Transforming growth factor-β induces vascular endothelial growth factor-C expression leading to lymphangiogenesis in rat unilateral ureteral obstruction

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Inflammation is recognized as an important contributor to lymphangiogenesis; however, in tubulointerstitial lesions in human chronic kidney diseases, this process is better correlated with the presence of myofibroblasts rather than macrophages. As little is known about the interaction between lymphangiogenesis and renal fibrosis, we utilized the rat unilateral ureteral obstruction model to analyze inflammation, fibrosis, lymphangiogenesis, and growth factor expression. Additionally, we determined the relationship between vascular endothelial growth factor-C (VEGF-C), an inducer of lymphangiogenesis, and the profibrotic factor, transforming growth factor-β1 (TGF-β1). The expression of both TGF-β1 and VEGF-C was detected in tubular epithelial and mononuclear cells, and gradually increased, peaking 14 days after ureteral obstruction. The kinetics and localization of VEGF-C were similar to those of TGF-β1, and the expression of these growth factors and lymphangiogenesis were linked with the progression of fibrosis. VEGF-C expression was upregulated by TGF-β1 in cultured proximal tubular epithelial cells, collecting duct cells, and macrophages. Both *in vitro* and *in vivo*, the induction of VEGF-C along with the overall appearance of lymphatics *in vivo* was specifically suppressed by the TGF-β type I receptor inhibitor LY364947. Thus, TGF-β1 induces VEGF-C expression, which leads to lymphangiogenesis.

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Blood vessels have a continuous basal lamina with tight inter-endothelial junctions and are supported by pericytes and smooth muscle cells. In contrast, lymphatic endothelial cells have a thin discontinuous basement membrane and have gaps between the cells that open to the adjacent connective tissues.[1] In edematous tissue, the lymphatic endothelial cells are pulled by anchoring filaments and bileaflet valves to prevent the backflow of lymphatic fluid.[2] These structures remove tissue fluid from the interstitium and transfer extravasated plasma protein and cells back into circulation. In this respect, lymphatic vessels are essential for body fluid balance and immunological surveillance.[3]

Increases in lymphatic vessels have recently been reported in several disease conditions, including tumor metastasis,[4–7] chronic respiratory inflammatory diseases,[8] wound healing,[9] renal transplant rejection,[10,11] and granulation tissues in myocardial infarction.[12] Inflammation is recognized as an important contributor to lymphangiogenesis in human diseases[10,13] and in animal models.[14,15] In particular, macrophages are involved in lymphangiogenesis in the production of vascular endothelial growth factor (VEGF)-C and -D, which are recognized as potentially important mediators for lymphangiogenesis.[16] Interleukin-1β and tumor necrosis factor-α have been shown to induce the upregulation of VEGF-C.[17] In addition, CD11b+ macrophages may form lymphatic-like vessels *in vitro*.[14,18] A recent report showed that inflammation induced lymphangiogenesis through the upregulation of VEGF receptor-3 mediated by nuclear factor-kappa-B and prospero-related homeobox 1.[19]

In chronic kidney disease, we recently reported an increase in the number of lymphatics observed at the site of tubulointerstitial lesions and this increase was correlated with

degree of tissue damage. In addition, lymphangiogenesis was more strongly correlated with fibrosis than inflammation on analysis of human glomerular diseases and acute and chronic tubulo-interstitial nephritis.[20] We also demonstrated strong expression of VEGF-C in the proximal tubules.[20] Our results indicated that lymphangiogenesis is a common feature in the progression of tubulo-interstitial fibrosis, and the fibrotic process may play a role in the development of lymphatics.[20] However, there has been little focus on the role of transforming growth factor (TGF)-β, one of the most important mediators for tissue fibrosis, in lymphangiogenesis to date.

In this study, we investigated the roles of TGF-β and VEGF-C in the development of lymphangiogenesis in the unilateral ureteral obstruction (UUO) model. In addition, we studied the relationship between TGF-β and VEGF-C in cultured proximal and collecting tubules, macrophages and fibroblasts, which are involved in tubulo-interstitial fibrosis. This is the first report to examine the mechanisms and roles of TGF-β in lymphangiogenesis.

RESULTS

Basic characteristics of the rat UUO model

Three days after ligation, dilation of the tubules and infiltration of inflammatory cells were seen in both the cortex and the medulla. On day 14, tubular degeneration, dilation, and atrophy became severe and intense interstitial fibrosis was also observed (Figure 1). Morphological analysis demonstrated strong infiltration of CD68 (ED-1)-positive macrophages in both the cortex and medulla on day 3 that was more prominent than the expression of α-smooth muscle actin (α-SMA)-positive fibroblasts (Figure 2a and b). Expression of α-SMA-positive fibroblasts and type III collagen deposition in the tubulo-interstitial area became conspicuous and peaked on day 14 (Figure 2).

Lymphatic vessels in control and UUO model

In the control kidney, podoplanin-positive and lymphatic endothelial hyaluronan receptor-1 (LYVE-1)-positive lymphatic vessels were not encountered in the normal cortical tubulo-interstitial area and were observed only

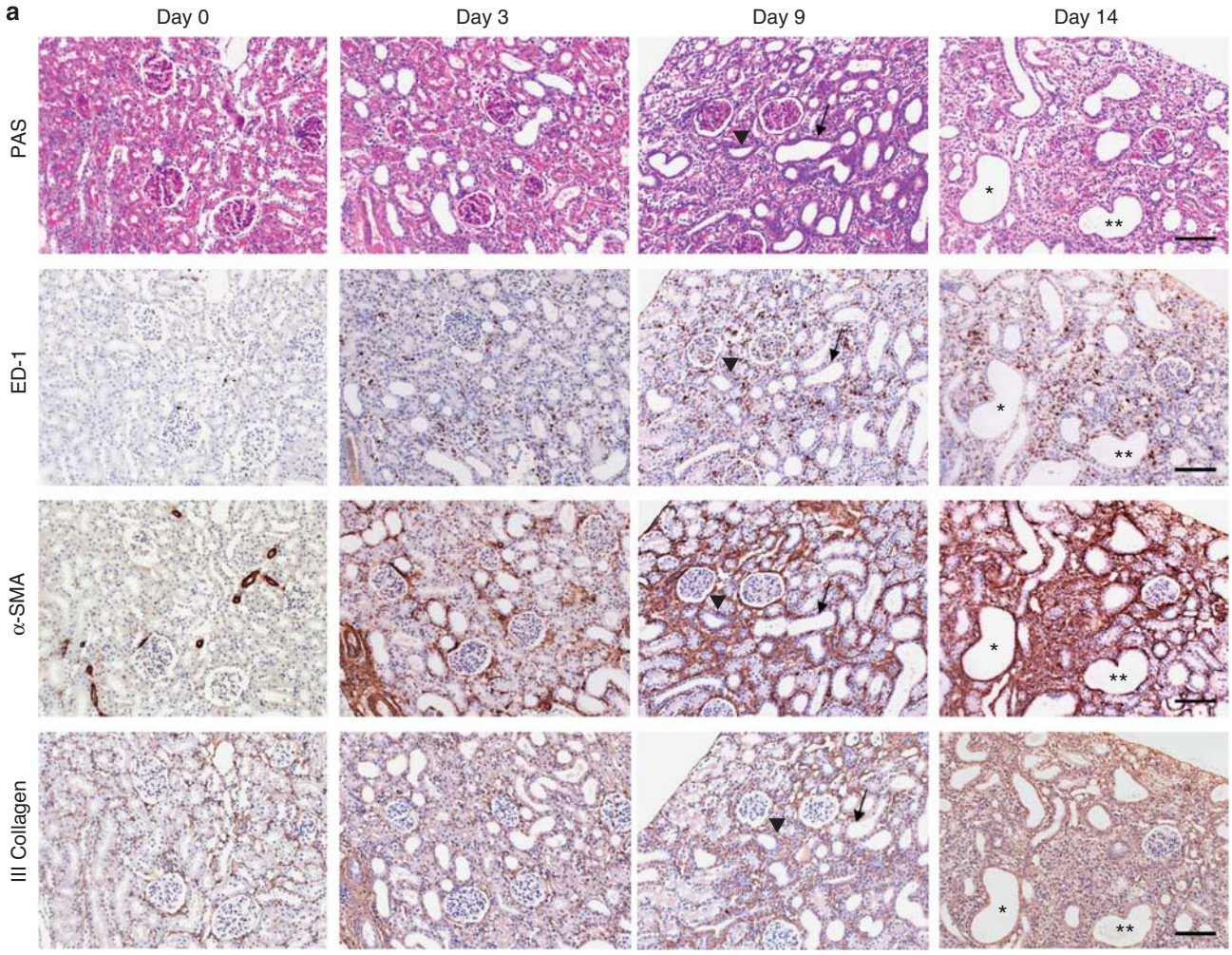

Figure 1 | Rat unilateral ureteral obstruction (UUO) model. (**a**) Renal cortex of rat UUO model. (**b**) Renal medulla of rat UUO model. Arrows, arrowheads, asterisks, triangles, and stars indicate the same dilated tubules. Bar = 100 μm. ED-1, CD68; P, renal pelvis; PAS, periodic acid–Schiff; α-SMA, α-smooth muscle actin.