized group elevated significantly from 4 weeks as compared to the sham-operated group and thereafter the value reached a plateau (**Table 1**). At week 8, significantly high blood pressure was seen in the 3/4 nephrectomized group. The glomerulosclerosis index (19.0 ± 13.38) of the 3/4 nephrectomized group was significantly increased as compared to that (0.1 ± 0.26) of the sham-operated group (**Table 2**). As shown in Figs. 1 and 2, there was a positive correlation between urinary protein and glomerulosclerosis index, while there was a negative correlation between the number of glomeruli per section and glomerulosclerosis index. Glomerulosclerotic lesions were segmental, and focally observed in the 3/4 nephrectomized group (Fig. 3), accompanying with capillary obsolescence, mesangial matrix increase (proliferation of collagen fibers indicated by Azan staining) and PAS positive granule deposits in the glomerular tuft. Significant increases in number of glomerular cells (including mesangial cells) were seen in the 3/4 nephrectomized group and their PCNA labeling indices also elevated (**Table 2** and Fig. 4). The expression of α -SMA and desmin was mainly localized to mesangial cytoplasm and some was also observed in the mesangial matrix. The staining score of α -SMA and desmin in the 3/4 nephrectomized group was 0.81 ± 0.43 and 1.99 ± 0.83 , respectively, attaining a significant increase in comparison with those values (0.01 ± 0.04 and 0.03 ± 0.05) in the sham-operated group (**Table 3** and Figs. 5, 6). There

Table 1. Data for Urinary Protein and Blood Pressure in Rats Receiving 3/4 Nephrectomy

Group	No. of animals	Urinary protein (mg/kg/day)				Blood pressure (mmHg)
		2	4	6	8 (weeks)	
Sham operation	7	38 ± 7.8^a	41 ± 4.0	34 ± 4.0	29 ± 4.2	129.8 ± 5.9
3/4 nephrectomy	7	41 ± 9.7	$184 \pm 90.3^*$	$174 \pm 52.9^*$	$186 \pm 63.6^*$	$152.6 \pm 5.5^{**}$

^a): Mean $\pm$ S.E.

*, **: Significantly different from the sham-operated group at $P < 0.05$ and 0.01 , respectively.

Table 2. Glomerulosclerosis Index, and Number and PCNA Labeling Index of Composing Cells per Glomerulus in Rats Receiving 3/4 Nephrectomy

Group	No. of animals	Glomerulosclerosis index (%)	No. of composing cells per glomerulus	PCNA labeling index (%)
Sham operation	7	0.1 ± 0.26^a	91.3 ± 5.62	0.5 ± 0.15
3/4 nephrectomy	7	$19.0 \pm 13.38^*$	$118.8 \pm 8.80^*$	$1.95 \pm 0.58^*$

^a): Mean $\pm$ S.D.

*: Significantly different from the sham-operated group at $P < 0.01$.

Fig. 1. Correlation between glomerulosclerosis index and urinary protein in rats receiving 3/4 nephrectomy.

Fig. 2. Correlation between glomerulosclerosis index and number of glomeruli in rats receiving 3/4 nephrectomy.

Fig. 3.

Fig. 3. Photomicrograph of the glomerulus in the 3/4 nephrectomized group. Note segmental glomerulosclerosis with obliteration of capillaries and mesangial matrix increase. Azan staining, $\times 400$.

Fig. 4.

Fig. 4. Immunohistochemistry of the glomerulus in the 3/4 nephrectomized group. Note a few PCNA positive cells within the glomerulus (arrows). PCNA immunostaining, $\times 400$.

Table 3. The Staining Score of α -Smooth Muscle Actin (SMA), Desmin, and Platelet-derived Growth Factor (PDGF) Receptor, and the Number of ED-1 Positive Cells per Glomerulus in Rats Receiving 3/4 Nephrectomy

Group	No. of animals	α -SMA staining score	Desmin staining score	PDGF receptor staining score	No. of ED-1 positive cells
Sham operation	7	0.01 ± 0.04^a	0.03 ± 0.05	0.83 ± 0.21	0.13 ± 0.15
3/4 nephrectomy	7	$0.81 \pm 0.43^{**}$	$1.99 \pm 0.83^{**}$	$2.20 \pm 0.13^{**}$	$0.97 \pm 0.76^*$

^{a)}: Mean $\pm$ S.D.

*, **: Significantly different from the sham-operated group at $P < 0.05$ and 0.01 , respectively.

was no apparent correlation between glomerulosclerosis index and the degree of α -SMA or desmin expression. The staining score of PDGF receptor in the glomerular tuft in the 3/4 nephrectomized group was 2.20 ± 0.13 , attaining a significant increase as compared to that (0.83 ± 0.21) in the sham-operated group (Table 3 and Fig. 7). The expression of α -SMA, desmin, and PDGF receptor was all segmental. In addition, intraglomerular macrophage influx (proven by

ED-1 staining) was seen in the 3/4 nephrectomized group (Table 3 and Fig. 8).

Discussion

Glomerulosclerosis has been known to be a disorder evoked through a complex pathologic process after tissue injury. In the present study, glomerulosclerotic changes

Fig. 5-A.

Fig. 5-B.

Fig. 5. Immunohistochemistry of the glomerulus in the sham-operated or 3/4 nephrectomized group. (A) α -SMA positive reactions within the glomerulus are absent in the sham-operated group. (B) Note segmental α -SMA positive reactions in the mesangial cytoplasm and matrix in the 3/4 nephrectomized group. α -SMA immunostaining, $\times 400$.

Fig. 6-A.

Fig. 6-B.

Fig. 6. Immunohistochemistry of the glomerulus in the sham-operated or 3/4 nephrectomized group. (A) No desmin positive reactions within the glomerulus are seen in the sham-operated group. (B) Note segmental desmin positive reactions in the mesangial cytoplasm and matrix in the 3/4 nephrectomized group. Desmin immunostaining, $\times 400$.

were induced by 3/4 nephrectomy and associated with proteinuria, hypertension, capillary obsolescence, increases of mesangial cells and matrix, expression of α -SMA and desmin in the glomerular tuft (mainly mesangial cells) and PDGF receptor in the glomerular tuft, and macrophage influx. There was a negative correlation between the number of glomeruli and glomerulosclerosis index, demonstrating that impaired endothelial cells of glomerular capillaries by rapidly increased blood flow into the remaining nephrons and hypertension would play a key role in the development of glomerulosclerosis. This model has been widely used for assessing the therapeutic effects of the test drug against renal diseases^{3,4} as well as for investigating the mechanisms of renal diseases²³. Considering that individual glomerulosclerosis

index in the 3/4 nephrectomized group varied in degree and fell within the ranges of 2.5 to 41.6 (Fig. 1), it is speculated that development of glomerulosclerotic lesions is strongly dependent on the extent of renal cortical ablation. Therefore, it would be necessary to monitor the urinary protein level for preparing the animals with glomerulosclerotic lesions effectively although glomerulosclerosis index in the present experiment was similar to that documented by other investigators³.

PDGF is one of the most potent mesangial mitogens *in vitro*, and it can be released not only from activated platelets, but also from macrophages, endothelial cells, and mesangial cells themselves²⁴⁻²⁷. Indeed, the intraglomerular expression of PDGF receptor, indicative of excessive production of

Fig. 7-A.

Fig. 7-B.

Fig. 7. Immunohistochemistry of the glomerulus in the sham-operated or 3/4 nephrectomized group. (A) Little PDGF receptor positive reactions within the glomerulus are seen in the sham-operated group. (B) Note segmental PDGF receptor positive reactions within the glomerulus in the 3/4 nephrectomized group. PDGF receptor immunostaining, $\times 330$.

Fig. 8. Immunohistochemistry of the glomerulus in the 3/4 nephrectomized group. Note a few ED-1 positive cells within the glomerulus (arrows). ED-1 immunostaining, $\times 400$.

PDGF within the glomerulus, was induced by partial nephrectomy. The present study thus demonstrates that growth factors such as PDGF would be responsible for mesangial proliferation leading to glomerulosclerotic changes. In contrast, it has been documented that the expression of PDGF and PDGF receptor after partial nephrectomy in rats was observed from weeks 1 and 2, respectively², suggesting that the expression of PDGF and PDGF receptor would also have occurred from earlier stages after treatment in the present study. Furthermore, the present results indicate that intraglomerular macrophage influx may possibly contribute to the development of glomerular sclerotic changes.

A few investigators have reported that in kidney, lung or liver fibrosis, myofibroblasts expressing α -SMA are constant-

ly present and in addition, a proportion of them also express desmin^{28,29}. In contrast, in the normal rat kidney, expression of α -SMA and desmin can be noted only in vascular smooth muscle cells, but not in mesangial cells. Previous investigator has reported that intraglomerular expression of α -SMA was observed from day 3 after partial nephrectomy, and in addition the number of mesangial cells increased from week 2². To our knowledge, there are no reports that desmin expression in mesangial cells was demonstrated on glomerulosclerosis in rats. However, taking into account that both α -SMA and desmin have been known to be present in rat mesangial cells³⁰, we attempted to investigate the expression of these myofilaments and demonstrated their positive reactions in mesangial cells. Accordingly, our results and previous documentation² speculate that in the present study α -SMA and desmin would have expressed in mesangial cells at earlier stages after partial nephrectomy. In addition, urinary protein levels increased after 4 weeks of partial nephrectomy, suggesting that glomerular changes began to develop after 2 weeks. These facts indicate that the expression of such myofilaments precedes the development of glomerulosclerosis. In contrast, normal mesangial cells have the capability for producing the matrix, but never produce it excessively. Also, there is evidence that the process of fibrosis represents an imbalance between extracellular matrix protein synthesis and degradation³¹. Taking together the above facts, the present expression of α -SMA and desmin in mesangial cells suggests that the upregulation of these filaments serves as indicators of mesangial cell modulation within the glomerulus, although the precise mechanisms that lead to the proliferation of glomerular cells resulting in excessive matrix production remain unclear. Also, there was no apparent correlation between glomerular lesions and the degree of α -SMA or desmin expression, and it is thus unlikely that the expression of these myofilaments at later stages after partial nephrectomy may indicate the progression of glomerulosclerosis. Based on the findings that urinary protein levels reached a plateau after 4 weeks and glomerulosclerotic lesions after 8 weeks were not marked, it is considered that the

present glomerulosclerosis may not be of progressive nature.

In conclusion, the present results suggest that the key pathologic changes for glomerulosclerosis are impaired endothelial cells of glomerular capillaries by rapidly increased blood flow into the remaining nephrons and the resultant production of growth factors such as PDGF leading to mesangial cell proliferation. Furthermore, the present expression of α -SMA and desmin in mesangial cells suggests a phenotype switch of mesangial cells responsible for the induction of glomerulosclerosis.

Acknowledgments: We are grateful to Mr. Kazuhiro Nakamura and Mmes. Sachiyo Maeda and Setsuko Tanaka for their technical assistance.

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