

Original

## Thy-1 Expressing Mesenchymal Cells in Rat Nephrogenesis in Correlation with Cells Immunoreactive for $\alpha$ -Smooth Muscle Actin and Vimentin

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**Abstract:** Thy-1 expression may influence myofibroblast development. Through the epithelial-mesenchymal transition (EMT), injured renal epithelial cells undergo regression to the metanephric mesenchymal phenotype and then acquire a myofibroblastic nature (expressing  $\alpha$ -smooth muscle actin;  $\alpha$ -SMA). Because the metanephric blastema differentiates into mesenchymal and renal epithelial cells, we investigated Thy-1 immunoexpression during nephrogenesis in F344 rats in correlation with vimentin and  $\alpha$ -SMA expressions. Kidney samples were obtained from fetuses on gestation days 18 and 21, neonates on days 1–18 and adults at 6 weeks of age. Mesangial cells in S-shaped bodies and immature and mature glomeruli continuously expressed both Thy-1 and  $\alpha$ -SMA during early nephrogenesis (fetuses and neonates on days 1–9). During early nephrogenesis, loosely-arranged blastemal cell-derived mesenchymal cells in the cortex and medulla also exhibited Thy-1 and  $\alpha$ -SMA, although the  $\alpha$ -SMA expression was weaker than that of Thy-1. Vimentin expression coincided with that of Thy-1. These findings indicate that the derivation of  $\alpha$ -SMA-expressing myofibroblastic cells may be related to mesangial or blastemal cells expressing both Thy-1 and  $\alpha$ -SMA. Interestingly, there was a difference in Thy-1 expression between cortical and medullary tubulointerstitial cells from late nephrogenesis (neonates on days 12–18) and those from adults in that the cortical cells reacted faintly or negatively to Thy-1, whereas the medullary cells reacted strongly to Thy-1; additionally, bundle-arranged mesenchymal cells that were only observed in the neonates on days 1–12 reacted strongly to  $\alpha$ -SMA, but faintly to Thy-1. Blastemal cell-derived mesenchymal cells seem to alter the immunoexpressions of Thy-1 and  $\alpha$ -SMA, depending on the conditions which they develop. Thy-1 immunoexpression would be useful for investigation of reverse embryogenesis, which might occur in fibrotic kidneys. (*J Toxicol Pathol* 2010; 23: 1–10)

**Key words:**  $\alpha$ -smooth muscle actin, blastemal cells, myofibroblasts, nephrogenesis, rat, Thy-1

### Introduction

Metanephric development begins with mutual induction between the mesenchymal cells of the metanephric blastema and epithelial cells of the ureteric bud. After induction, some blastemal cell-derived mesenchymal cells form epithelial cells of the renal tubules and glomeruli through mesenchymal-epithelial transition (MET); others persist in the interstitium, maintaining the features of mesenchymal cells<sup>1</sup>. The blastemal cells have potential to differentiate toward both epithelial and mesenchymal cells during nephrogenesis. In renal interstitial fibrosis, which has been regarded as the common and ultimate pathway in

chronic renal diseases regardless of the etiology, myofibroblasts play an important role by producing excessive extracellular matrices such as collagens<sup>2,3</sup>. The myofibroblasts have an intermediate nature between fibroblasts and smooth muscle cells and are also called contractile cells<sup>4</sup>. Besides the pre-existing interstitial fibroblasts, in the fibrotic kidney, it has been considered that renal tubular epithelial cells may be a possible origin of myofibroblasts<sup>3,5,6</sup>. In the process known as the epithelial-mesenchymal transition (EMT), mature epithelial cells undergo regression to the metanephric mesenchymal phenotype in response to injury and then acquire myofibroblastic characteristics<sup>7–10</sup>. This suggests that reverse embryogenesis occurs in fibrotic kidneys. However, the relationship between blastemal cells and myofibroblasts remains to be investigated.

Thy-1 (CD90) is a 25–37-kDa GPI-anchored cell surface protein<sup>11</sup>. Hematopoietic and mesenchymal stem cells have been reported to react with the Thy-1 antibody based on findings from immunohistochemistry<sup>12, 13</sup>.

Received: 3 August 2009, Accepted: 30 September 2009

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Additionally, Thy-1 is heterogeneously expressed in fibroblastic cells; its expression may correlate with myofibroblast differentiation in the uterus, lungs, skin, orbit and cornea<sup>14–18</sup>. Thy-1 immunohistochemistry appears to be useful for exploration of the origin and nature of myofibroblasts. Although it is well known that glomerular mesangial cells specifically express Thy-1<sup>19, 20</sup>, the distribution of mesenchymal cells expressing Thy-1 has not yet been investigated during rat renal development. The myofibroblasts seen in renal fibrotic lesions are characterized fundamentally by expressions of cytoskeletons such as  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and vimentin<sup>3,4</sup>. In the present study, therefore, the detailed distribution of Thy-1 expressing cells was investigated in developing kidney tissues of rats obtained from fetuses, neonates and adults, in correlation with cells expressing  $\alpha$ -SMA and vimentin, by immunohistochemistry. The present study showed that along with glomerular mesangial cells, blastemal cells expressed Thy-1 and vimentin during early nephrogenesis; the Thy-1-positive cells partly agreed with  $\alpha$ -SMA-positive cells. There may be a close relationship between blastemal cells and cells with myofibroblastic features.

## Materials and Methods

### Animals

The following experiments were conducted in full compliance with our institutional guidelines for animal care. Pregnant F344 rats were obtained from Charles River Japan (Hino, Shiga, Japan). They and their neonates after birth were housed in an animal room at  $22 \pm 3^\circ\text{C}$  with a 12 h light-dark cycle and allowed free access to a standard commercial diet (MF, Oriental Yeast Co, Ltd., Tokyo, Japan) and tap water. Animals were euthanized by exsanguination under ether anesthesia, and kidney samples were obtained from fetuses on gestation days (GDs) 18 and 21, neonates on days 1, 3, 6, 9, 12, 15 and 18, and adults at 6 weeks of age ( $n=3-6$  at each examination point). The adult rats at 6 weeks of age were all male, and both sexes were used for fetuses and neonates.

### Histopathology and Immunohistochemistry

Renal tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 3–4  $\mu\text{m}$  thickness and stained with hematoxylin and eosin (HE) for morphological observations.

For the immunohistochemical analyses, renal samples were fixed in periodate-lysine-paraformaldehyde (PLP) fixative, embedded in paraffin by the AMeX method (PLP-AMeX method) and sectioned at 2–3  $\mu\text{m}^2$ . The peroxidase-labeled amino acid polymer method was used with the following primary antibodies: anti-Thy-1 monoclonal antibody ( $\times 100$ ; Cedarlane Laboratories Limited, Burlington, ON, Canada), anti- $\alpha$ -SMA monoclonal antibody ( $\times 200$ ; Dako, Corp., Carpinteria, CA, USA) and anti-vimentin monoclonal antibody ( $\times 200$ ; Dako). For antigen retrieval, deparaffinized sections for Thy-1 and vimentin

were pretreated with a microwave for 10 min, and sections for  $\alpha$ -SMA were incubated with 0.1% trypsin for 10 min at  $37^\circ\text{C}$ . After pretreatment, sections were incubated with 3%  $\text{H}_2\text{O}_2$  for 10 min to quench endogenous peroxidase and then with 5% skimmed milk in phosphate-buffered saline (PBS) for 30 min; they were then incubated with each primary antibody overnight at  $4^\circ\text{C}$ . Next, incubation with a Histofine Simple Stain MAX-PO Kit (Nichirei, Tokyo, Japan) as the secondary antibody for monoclonal antibodies was performed for 1 h. Positive reactions were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Vector Laboratories, Burlingame, CA, USA). Serial sections were made for co-expression to Thy-1 and  $\alpha$ -SMA or vimentin. Non-immunized mouse serum in place of the primary antibody served as negative controls. Sections were counterstained lightly with hematoxylin.

### Evaluation

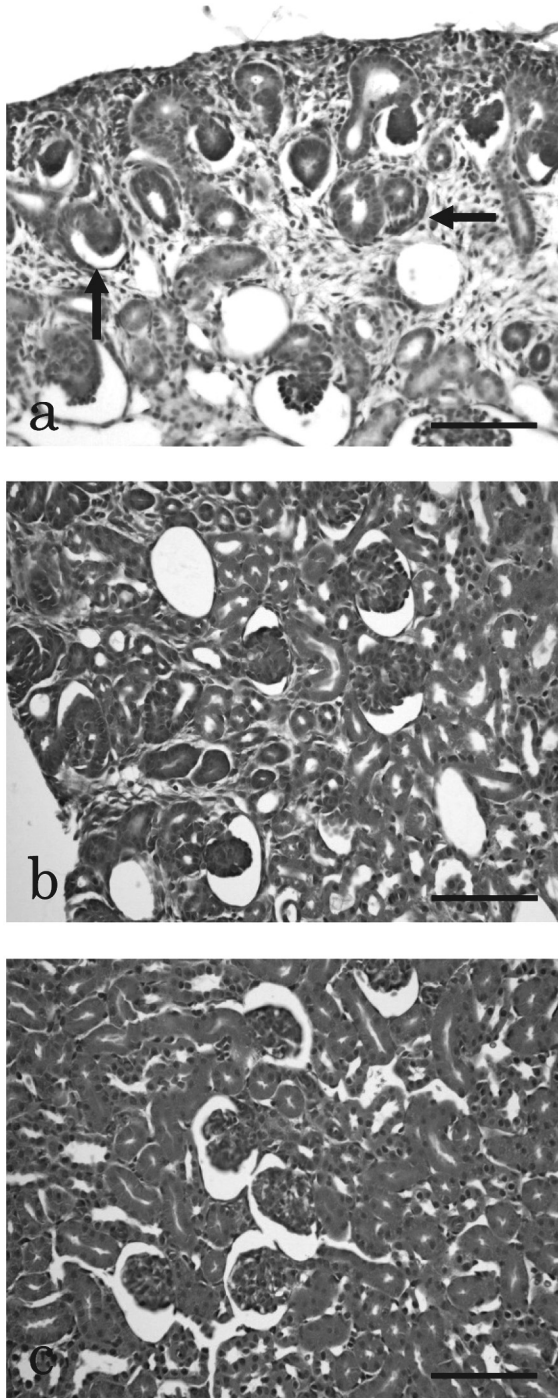
Based on the immunohistochemical reactivity to Thy-1,  $\alpha$ -SMA or vimentin in the glomeruli, cortex and medulla, the degree was evaluated as follows: –, negative;  $\pm$ , faintly positive; +, moderately/strongly positive.

## Results

### Nephrogenesis

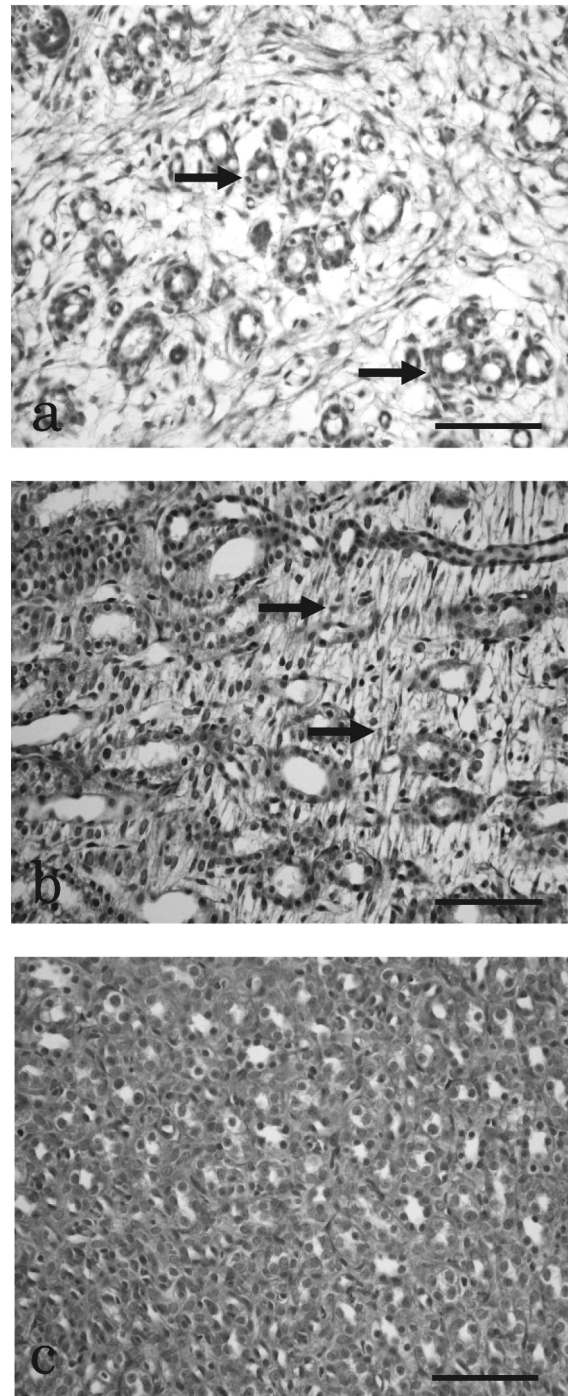
To confirm nephrogenesis in the rat samples used in this study, we observed the histology in HE-stained sections. In the cortical areas of the fetuses on GDs 18 (Fig. 1a) and 21, loosely-arranged blastemal cell-derived mesenchymal cells were abundantly seen among developing renal tubules and glomeruli formed through the MET; the developing glomeruli consisted of round-, comma- and S-shaped bodies (Fig. 1a, arrows) generated from vesicles on the part of the condensed metanephric blastema around the tip of the ureteric bud (Fig. 1a). The mesenchymal cells were gradually decreased with age after birth (neonates), and instead, mature renal tubules and glomeruli became predominant (Fig. 1b); in neonates on 18 days, renal tissues were completely formed and had similar histologies to those of the adult rats (Fig. 1c); mesenchymal cells were only present in the tubulointerstitium of the completed kidney.

In the medullary areas of the fetuses on GDs 18 and 21 (Fig. 2a), loosely-arranged mesenchymal cells were seen surrounding the branched epithelial ureteric tubules (Fig. 2a, arrows); in the neonates on days 1–15, the loosely-arranged mesenchymal cells gradually decreased. In the neonates on day 1, mesenchymal cells arranged parallel to each other and perpendicular to the developing renal tubules appeared in the cortic-medullary junction; these bundle-arranged mesenchymal cells became predominant in the neonates on days 3–12 (Fig. 2b, arrows) and then disappeared in the neonates on days 15 and 18 and the adults at 6 weeks of age. In the neonates on day 18, mature renal tubules and collecting ducts were observed, and a small number of mesenchymal cells were present in the tubulointerstitium (Fig. 2c) in the medulla and around blood vessels in the



**Fig. 1.** Cortical areas during rat nephrogenesis. Loosely-arranged mesenchymal cells are prominently seen among the developing renal tubules, and there are round-, comma- and S-shaped bodies (arrows) as well as immature glomeruli in the fetus on gestation day (GD) 18 (a). In conjunction with nephrogenesis, the mesenchymal cells are decreased in neonates on days 6 (b) and 18 (c). HE stain. Bar=50  $\mu$ m.

cortico-medullary junction. These histological observations indicate that nephrogenesis finishes around 18 days of age in rats.



**Fig. 2.** Medullary areas during nephrogenesis. Loosely-arranged mesenchymal cells are seen mainly around the branched epithelial ureteric tubules (arrows) in the fetus on gestation day 21 (a). Mesenchymal cells arranged parallel to each other and perpendicular to the developing renal tubules (arrows) are present in the cortico-medullary junction of the neonate on day 3 (b). Mature renal tubules and tubulointerstitial cells are seen in neonate on day 18 (c). HE stain. Bar=50  $\mu$ m.

#### *Immunohistochemistry during nephrogenesis*

We evaluated the immunoreactivity of mesenchymal cells for Thy-1,  $\alpha$ -SMA and vimentin in the glomeruli

**Table 1.** Immunohistochemical Reactivity to Thy-1,  $\alpha$ -Smooth Muscle Actin ( $\alpha$ -SMA) and Vimentin During Rat Nephrogenesis

Cells	Antibody to	Gestation days (fetuses)		Days after birth (neonates)						Adults	
		18	21	1	3	6	9	12	15	18	6 weeks
Mesangial cells*	Thy-1	+	+	+	+	+	+	+	+	+	+
	$\alpha$ -SMA	+	+	+	±	±	±	±	±	±	±
	Vimentin	+	+	+	+	+	+	+	+	+	+
Mesenchymal cells in the cortex **	Thy-1	+	+	+	+	+	+	±	±	±	-
	$\alpha$ -SMA	±	±	±	+	+	+	+	+	+	-
	Vimentin	+	+	+	+	+	+	±	±	±	±
Mesenchymal cells in the medulla**	Thy-1	+	+	+	+	+	+	+	+	+	+
	$\alpha$ -SMA	±	±	±	+	±	±	-	-	-	-
	Vimentin	±	+	+	+	+	+	+	+	+	+
Bundle-arranged mesenchymal cells in the cortico-medullary junction***	Thy-1			±	±	±	±	±			
	$\alpha$ -SMA			+	+	+	+	+			
	Vimentin			±	±	±	±	±			

Immunoreactivity was evaluated as follows: -, negative; ±, faintly positive; +, more strongly positive. \*: Mesangial cells seen in the S-shaped body and immature and mature types of glomeruli. \*\*: Mesenchymal cells loosely-arranged in the cortex and medulla in fetuses and early stage neonates, and tubulointerstitial mesenchymal cells in late stage neonates and adults. \*\*\*: The unique and preferential arrangement was seen only in neonates on days 1-12.

(mesangial cells), cortex and medulla (Table 1).

#### *Immunoreactivity for Thy-1*

In the fetuses on GDs 18 (Figs. 3a and 3c) and 21 and the neonates on days 1-18, a few spindle-shaped cells reacting to Thy-1 were seen among the epithelial cells in the S-shaped bodies (Figs. 3a and 3c, large arrows); mesangial cells in the immature glomeruli, in which podocytes and blood vessels were being formed; and in the mature glomeruli, which reacted strongly to Thy-1 (+) (Figs. 3a and 3c, small arrows). In the adults at 6 weeks of age, the mesangial cells in all glomeruli showed a strong reaction to Thy-1 (+) (Fig. 6a).

In the cortical areas, loosely-arranged mesenchymal cells around the developing renal tubules and glomeruli in the fetuses on GDs 18 (Figs. 3a and 3c, arrowheads) and 21 and the neonates on days 1 and 3 gave a strong positive reaction to Thy-1 (+), as did interstitial mesenchymal cells close to the developed renal tubules in the neonates on days 6 and 9; however, the reactivity of interstitial mesenchymal cells to Thy-1 became faint in the neonates on days 12-18 (±), and Thy-1-positive cells were not seen in the cortex of adults (-) (Fig. 6a, Table 1).

In the medullary areas, loosely-arranged mesenchymal cells around the immature renal tubules and branching ureteric buds (collecting ducts) in the fetuses on GDs 18 (Fig. 4a) and 21 and the neonates on day 1 were strongly reactive to Thy-1 (+); interstitial mesenchymal cells around the developed renal tubules and collecting ducts in the neonates on days 3-18 (Fig. 5a and 5c, small arrows) and in the adults at 6 weeks of age (Fig. 6c) also reacted to Thy-1 (+) (Table 1). Interestingly, the bundle-arranged mesenchymal cells seen mainly in the cortico-medullary

junction in the neonates on days 1 to 12 showed a faint reaction to Thy-1 (±) (Figs. 5a and 5c, large arrows; Table 1).

As described above, in the cortex of the adults at 6 weeks of age, Thy-1 reacted only with glomerular mesangial cells and perivascular cells around arterioles (inset in Fig. 6a, arrow) (+), but did not react with tubulointerstitial cells (-) (Fig. 6a); interestingly, Thy-1 strongly reacted with tubulointerstitial cells in the medulla (+) (Fig. 6c, Table 1).

#### *Immunoreactivity for vimentin*

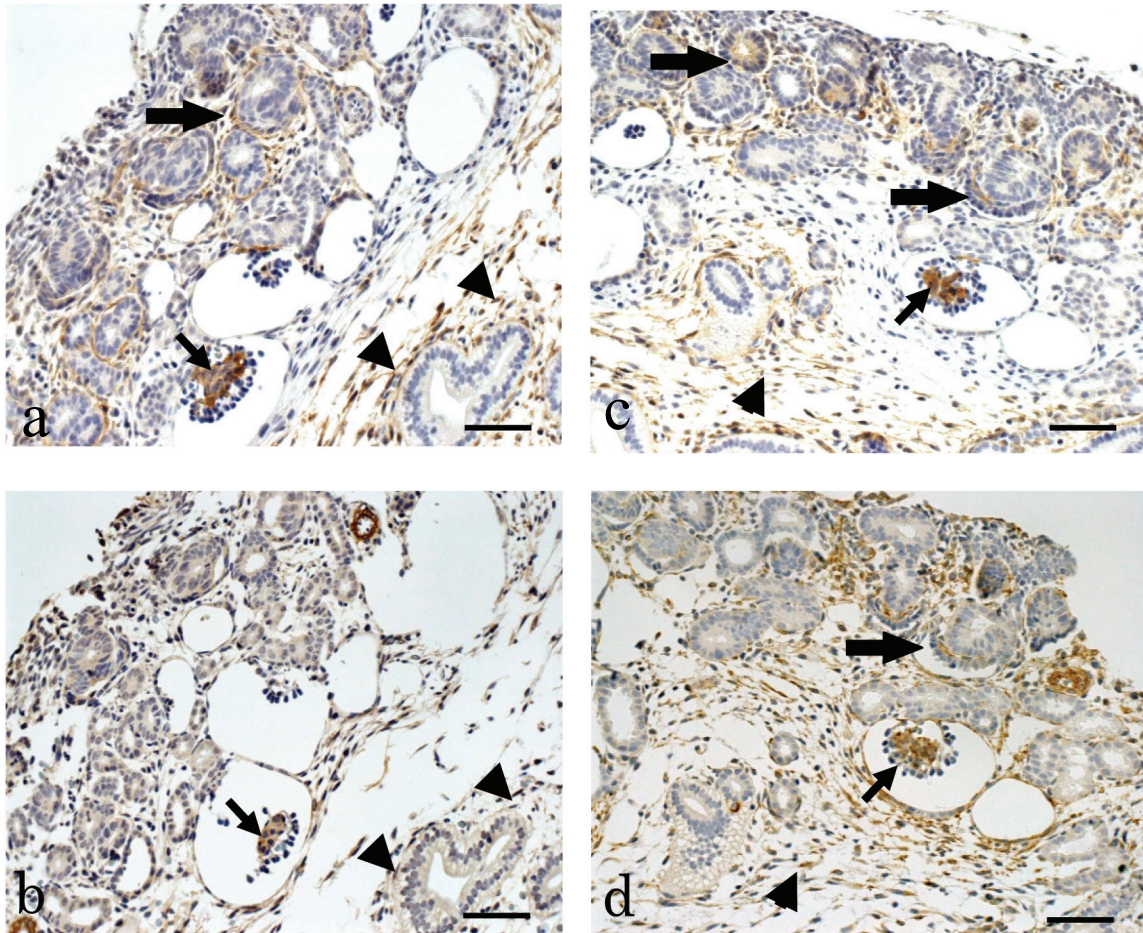
Mesangial cells in the S-shaped body (Fig. 3d, large arrow) and immature (Fig. 3d, small arrow) and mature types of glomeruli of the fetuses, neonates and adults consistently showed a positive reaction to vimentin; the immunoreactivity of the mesangial cells to vimentin was similar to that for Thy-1 (Table 1).

Loosely-arranged mesenchymal cells in the cortical and medullary areas in the fetuses (Fig. 3d, arrowhead), neonates (Fig. 5d, small arrows) and adults showed a positive reaction for vimentin, although the reactivity of the mesenchymal cells in the cortex became faint in the neonates on days 12-18 and in the adults at 6 weeks of age (Table 1); the reactivity was generally in agreement with that for Thy-1 (Figs. 3c and 3d for the cortex of the fetus; Fig. 5c and 5d for the cortico-medullary junction; Table 1).

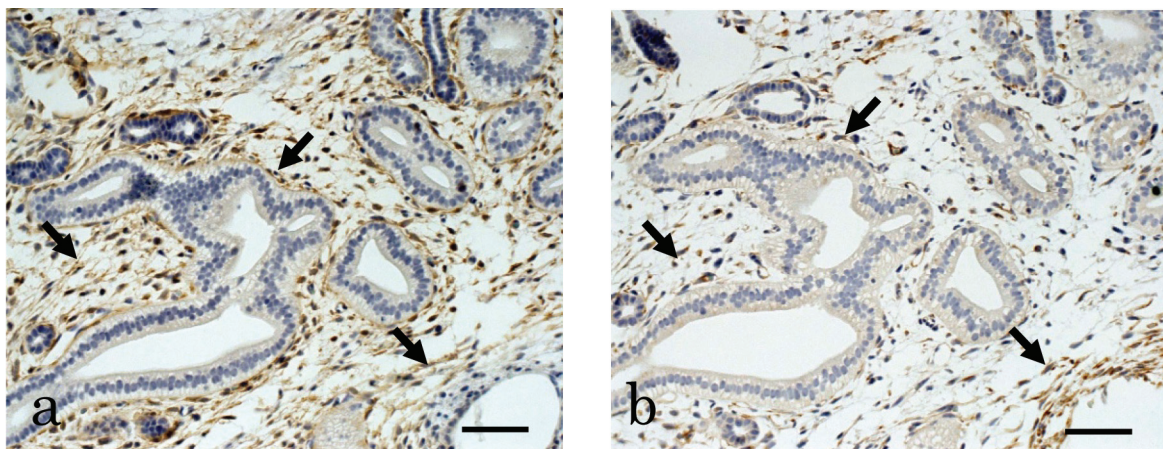
Bundle-arranged mesenchymal cells in the neonates on days 1-12 reacted faintly to vimentin (Fig. 5d, large arrow).

#### *Immunoreactivity for $\alpha$ -SMA*

Mesangial cells in the S-shaped body and immature type of glomeruli of the fetuses on GDs 18 (Fig. 3b, arrow) and 21 and of the neonates on day 1 reacted strongly to  $\alpha$ -SMA; the reactivity was similar to that for Thy-1 (Figs. 3a



**Fig. 3.** Immunohistochemical findings in cortical areas for Thy-1 (a, c),  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) (b), and vimentin (d) on GD 18. Thy-1-positive cells are seen in S-shaped bodies (a, c; large arrows) and mesangial cell in immature glomeruli (a, c; small arrows); additionally, loosely-arranged mesenchymal cells in the cortex react to Thy-1 (a, c; arrowheads). In serial sections (a, b; small arrows), Thy-1-positive mesangial cells (a) also react to  $\alpha$ -SMA (b); additionally, there are loosely-arranged mesenchymal cells faintly reacting to  $\alpha$ -SMA in the cortex (b, arrowheads). In serial sections (c, d), the vimentin-positive mesangial cells seen in immature glomeruli (c, d; small arrows) and loosely-arranged mesenchymal cells in the cortex (c, d; arrowheads) correspond to Thy-1-positive cells. Immunohistochemistry, counterstained with hematoxylin. Bar=50  $\mu$ m.



**Fig. 4.** Immunohistochemical findings in medullary areas for Thy-1 (a) and  $\alpha$ -SMA (b) in serial sections (a, b) on GD 18. Loosely-arranged mesenchymal cells mainly around developing renal tubules react strongly to Thy-1 (a, arrows), whereas these cells show a faint reaction to  $\alpha$ -SMA (b, arrows). Immunohistochemistry, counterstained with hematoxylin. Bar= 50  $\mu$ m.

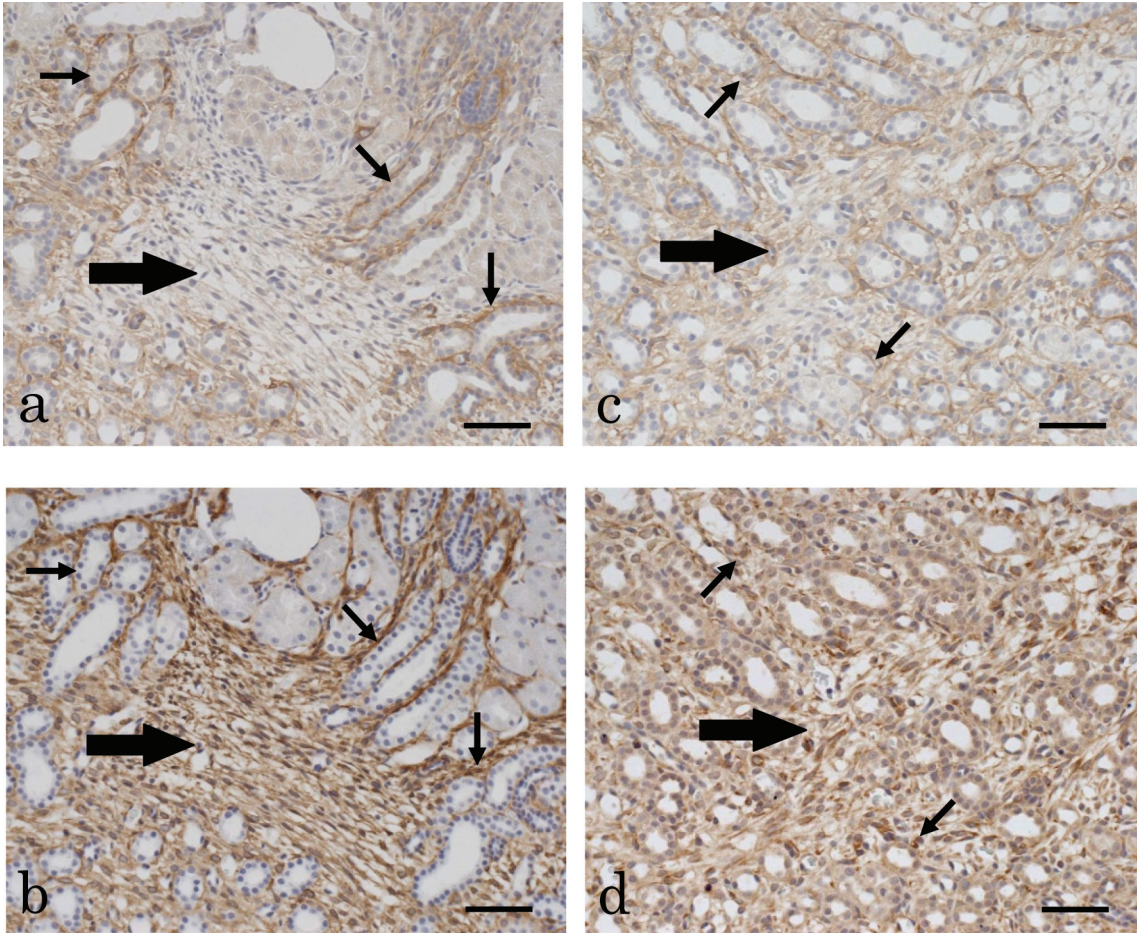


Fig. 5.

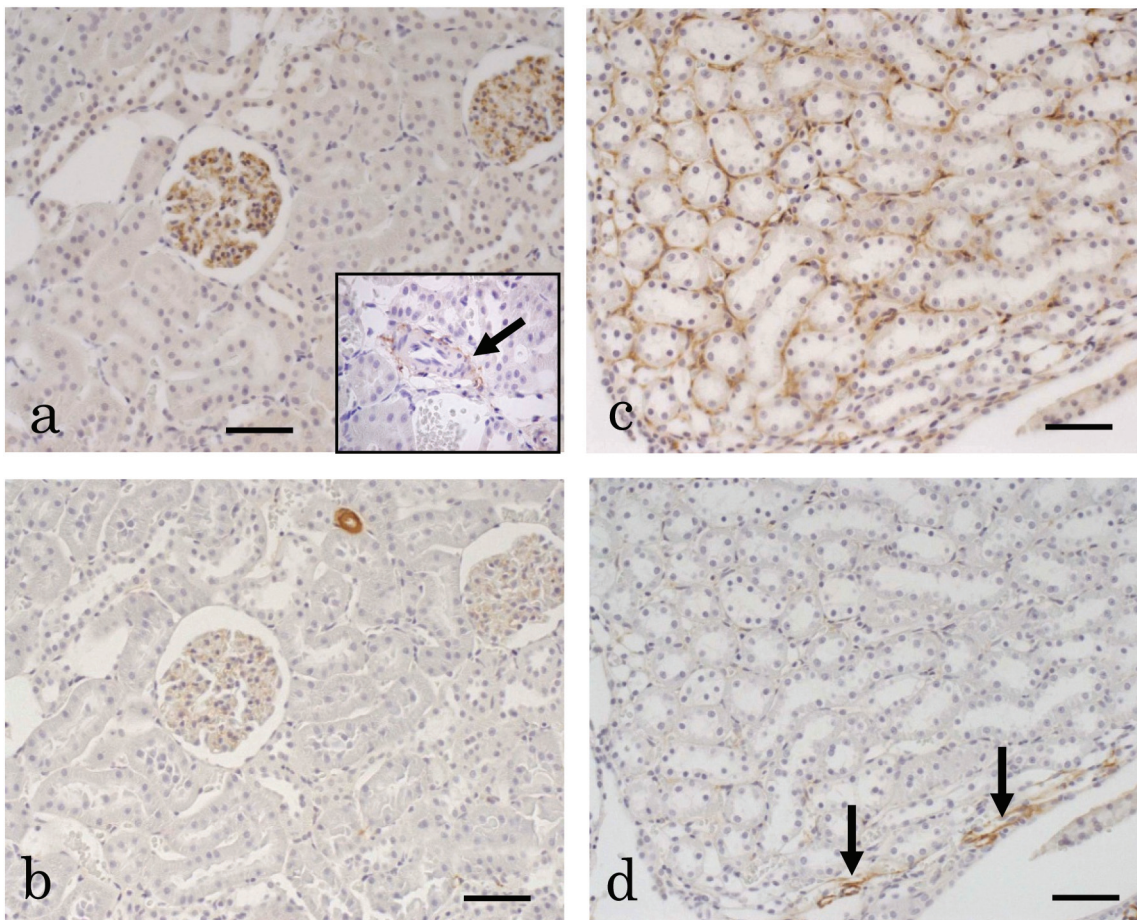


Fig. 6.

**Fig. 5.** Immunohistochemical findings in the cortico-medullary junction for Thy-1 (a, c),  $\alpha$ -SMA (b) and vimentin (d) 3 days after birth (neonate). Thy-1-positive cells are seen around the developing renal tubules (a, small arrows); the positive cells correspond to cells reacting to  $\alpha$ -SMA (b, serial section to a; small arrows) and vimentin (d, serial section to c, small arrows). Bundle-arranged mesenchymal cells seen in the cortico-medullary junction show a positive reaction for  $\alpha$ -SMA (b; large arrow), whereas they show a faint reaction for Thy-1 (a, b; large arrows); in serial sections (a for Thy-1 and d for vimentin), bundle-arranged mesenchymal cells reacted faintly to Thy-1 (a; large arrow) and vimentin (d; large arrow), in contrast to the strong reaction for  $\alpha$ -SMA in the mesenchymal cells (b; large arrow). Immunohistochemistry, counterstained with hematoxylin. Bar=50  $\mu$ m.

**Fig. 6.** Immunohistochemical findings for Thy-1 (a, c) and  $\alpha$ -SMA (b, d) in adults at 6 weeks of age. In serial sections (a, b) of the cortex, mesangial cells in mature glomeruli react strongly to Thy-1 (a) and faintly to  $\alpha$ -SMA (b). Tubulointerstitial cells in the cortex do not react to Thy-1 (a), whereas interstitial cells in the medulla show a strong reaction to Thy-1 (c). In serial sections (c, d) of the medulla, Thy-1-positive tubulointerstitial cells (c) do not react to  $\alpha$ -SMA (d); similarly, cortical tubulointerstitial cells are negative for  $\alpha$ -SMA (b). Spindle-shaped cells (so-called pericytes) surrounding arterioles show a positive reaction for Thy-1 (inset in a; arrow) and  $\alpha$ -SMA (d; arrows). Immunohistochemistry, counterstained with hematoxylin. Bar=50  $\mu$ m.

and 3b). However, the reactivity gradually decreased in the mature glomeruli of the neonates on days 3-18 and in the adults at 6 weeks of age (Fig. 6b); the reactivity was much weaker than that for Thy-1 (Table 1).

In the cortical areas, the reactivity of loosely-arranged mesenchymal cells to  $\alpha$ -SMA in the fetuses on GDs 18 and 21 and the neonates on day 1 was faint (Fig. 3b, arrowheads). On the other hand, the reactivity of the cells to Thy-1 was strongly positive (Fig. 3a, arrowheads). But tubulointerstitial mesenchymal cells close to the developing renal tubules and developed renal tubules in the neonates on days 3-18 exhibited a strong reaction for  $\alpha$ -SMA (Fig. 5b, small arrows); in the neonates on days 3-9, the distribution of the  $\alpha$ -SMA-positive cells coincided with that of Thy-1-positive cells (Fig. 5a and 5b, small arrows). Interestingly, no tubulointerstitial mesenchymal cells reacted to  $\alpha$ -SMA in the adults (Fig. 6b). In the medullary areas of the fetuses on GDs 18 and 21 and the neonates on days 1-9, except on day 3, the reactivity of loosely-arranged mesenchymal cells to  $\alpha$ -SMA was weaker than that for Thy-1 (Figs. 4a and 4b, Table 1), but the distribution of the cells reacting to Thy-1 appeared to correspond partly to that to  $\alpha$ -SMA (Figs. 4a and 4b, arrows). No interstitial mesenchymal cells reacting to  $\alpha$ -SMA were seen in the medulla in the neonates on days 12-18 or the adults at 6 weeks of age (Fig. 6d), although perivascular mesenchymal cells reacted to  $\alpha$ -SMA (Fig. 6d, arrows).

Bundle-arranged mesenchymal cells in the cortico-medullary junction in the neonates on days 1-12 reacted strongly to  $\alpha$ -SMA (Fig. 5b, large arrow), although these cells showed a very faint reaction for Thy-1 (Figs. 5a and 5c, large arrows) and vimentin (Fig. 5d, large arrow). There was a difference in the immunoreactivity of bundle-arranged mesenchymal cells between Thy-1/vimentin (faint) and  $\alpha$ -SMA (strong; Table 1).

## Discussion

### Mesangial cells

Thy-1 expression in glomerular mesangial cells has been well characterized<sup>19, 20</sup>; in particular, nephropathy induced in rats or mice by anti-Thy-1 antibody

administration has been used to investigate the pathogenesis of glomerulonephritis<sup>22</sup>. The present study confirmed Thy-1 expression in the mesangial cells of mature glomeruli in adults. Developing glomeruli during nephrogenesis consist of round-, comma- and S-shaped bodies and immature and mature types of glomeruli<sup>1</sup>. Mesangial cells in the immature type of glomeruli seen in the fetuses and neonates reacted strongly to Thy-1. Additionally, spindle cells present in the S-shaped body showed a reaction for Thy-1, however, there were no cells showing a clear reaction for Thy-1 in the round- and comma-shaped bodies. These findings indicated that mesangial cells started to be formed from the S-shaped body in conjunction with Thy-1 expression; because podocyte folding and vascularization began to be seen in the S-shaped body stage, formation of mesangial cells may be related to podocytes and endothelial cells<sup>23</sup>. Here, we must take into consideration the difference in immunoreactivity of Thy-1 between rats and humans; human Thy-1 expression in mesangial cells is lost with renal development<sup>24</sup>. The expression patterns of mesangial cells for vimentin were similar to those for Thy-1 (Table 1), indicating the mesenchymal nature of the mesangial cells. Mesangial cells in the S-shaped body and immature glomeruli in the fetuses on GDs 18 and 21, as well as in the neonates on day 1, reacted strongly to  $\alpha$ -SMA, and the reactivity gradually reduced until adulthood. In glomerular injury, such as IgA and anti-Thy-1 antibody nephropathy, mesangial cells have been considered to undergo transdifferentiation into myofibroblasts (expressing  $\alpha$ -SMA), culminating in glomerulosclerosis<sup>20, 25</sup>. Because the Thy-1-reacting mesangial cells during early nephrogenesis also expressed  $\alpha$ -SMA, transdifferentiation in glomerulosclerosis implies that glomerular mesangial cells undergo regression, thus showing the nature of immature mesangial cells.

### Loosely-arranged mesenchymal cells in the cortex and medulla

The present study showed that blastemal cell-derived mesenchymal cells loosely-arranged in the cortex and medulla reacted strongly to Thy-1 in the fetuses on GDs 18 and 21, as well as in the early stages in the neonates on days 1-9. The continuous expression of Thy-1 in the cortical and

medullary mesenchymal cells during early nephrogenesis was similar to that of glomerular mesangial cells. Additionally, the present study showed for the first time that there was a difference in Thy-1 immunoreactivity between cortical and medullary interstitial cells of the late stage in neonates and adults in that the immunoreactivity of tubulointerstitial cells in the cortex for Thy-1 gradually decreased in the late stage in the neonates on days 12-18 and then was completely lost in the adults, whereas the reactivity of medullary interstitial cells remained intensive in the neonates on days 12-18 and in the adults (Table 1). Previously, it has been reported that cortical interstitial fibroblasts in adults might differ in nature from medullary interstitial fibroblasts<sup>26</sup>. The difference of Thy-1 expression in the present study supports this previous notion. It has been reported that interstitial fibroblastic cells in the uterus, orbit, lung and skin heterogeneously show Thy-1<sup>14, 15, 17, 27</sup>. Since Thy-1 expression is related to cytokine secretion, response to cytokines and differentiation<sup>14-18</sup>, it is likely that the difference in Thy-1 expression between cortical and medullary tubulointerstitial cells reflects diversity in their functions.

Thy-1 immunoreactivity in loosely-arranged mesenchymal cells in the cortex and medulla during nephrogenesis coincided mostly with vimentin immunoreactivity. Vimentin antibody is used to identify mesenchymal cells<sup>28</sup>. These findings indicate that Thy-1-expressing cells are blastemal cell-derived mesenchymal cells.

Immunoreactivity of  $\alpha$ -SMA is a marker for myofibroblasts, which have been considered to be derived from pre-existing fibroblasts or undifferentiated mesenchymal cells in fibrotic tissues<sup>8, 10, 29</sup>. Although the  $\alpha$ -SMA immunoreactivity was weaker than the Thy-1 reactivity, the distribution of Thy-1-expressing mesenchymal cells in the cortex and medulla in fetuses and neonates on days 1-9 coincided partly with that of  $\alpha$ -SMA-reacting cells. Furthermore, in the cortex of the neonates on days 12-18, some  $\alpha$ -SMA-reacting mesenchymal cells corresponded to Thy-1-positive cells. These findings indicated that  $\alpha$ -SMA-expressing cells might have been generated from Thy-1-positive blastemal cells. Blastemal cells have the capacity to differentiate into renal epithelial cells and mesenchymal cells<sup>1</sup>. In the fibrotic kidney, some myofibroblasts arise from regressed tubular epithelial cells through the EMT<sup>7-10</sup>. Taken together, the  $\alpha$ -SMA-expressing myofibroblasts seen in the fibrotic kidney may be derived from metanephric blastemal cells expressing both Thy-1 and  $\alpha$ -SMA. However, no  $\alpha$ -SMA-positive interstitial cells were observed in the medullas of the neonates on days 12-18 or in those the adults, irrespective of retained Thy-1 expression. It seems likely that Thy-1 and  $\alpha$ -SMA expressions are changeable depending on microenvironmental conditions in neonate and adult kidneys. The expression patterns of Thy-1 and  $\alpha$ -SMA in fibrotic kidneys should be investigated to clarify the relationship between myofibroblasts and blastemal cells in

more detail; additionally, the factors that influence Thy-1 or  $\alpha$ -SMA expression need to be clarified.

It is worth noting that spindle-shaped cells (so-called pericytes) surrounding arterioles in the adults reacted to both Thy-1 and  $\alpha$ -SMA<sup>17, 30</sup>. Immature blastemal cell-derived mesenchymal cells may be present in adults, suggesting another possible origin of myofibroblasts<sup>31-33</sup>.

#### *Bundle-arranged mesenchymal cells*

In the present study, mesenchymal cells, arranged parallel to each other and perpendicular to the tubular basement, were found in the cortico-medullary junction; these unique histological structures were only seen in the neonates on days 1-12. Although the reactivities were faint, the cells reacted to Thy-1 and vimentin, indicating that they were blastemal cell-derived mesenchymal cells. Interestingly, the cells showed a strong reaction to  $\alpha$ -SMA. A similar histological arrangement of mesenchymal cells has been observed in the unilateral ureteral obstruction rat model<sup>34</sup>; the authors speculated that the preferential arrangement might be caused by continuous mechanical force against urine retention in the pelvis and renal tubules. The myofibroblasts observed in myocardial infarction have been reported to be formed in association with hemodynamic stimulus<sup>35</sup>. Because  $\alpha$ -SMA is expressed in myofibroblasts (so-called contractile cells)<sup>4, 36</sup>, the  $\alpha$ -SMA expression of bundle-arranged mesenchymal cells might be related to increased contractility for formation of renal tubules during rat nephrogenesis; under the increased contractility,  $\alpha$ -SMA expression might be increased and Thy-1 expression might be reduced. As mentioned above, it would be interesting to investigate the altered expression of Thy-1 and  $\alpha$ -SMA in blastemal cell-derived cells and factors influencing the alteration.

In conclusion, the present study showed that in addition to glomerular mesangial cells, blastemal cell-derived mesenchymal cells arranged loosely in the cortex and medullas of fetuses and neonates expressed Thy-1. Thy-1-positive cells also reacted to vimentin, indicating their mesenchymal nature. In addition, the Thy-1-expression pattern corresponded generally to  $\alpha$ -SMA-positive cells, particularly in the fetuses and early stage neonates. These findings support the EMT theory (reverse embryogenesis) in fibrotic kidneys. Furthermore, in adults, tubulointerstitial cells (expressing Thy-1) in the medulla differed from cortical interstitial cells (negative to Thy-1); the bundle-arranged mesenchymal cells seen in the neonates reacted strongly to  $\alpha$ -SMA, but faintly to Thy-1. Thy-1 immunoreactivity has been used as a marker of pluripotential stem cells<sup>12, 37</sup>. Blastemal cells have the capacity to differentiate into renal epithelial cells and mesenchymal cells. In addition to the association of myofibroblasts (expressing  $\alpha$ -SMA in fibrotic kidney) with blastemal cells (expressing both Thy-1 and  $\alpha$ -SMA), it would be interesting to investigate the bone-marrow stem cell derivation of blastemal cells.



**Acknowledgments:** The authors would like to thank Drs. M. Suzuki and C. Kato of Chugai Pharmaceutical Co., Ltd. for their kind suggestions concerning immunohistochemistry with Thy-1.

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