CTGF silencing suppresses lymphangiogenesis in kidney disease

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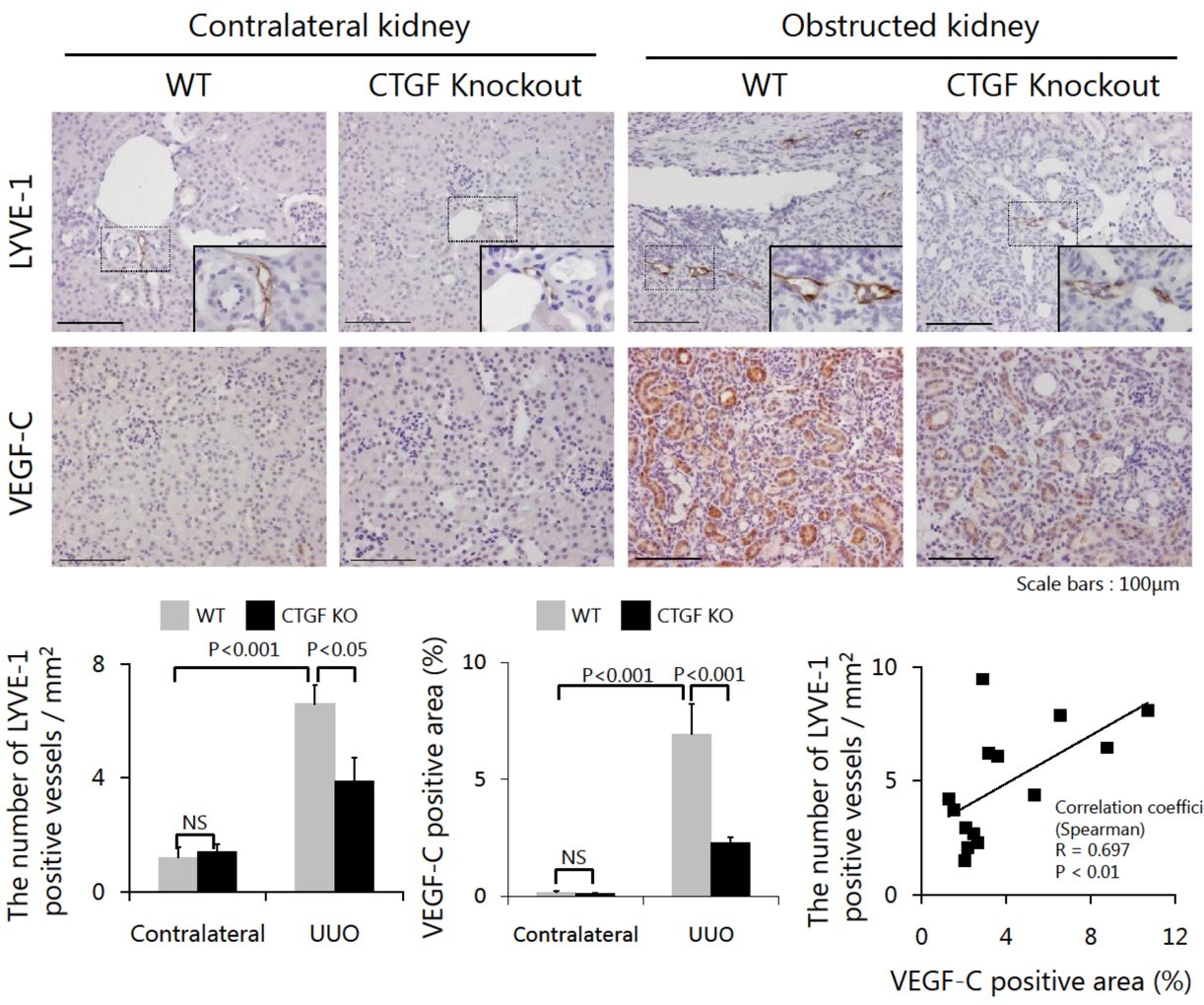
Introduction

Lymphatic vessels play important functions in drainage of interstitial fluid, macromolecules and cells. Lymphangiogenesis has been reported in several human and experimental kidney diseases and correlated with the degree of kidney fibrosis.
Connective tissue growth factor (CTGF) is thought to be an important player in the pathogenesis of chronic fibrotic disorders. The role of CTGF in angiogenesis may have some tissue specificity in previous reports, and there are no reports suggesting the effect of CTGF in lymphangiogenesis.
In this study, we aim to clarify the effect of CTGF gene knockdown in lymphangiogenesis of kidney disease by using CTGF knockout mice and a human renal tubular epithelial cell line (HK-2).

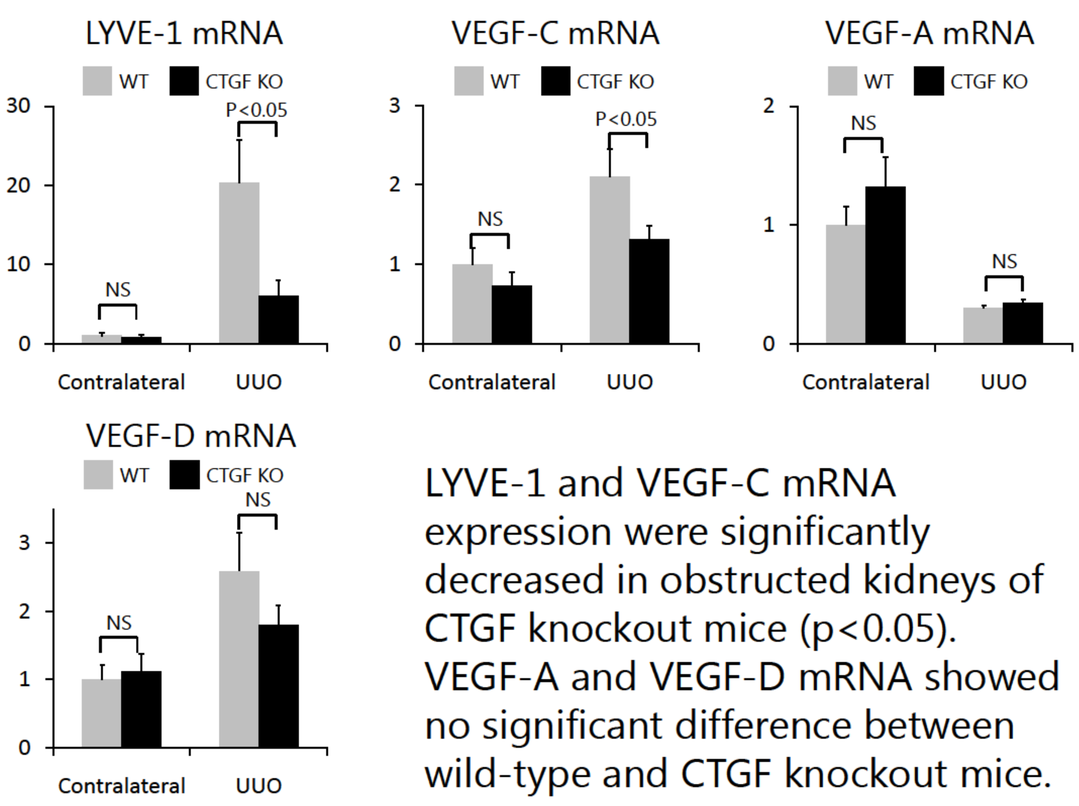
Methods

Wild-type (WT) mice (n=5) and CTGF knockout (KO) mice (n=9) underwent unilateral ureteral obstruction (UUO), and both the obstructed kidney and contralateral kidney were collected on day 14 after UUO.
We analyzed the expression of lymphatic vessels and vascular endothelial growth factor-C (VEGF-C) which is one of the main lymphangiogenic growth factors by immunohistochemistry and quantitative polymerase chain reaction (PCR).
We checked VEGF-C mRNA expression of human-derived renal proximal tubular cells after CTGF gene knockdown by siRNA together with TGF-β1 stimulation (n=4).

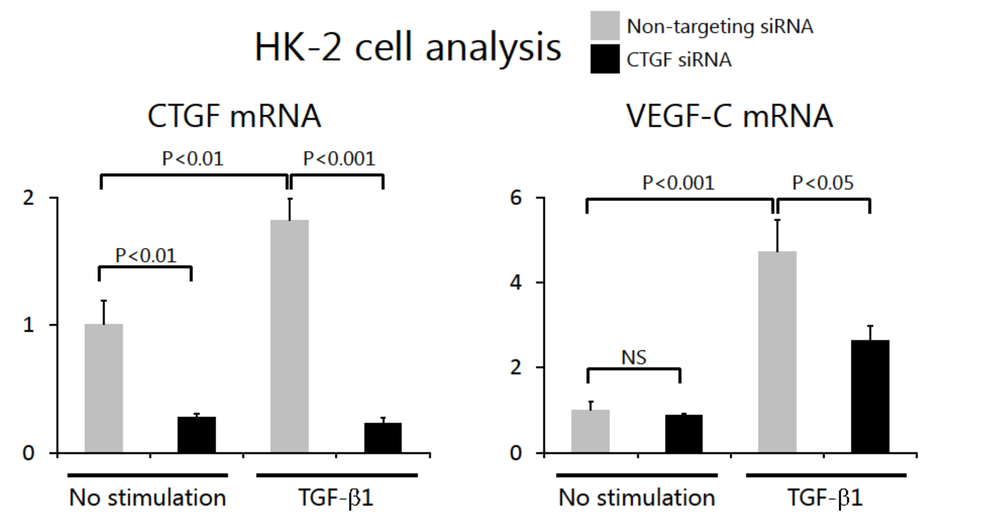
Results



Immunohistochemical staining for lymphatic endothelial hyaluronan receptor-1 (LYVE-1) and VEGF-C showed an increased expression of LYVE-1 positive lymphatic vessels and VEGF-C in obstructed kidneys. Quantification of immunohistochemical staining showed that the number of LYVE-1 positive vessels ($p<0.05$) and VEGF-C positive area ($p<0.001$) were significantly smaller in obstructed kidneys of CTGF knockout mice compared to wild-type obstructed kidneys. Quantification of staining showed a positive correlation between the number of LYVE-1 positive vessels and VEGF-C positive area in obstructed kidneys ($r=0.697$; $p<0.01$).



LYVE-1 and VEGF-C mRNA expression were significantly decreased in obstructed kidneys of CTGF knockout mice ($p<0.05$). VEGF-A and VEGF-D mRNA showed no significant difference between wild-type and CTGF knockout mice.



CTGF and VEGF-C mRNA expression were significantly upregulated in HK-2 cells by 10 ng/ml of TGF-β1 stimulation for 24h. CTGF siRNA significantly reduced VEGF-C upregulation induced by TGF-β1 compared to Non-targeting siRNA.

Conclusion

Expression of renal lymphatics and VEGF-C were significantly decreased in obstructed kidneys of CTGF knockout mice compared to wild-type obstructed kidneys by immunohistochemistry and quantitative PCR analysis.
CTGF gene knockdown suppressed VEGF-C upregulation in renal tubular cells induced by TGF-β1. CTGF is involved in VEGF-C production during kidney fibrosis. Clarification of the mechanism of lymphangiogenesis in kidney fibrosis might lead to find identification of novel intervention parts for treatment of chronic kidney disease.

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