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Original Article

Injured kidney cells express SM22α (transgelin): Unique features distinct from α-smooth muscle actin (αSMA)

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SUMMARY AT A GLANCE

This manuscript describes de novo expression of the actin-associated protein SM22α (also known as transgelin) by injured podocytes in a rat model of crescentic glomerulonephritis. Immuno-EM localized SM22α to effaced, but not intact, foot processes in podocytes suggest a role for this molecule in reorganization of the actin cytoskeleton following podocyte injury.

ABSTRACT:

Aim: **SM22α (transgelin) has been focused upon as a player in the process of phenotypic changes of types of cells. The SM22α expression in the rat anti-glomerular basement membrane (GBM) nephritis model and differences from an established phenotypic marker for the myofibroblast, α-smooth muscle actin (αSMA), were investigated.**

Methods: **The rat kidney tissues were processed for histological studies, immunohistochemical and immunoelectronmicroscopy analyses on days 0, 7, 28, 42 and 56 after injection of rabbit anti-GBM serum for the disease induction.**

Results: **Immunohistochemistry with anti-SM22α antibodies (Ab) revealed that kidneys of the nephritic rats on day 7 expressed SM22α in podocytes, crescentic cells and epithelial cells of Bowman's capsule. After 28 days, SM22α was also expressed in peritubular interstitial cells. Double immunofluorescence with anti-SM22α Ab and anti-αSMA Ab showed that SM22α was preferentially expressed in podocytes, whereas αSMA was positive in mesangial cells on day 7. After day 28, both molecules became positive in peritubular interstitial cells.**

Conclusion: **SM22α was expressed in epithelial cells of inflamed glomeruli in the early phase, and then also in peritubular interstitial cells in the later phase of anti-GBM nephritis model. SM22α presented unique kinetics of expression distinct from αSMA.**

SM22α was first identified as a 22 kDa protein of unknown function in smooth muscles.[1] It has variably been designated as transgelin,[2] p27[3] and WS3-10.[3] It is abundantly expressed in smooth muscle cells (SMC) and is well known as a differentiation marker of the contractile SMC.[4,5] It is localized in the cytoskeletal apparatus[6] and is a member of the calponin family.[7,8] The function of SM22α has not been yet completely elucidated. SM22α-deficient mice normally develop and appear similar to control mice histologically.[9] However, SM22α is downregulated in atherosclerotic SMC, and its gene ablation in apoE-deficient mice results in the extension of atherosclerotic lesions and increases the proportion of proliferating SMC in plaque, indicating that SM22α may be involved in controlling the phenotypic modulation of SMC, from contractile to proliferative, in the sclerotic artery.[10] Its expression has also been detected in several epithelial cells.[7,11,12] Upon tissue injuries, SM22α is increasingly expressed in alveolar epithelial type II cells of pulmonary fibrosis, and it may contribute to epithelial-to-mesenchymal transition (EMT) in lung fibrosis.[13] Moreover, SM22α acts to suppress expression of the matrix metalloproteinase (MMP)-9,[14] which is involved in the tissue remodelling. SM22α could act as a tumour suppressor. Loss of its expression could account in part for the development of cancer.[7,11,12,15] Thus, the functional and pathological significance of SM22α expression has recently been focused on.

The appearance of modified fibroblasts with smooth muscle-like features, called myofibroblasts, is the hallmark of

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biological response for kinds of injuries, and was first observed in granulation tissue of healing wounds.[16] Most myofibroblasts express α-smooth muscle actin (αSMA), which has been established as a marker of EMT or transdifferentiation of various cells. αSMA is an actin isoform, typically found in the vascular SMC.[16,17] Myofibroblasts expressing αSMA were also noticed in kidney diseases such as crescentic glomerulonephritis (GN),[18] mesangial proliferative GN,[19] including immunoglobulin (Ig)A nephropathy,[20] and tubulointerstitial (TI) injury and fibrosis.[21] αSMA has been deemed to be a predictor of mesangial proliferation and sclerosis, TI scarring, and ultimately renal dysfunction.[20,21] In a rat anti-glomerular basement membrane (GBM) nephritis, it was reported that αSMA-positive cells were detected in a mesangial distribution.[21]

We reported in the previous study[22,34] that SM22α might be inducibly expressed in injured glomerular epithelial cells in the early phase of the rat anti-GBM nephritis model and it could be a novel marker of glomerular epithelial cell injury. However, we did not show the ultrastructure of SM22α-expressing cells or its intracellular localization. Nor did we show how it was detected in the later phase of the nephritis model. In this present study, we investigated these undetermined issues on the inducible SM22α expression using a long-term model of anti-GBM nephritis, which showed TI injury as well as the glomerular inflammation. We also demonstrate notable differences from αSMA as a tissue injury marker in the kidney disease.

METHODS

Animals

Male Wistar-Kyoto rats (7 weeks old) were purchased from Charles River Japan (Yokohama, Japan), and maintained in our animal facility. Animal care was performed in accordance with the guidelines of Niigata University. For 24 h urine collections, individual animals were placed in metabolic cages following the methods of our previous works.[23,24]

Experimental protocols

On day 0, rats ($n = 17$), except negative controls ($n = 5$), were injected with rabbit anti-GBM serum (200 μL), which was prepared as previously described.[25] On days 7, 28, 42 and 56 (each $n = 3$–5), after collecting urine and blood samples, the rats were killed after removal of their kidneys.

Antibody

The protocol to obtain the SM22α antibody (Ab) was previously described.[22] Goat anti-SM22α polyclonal Ab (Abcam, Cambridge, UK) was also used for immunochemistry. Mouse monoclonal anti-αSMA Ab (ASM-1) was purchased from Chemicon (Millipore, Billerica, MA, USA).

Immunohistochemistry

Studies of immunohistochemistry were carried out following the methods of our previous works.[26,27] Kidney tissues were fixed in Carnoy's fluid. Sections were incubated with rabbit anti-rSM22α Ab (2 μg/mL) or goat anti-SM22α Ab (4 μg/mL), and then peroxidase-conjugated goat anti-rabbit IgG Ab (DAKO, Glostrup, Denmark) or peroxidase-conjugated rabbit anti-goat IgG Ab (DAKO) as secondary Ab. The immune complex was detected with 3,3'-diaminobenzidine tetrahydrochloride (DAKO), and counter staining was performed with hematoxylin–eosin. Control sections were incubated with normal rabbit IgG (Sigma-Aldrich, St Louis, MO, USA) or normal goat IgG (R&D Systems, Minneapolis, MN, USA).

Immunofluorescence

For double labelling, rSM22α Ab and normal rabbit IgG were biotinylated by using biotin-labelled Kit-NH2 (Dojindo Laboratories, Kumamoto, Japan). Frozen tissues were fixed and blocked with a Biotin Blocking System (DAKO), incubated with biotinylated rabbit anti-rSM22α Ab (50 μg/mL) and mouse anti-αSMA Ab (ASM-1, IgG2a), and then treated with fluorescein isothiocyanate (FITC)-conjugated streptavidin (Beckton-Deckinson, Franklin Lakes, NJ USA) and tetramethyl rhodamine isothiocyanate (TRIT)-conjugated goat anti-mouse IgG2a (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Control sections were incubated with biotinylated normal rabbit IgG or mouse IgG2a (Ancell, MN, USA).

Immunoelectronmicroscopy

Immunoelectronmicroscopic technique was previously described.[28] Because rabbit Ig (anti-GBM) reacted with rat glomeruli in this model, goat anti-SM22α Ab was used in this experiment. Ultrathin sections were incubated with goat anti-SM22α polyclonal Ab (4 μg/mL) followed by 10 nm colloidal gold EM rabbit anti-goat IgG (H+L) (BBInternational, Cardiff, UK). The gold particles were enhanced using an IntenSEM silver staining kit (Janssen Pharmaceutica NV, Beerse, Belgium). After fixing, the sections were contrasted and viewed with a Hitachi H-600A electron microscope (Tokyo, Japan).

RESULTS

Immunohistochemistry

The development of anti-GBM nephritis in the present experiments is summarized by the parameters as shown in Table 1. The periodic acid-Schiff (PAS)-stained kidney specimens are shown in Figure 1. On day 7, the cellular crescent formation and mesangial cell proliferation with fibrinoid changes were observed in most glomeruli (Fig. 1b). After day 28, crescents were fibrocellular or fibrous, and glomeruli were sclerosing. The mononuclear cell infiltration, tubular atrophy and fibrosis became remarkable in the interstitium (Fig. 1c,d).

Kidneys on day 7 expressed SM22α in glomerular epithelial cells and crescentic cells (Fig. 2b), whereas only the

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Table 1 Parameters of the development of rat anti-glomerular basement membrane (GBM) nephritis

	Urinary protein (g/g creatinine)		Blood urea nitrogen (mg/dL)		Serum creatinine (mg/dL)		Creatinine clearance (mL/min)	
	Anti-GBM	Control	Anti-GBM	Control	Anti-GBM	Control	Anti-GBM	Control
Day 7	20.6 ± 1.99	1.1 ± 0.10	17.5 ± 2.35	14.9 ± 0.00	0.17 ± 0.01	0.17 ± 0.00	2.87 ± 0.05	1.44 ± 0.00
Day 28	51.0 ± 3.26**	1.6 ± 0.02	56.2 ± 9.27**	17.9 ± 0.00	0.98 ± 0.43	0.23 ± 0.00	0.63 ± 0.28**	3.07 ± 0.00
Day 42	45.8 ± 3.79**	1.3 ± 0.08	133.1 ± 35.8*	18.7 ± 0.00	2.60 ± 0.57**	0.24 ± 0.00	0.17 ± 0.10**	3.46 ± 0.00
Day 56	39.7 ± 0.09**	1.0 ± 0.00	N.D.	6.2 ± 0.00	N.D.	0.22 ± 0.00	N.D.	4.22 ± 0.00

*$P < 0.05$ *vs* day 7, **$P < 0.01$ *vs* day 7. Urine protein standardized by urine creatinine (g/g creatinine). Anti-GBM nephritis rats were all dead by day 57. N.D., not done.

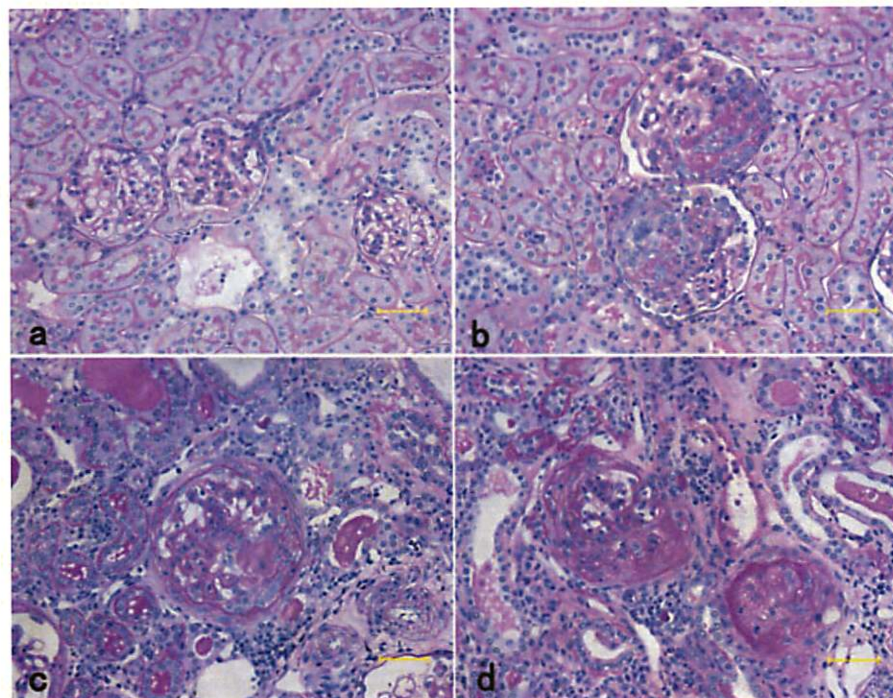

Fig. 1 Histology of anti-glomerular basement membrane nephritis. Periodic acid-Schiff staining of specimens on days 0 (a), 7 (b), 28 (c) and 42 (d) were performed. (Bars = 50 μm, original magnification ×400.)

vessel walls expressed SM22α in control rat kidneys (Fig. 2a). On day 7, SM22α expression in the interstitium was only seen in some periglomerular cells and a few peritubular cells. On day 28, in crescents and interstitial areas, SM22α-positive cells became apparent. Glomerular epithelial cells and periglomerular cells obviously expressed SM22α (Fig. 2c). On day 42, the SM22α-positivity in crescents and interstitial areas was sustained, whereas that in glomerular epithelial cells decreased (Fig. 2d). The stainings with two different specific Ab (rabbit anti-rSM22α Ab and goat anti-SM22α Ab) showed the same results (data not shown).

Immunoelectronmicroscopy

To investigate the SM22α expression in the cells in detail, we performed immunoelectronmicroscopy by immuno-gold technique with the goat anti-SM22α Ab. In control normal rats, SM22α was negative in glomerular epithelial cells (Fig. 3a). In rats on day 7, podocytes showed the foot process effacement in part. SM22α was preferentially detected in such foot processes (Fig. 3b). The parietal epithelial cells of Bowman's capsule were also positive for SM22α (Fig. 3c). SM22α was preferentially expressed along the dense basal microfilaments of both types of epithelial cells. After day 28 (Fig. 3d), as well as the glomerular epithelial cells, the periglomerular interstitial cells became positive for SM22α diffusely in the cytoplasm.

Double immunofluorescence with anti-SM22α and anti-αSMA Ab

It has been reported that the positivity for αSMA is a marker of tissue injury and a predictor for progression of glomeru-

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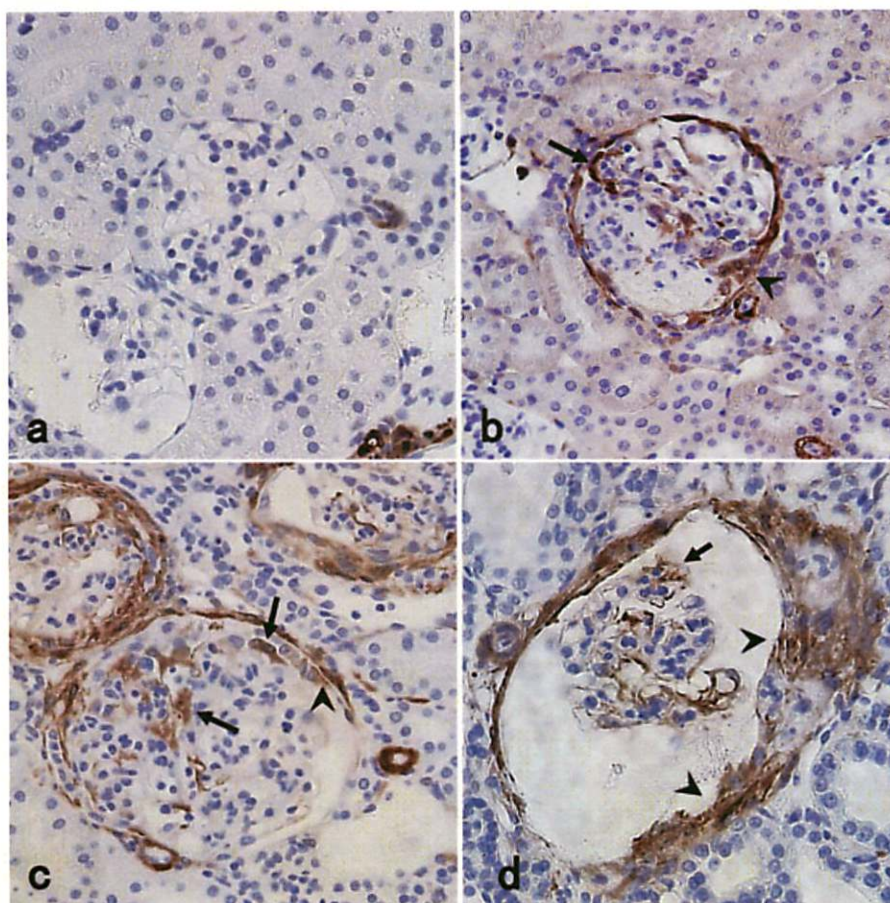

Fig. 2 Immunohistochemistry of SM22α. Specimens at days 0 (a), 7 (b), 28 (c) and 42 (d) were immunostained by anti-SM22α. SM22α was expressed in podocytes (arrow), crescentic cell and epithelial cells of Bowman's capsule (arrowhead) after day 7, and gradually expressed in periglomerular or peritubular interstitial cells. (Original magnification ×400.)

losclerosis,[20,21] TI fibrosis and renal insufficiency.[20] Because SM22α could be another injury marker of renal cells, we performed the double immunofluorescence staining with anti-SM22α and anti-αSMA Ab to investigate whether or not SM22α and αSMA co-expressed in the same series of cells during the development of the nephritis. The expression profiles of these in glomeruli were clearly different (Fig. 4). SM22α was expressed partly in a podocytic pattern (Fig. 4d; arrow), while αSMA was expressed in a mesangial pattern (Fig. 4e), and rarely expressed in the interstitium on day 7. On day 28, SM22α was also expressed in a parietal epithelial pattern along Bowman's capsule as well as in a podocytic pattern (Fig. 4l; arrowhead), in which αSMA was negative. In parts of crescentic cells, both molecules were expressed together (Fig. 4i; arrow). After day 28, both molecules were detected not only in the glomeruli, but also in interstitial cells, especially in the periglomerular area. In crescents of glomeruli or in sclerosing glomeruli, both SM22α and αSMA were expressed almost in the same cells (Fig. 4p–u). These data clearly indicated that the expression profile of SM22α was distinct from that of αSMA in the early stage of the nephritis. However, in the later phase, both were co-expressed in cells of crescents and interstitial area.

DISCUSSION

In the present study, we demonstrated that SM22α was expressed also in TI cells, especially of the periglomerular and peritubular area, in the later stage of the anti-GBM nephritis, as well as in glomerular epithelial cells. In our previous study,[22] we could not surely determine whether SM22α-expressing cells were podocytes or not, because those cells were not co-stained with podocyte-specific markers, nephrin or podocalyxin. The immunoelectronmicroscopy revealed that SM22α was expressed in the basal microfilament layer of podocytes, which showed foot process effacement. The epithelial cells of Bowman's capsule were also positive for SM22α in the basal microfilament layer. On the other hand, it was detected diffusely in the cytoplasm of TI cells. The inducible expression of SM22α might imply the acquisition

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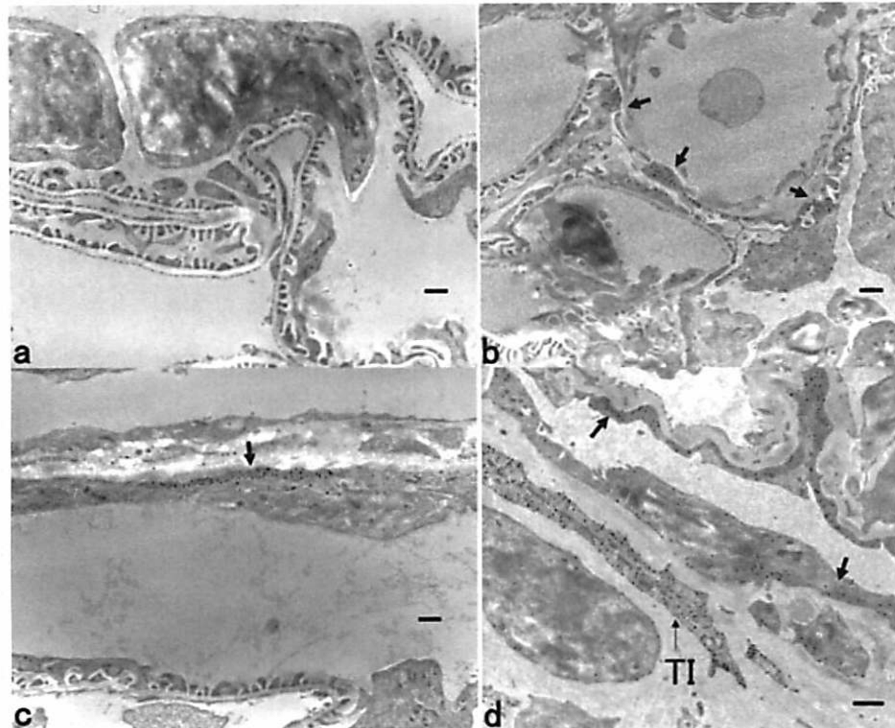

Fig. 3 Immunoelectronmicroscopy for SM22α. Specimens at days 0 (a), 7 (b–c) and 28 (d) were electron-microscopically stained by anti-SM22α with immuno-gold technique (bars = 1 μm, original magnification ×9450). (a) On day 0, the gold particle was not detected in the podocyte. (b) On day 7, the foot processes of podocytes almost disappeared, and the immuno-gold label was observed in such podocytes, especially in the dense basal microfilaments layer (arrows). (c) Immuno-gold labelling was also positive in the parietal epithelial cells of Bowman's capsule, which was mostly distributed in the dense microfilament basal layer (arrow). (d) On day 28, the gold particles were detected not only in the epithelial cells (arrows), but also diffusely in the cytoplasm of tubulointerstitial (TI) cells.

of contractile or other inflammatory properties by the kidney cells. Because SM22α is originally localized in the cytoskeletal apparatus of SMC, the difference of its distribution between in glomerular epithelial cells and in TI cells might be derived from the anatomical relationship of the cells to surrounding extracellular matrices (ECM). The modified environment for the attachment of cells to ECM and tension generation might lead to the SM22α expression in accordance with the actin-reorganization under the inflammatory conditions.

α-Smooth muscle actin, also originally expressed in SMC, is well established as a marker of kidney cell injuries. The de novo expressions of αSMA in mesangial cells, periglomerular cells and peritubular TI cells represent myofibroblastic changes and are related to TI fibrosis and renal dysfunction.[20,21,29,30] Because the inducible SM22α expression in TI cells of injured kidneys mostly coincided with αSMA expression, SM22α expression in the interstitial cells might be correlated with the later renal functional outcome, as well as αSMA. On the other hand, in the early stage, inducible SM22α expression was observed in the podocytes and parietal epithelial cells of Bowman's capsule of injured glomeruli. These SM22α-expressing cells were completely distinguished from αSMA-positive cells. The parietal epithelial cells are also known to undergo transdifferentiation upon the crescent formation, and to express αSMA.[31,32] Our studies demonstrated that SM22α-expression in the parietal epithelial cells might precede the following αSMA expression and crescent formation. The SM22α expression might be an earlier marker of phenotypic changes of the epithelial cells, and might be one transdifferentiation sequence upon the crescent formation.

The functional consequence of SM22α expression in the glomerular cells, including injured podocytes, remains unclear. In podocytes upon the glomerular injury, actin fibres might be reorganized and form dense microfilament bundles containing SM22α. The cytoskeletal changes in podocytes in this model were precisely investigated in previous studies,[33] reporting that the dense microfilament network appeared along the basal cell membrane of podocytes in this model. The dense bundles contained α-actinin, which might serve as a cross-linker for the microfilaments and represent the contractile phenotypic change of podocytes upon glomerular injury. Because SM22α is known to be associated with actin-stress fibres and stabilize actin gels *in vitro*,[7] it might also work as a factor that promoted the reorganization of the actin cytoskeleton of

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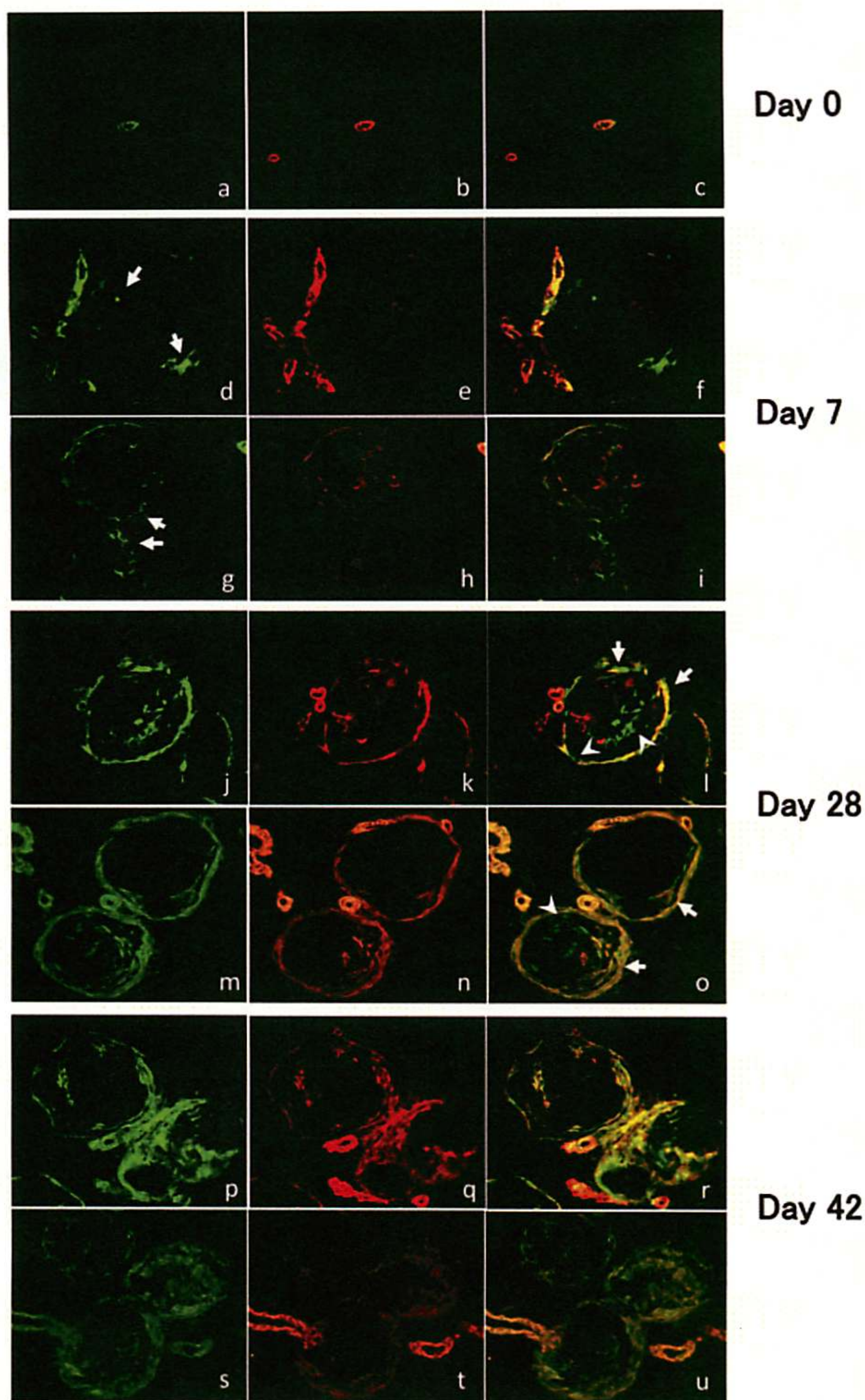

Fig. 4 Double immunofluorescence of SM22α and α-smooth muscle actin (αSMA). Specimens at days 0 (a–c), 7 (d–i), 28 (j–o) and 42 (p–u) were reacted with biotinylated-anti rSM22α antibody (Ab) (a,d,g,j,m,p,s) and anti-αSMA Ab (b,e,h,k,n,q,t), followed by incubating with fluorescein isothiocyanate-conjugated streptavidin for SM22α and tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse immunoglobulin G2a for αSMA. The images were merged (c,f,i,l,o,r,u). (a–c) On day 0, both SM22α (green) and αSMA (red) were expressed only in vessels. (d–i) On day 7, SM22α (green; d: arrow) and αSMA (red) were de novo expressed in glomeruli. (j–o) On day 28, both were positive in crescentic cells (l,o: arrow) and interstitial cells. SM22α (green) was expressed in podocytes and the parietal epithelial cells of Bowman's capsule (l,o: arrow head), whereas αSMA (red) was expressed in mesangial cells. (p–u) On day 42, in the advanced stage, both were expressed almost in the same cells.

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podocytes and supported some functions of podocytes under the inflammatory conditions. The functional significance of SM22α in kidney diseases should be further investigated, so that the pathophysiology, or the mechanism of the development, of the diseases could be well understood. Moreover, revealing the functional role of this molecule might lead to the therapeutic application for renal diseases by controlling its function.

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