SCUBE1, a novel developmental gene involved in renal regeneration and repair

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Abstract

Background. We have identified that a novel developmental gene and protein, SCUBE1, is expressed in endothelial cells and may play an important role in kidney regeneration.

Methods. The temporal and spatial expression of SCUBE1 was determined in a mouse model of ischaemia–reperfusion (IR) injury at 3 days and 1, 3 and 6 weeks post-injury by immunofluorescence microscopy. In vitro analysis was used to examine SCUBE1 signalling in endothelial cells under conditions of cell stress using quantitative real-time polymerase chain reaction and immunofluorescence labelling. The media from cultured endothelial cells following SCUBE1 small interfering RNA (siRNA) transfection was used to assess the proliferation capacity of epithelial cells.

Results. Immunofluorescence confocal microscopy demonstrated that the SCUBE1 protein was localized to CD31-positive endothelial cells in IR kidneys during the resolution of tissue damage (3 weeks), but not in control animals. The peak expression of SCUBE1 following 3 weeks of IR injury was confirmed by reverse transcription–polymerase chain reaction. SCUBE1 mRNA and protein expression were detected in cultured endothelial cells under hypoxic conditions or serum starving. Furthermore, there was a significant decrease in epithelial cell proliferation following the addition of a supernatant derived from cultured endothelial cells following SCUBE1 siRNA gene silencing compared to control media.

Conclusions. Our results indicate that SCUBE1 may be involved in the regulation of tubular cell proliferation and re-epithelialization during the resolution of kidney injury.

Keywords: endothelial cells; ischaemia–reperfusion injury; kidney development; kidney regeneration; proximal tubular epithelial cells

Introduction

The adult kidney has an inherent ability to repair and regenerate following acute renal damage. The regenerative capacity of the kidney is multifactorial and has been extensively studied using experimental models of ischaemia–reperfusion (IR) injury where tubules undergo an endogenous cell replacement and re-epithelialization [2,16,24]. Moreover, the integrity of the renal vasculature can have a profound influence on tubular re-epithelialization following damage. The extent of injury to endothelial cells of the glomerulus and tubulointerstitial capillary network is an important determinant for the degree of overall structural damage following hypoxia and the subsequent ability of the kidney to recover from insult [22,23].

There is also growing evidence that links renal repair with the recapitulation of embryonic development, such that genes vital during nephrogenesis may regulate renal cellular replacement and growth following adult injury [28,30]. Understanding the molecular basis of kidney development is, therefore, a key to the regenerative process and promotion of kidney repair in adult disease [26]. The SCUBE family consists of a novel class of secreted, extracellular proteins that are important in organogenesis [11,12]. To date, three isoforms of the protein have been identified: SCUBE1, SCUBE2 and SCUBE3. SCUBE1 is expressed in the developing gonad as well as the nervous system, somites, surface ectoderm and limb buds [11,12]. In addition, it is specifically expressed in the mesenchyme during branching morphogenesis and was initially detected in the male and female urogenital ridge at day 12.5 (E12.5) of embryonic development in the mouse [11,40]. SCUBE1 and SCUBE3 have since been identified within the vasculature of various organs and are selectively expressed in endothelial cells [25,41,42] and platelets [37,38]. The zebrafish homolog of SCUBE2 has been implicated in hedgehog (HH) signalling [15]. SCUBE3 has recently been detected in the myocardium where it may be involved in myocyte integrity and cell growth [41].

The importance of SCUBE1 in adult kidney injury and endogenous repair has not been previously reported. The present study determines the spatial and temporal expression of SCUBE1 in a mouse model of IR injury. In addition, the regulatory role of SCUBE1 on endothelial cell integrity and tubular cell proliferation was explored in vitro.
In these studies, the effects of downregulating SCUBE1 via gene silencing using small interfering RNA (siRNA) transfection of cultured endothelial cells was determined. Mouse cortical tubular (MCT) cells were cultured with the media from siRNA-transfected endothelial cells and tubular epithelial cell proliferation was assessed. We provide evidence that the SCUBE1 protein may constitute an important regulatory protein operating within the renal vascular network exerting reparative effects on adjacent renal tubules.

Materials and methods

Experimental animals
 Male C57Bl/6 mice (20–25 g body weight) were anesthetized with 2.0–4.0% isoflurane and the left renal artery and vein were clamped via a flank incision to induce IR injury for 45 min (n=5 mice/group). The right kidney in these mice served as a contralateral control. The sham-operated control group (n=3/group) consisted of mice that underwent the same procedure excluding the left renal artery clamping. The kidneys from all groups were immersion fixed in 4% paraformaldehyde (PFA) for hematoxylin and eosin staining or transferred into 30% sucrose phosphate-buffered saline (PBS) for embedding in OCT medium (ProSciTech, Australia) for frozen sections using a cryostat (Leica, Nussloch Germany).

Immunofluorescence microscopy
 Frozen sections were treated with 2% bovine serum albumin and incubated with goat anti-mouse IgG antibody (Invitrogen, Eugene, OR, USA). Sections were stained with the primary mouse monoclonal anti-SCUBE1 IgG antibody (1:200; [37]) and incubated at 4°C overnight. A secondary TRITC-conjugated rabbit anti-mouse IgG antibody (1:2000; Invitrogen, Eugene, OR, USA) was added for 30 min before the addition of a primary rat anti-CD31 antibody (Cymbus Biotechnology Ltd, Chandlers Ford, UK), and an Alexa 647-conjugated goat anti-rat secondary antibody before counterstaining with DAPI and mounting with DakoCytomation fluorescent mounting medium (DakoCytomation, Glostrup, Denmark).

For proliferating cell nuclear antigen (PCNA) staining, paraffin sections were boiled in 10 mM citrate buffer (10 mM citric acid, pH 6.0) for 30 min. The membranes were washed in PBS and exposed to a high-per- formance liquid chromatography (HPLC) column containing 25 µM of primary antibody (1:3000) at 4°C overnight and a peroxidase-conjugated rabbit anti-mouse antibody (1:40 000; Chemicon, Temecula, CA, USA) for 30 min. The sections were then washed with 1× PBS and counterstained with DAPI and mounted with DakoCytomation fluorescent mounting medium (DakoCytomation, Glostrup, Denmark).

ForCell proliferation analysis using MCT cultures with post-siRNA-transfected media, we used a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA). MCT cells (n=4) were cultured in 10 µl DMEM media with 50 µl conditioned media removed from siRNA-transfected cells in 96-well plates for 24 h. The cell proliferation assay was performed in an ELISA microplate spectrophotometer plate reader using a Microplate Manager (Bio-Rad, Hercules, CA, USA) at 490 nm.

Reverse transcription and PCR
 Total RNA was extracted from the renal cortices of IR and control kidneys using an RNeasy Protect Mini Kit (QIAGEN, Clifton Hill, VIC, Australia). The first strand of full-length cDNA was obtained by reverse transcription–polymerase chain reaction (RT–PCR) from total RNA using the Superscript III First-Strand Synthesis System for RT–PCR (Invitrogen, Carlsbad, CA, USA). PCR was performed with the following primers: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (F): 5′-CTCACCACCTTGTGATTAG-3′ and (R): 5′-CAGCCGCATCTTGTGCAG-3′ [37]. For PCR reactions, 25 µl of total reaction containing 2.5 µl of 10× PCR buffer, 0.5 µl of dNTP mix, 0.8 µl of 50 mM MgCl2 (50 mM), 1 µl of each 10 µM forward and 10 µM reverse primer, 0.3 µl of Taq polymerase (Invitrogen, Carlsbad, CA, USA) and 2 µl of cDNA were incubated at 95°C for 2 min, then run for 28 cycles with the following steps: 30 s at 95°C, 30 s at 65°C and 45 s at 72°C and final extension at 72°C for 5 min in a type 2720 Thermal Cycler (Applied Biosystems, Mulgrave, VIC, Australia) and separated by SDS–PAGE on an 8% acrylamide gel.

Cell culture studies
 Mouse microvascular endothelial cells (MMEC cells) (ATCC log number: CRL-2279) were cultured in 5% FBS DMEM media (GIBCO, Invitrogen, Auckland, NZ) containing 4 mM L-glutamine, 4.5 g/L D-glucose and 1.5 g/L NaHCO3 and grown in incubators at 37°C in 5% CO2. In addition, differentiated SV40-transformed proximal tubular epithelial cells (MCT epithelial cell line [14]) were cultured in DMEM media (GIBCO, Invitrogen) containing 10% FBS and 4.5 g/L D-glucose under the same culture conditions as MMEC cells.

MMEC cells were cultured in either serum-free media to induce cell stress for 12 and 24 h or in standard media with the addition of 200 µM CoCl2 to induce hypoxic conditions for 3, 6, 12 and 24 h (n=5/time point). The cells cultured in standard media served as a control for the CoCl2-treated cells and processed for RNA extraction or fixed in 4% PFA for immunostaining. The chamber slides were then blocked with 10% goat serum PBS (v/v) and underwent the same protocol for SCUBE1 staining as detailed above.

siRNA transfection and proliferation assay
 For siRNA transfection, the sense and antisense strands of SCUBE1 siRNA were as follows: 5′-GGAAUGCCGUAUUACAAUAdTdT-3′ (sense) and 5′-pAUUGCUAAGGC-GAUUCCdTdTG-3′ (antisense). Control siRNA (5 mmol at 50 µM) was obtained from Ambion (Cambridge, MA, USA). When MMEC cells reached 60–70% confluence, they were serum starved for 12 h. The serum-free media was then replaced by 5% FBS DMEM before SCUBE1 siRNA and control siRNA transfection at a concentration of 80 nM was performed using Oligofectamine Reagent (Invitrogen, Carlsbad, CA, USA) media. Transfected cells were immunostained 24 h post-transfection with SCUBE1 antibody. Media from 24 h post-transfected cells was transferred to MCT cultures for gene-silencing experiments assessing tubular cell proliferation and for Western blot analysis.

Cell proliferation analysis using MCT cultures with post-siRNA-transfected media was performed by a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA). MCT cells (n=4) were cultured in 10 µl DMEM media with 50 µl conditioned media removed from siRNA-transfected cells in 96-well plates for 24 h. The cell proliferation assay was performed in an ELISA microplate spectrophotometer plate reader using a Microplate Manager (Bio-Rad, Hercules, CA, USA) at 490 nm.

Quantitative real-time PCR
 Total RNA was extracted from cultured cells using High Pure RNA Isolation Kit (Roche). The sequence of the self-designed primer for SCUBE1 was as follows: 5′-CGCAAAACTCTGCTGGACCTGTC-3′ and (R): 5′-GAACTCTCCCTGGACTTCG-3′ and GAPDH was (F): 5′-CTACCTCATCATTGATGTTAG-3′ and (R): 5′-CCAGCGGATCCTTGTGGCAG-3′. One-step quantitative real-time polymerase chain reaction (qPCR) analysis was performed with SYBR Green PCR Reagents (Sigma, St. Louis, MO, USA), using an Eppendorf Realplex4 Real-Time PCR machine. All reactions were performed in 96-well optical reaction plates (Applied Biosystems) in quadruplicate. Both SCUBE1 and GAPDH standard curves were generated at the end of the PCR cycles. In calculating the qPCR value, GAPDH amount served as a standard.

Western blotting
 MMEC cells underwent 12 h of serum starvation before siRNA transfection as described above and the supernatant was collected at 24 h thereafter. Five millilitres of supernatant were collected and snap frozen at 4000 g for 30 min to concentrate the solution to 300 µl. The supernatant proteins were separated by electrophoresis and the proteins electroblotted onto a polyvinylidene difluoride membrane (Roche, Castle Hill, Australia) and transferred onto a Pierce PVDF transfer membrane (Millipore, MA, USA) and spun at 4000 g for 30 min to concentrate the solution to 300 µl. The supernatant proteins were separated by electrophoresis and the proteins electroblotted onto a polyvinylidene difluoride membrane (Roche, Castle Hill, Australia). Transfer membranes were blocked and incubated with the SCUBE1 primary antibody (1:3000) at 4°C overnight and a peroxidase-conjugated rabbit anti-mouse antibody (1:40 000; Chemicon, Temecula, CA, USA) for 30 min. The membranes were washed in PBS and exposed to a high-performance chemiluminescent film (Amersham Biosciences, Buckinghamshire, UK).
Statistical analysis
Data were expressed as the mean ± SEM with statistical analyses performed using one-way and two-way analysis of variance (ANOVA) using GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Morphological alteration in IR injury
Histological staining was used to observe the morphological alterations in the IR kidneys at 1 and 3 weeks post-IR injury. At 1 week after IR, there was extensive damage throughout the kidney as evident by protein casts (arrows), a prominent inflammatory cell infiltrate and widespread necrosis in tubules as shown by denuded basement membranes; at 3 weeks after IR, endogenous repair was evident with reduced inflammatory cell infiltration, reduced numbers of protein casts or reabsorbing casts with tubules demonstrating widespread re-epithelialization (magnification A and C ×100; magnification E ×200; magnification B, D and F ×400).

SCUBE1 expression in kidney remodelling
SCUBE1 mRNA expression levels at different time points after IR injury were determined by PCR, using GAPDH standardization. Compared to the control contralateral kidney, SCUBE1 expression was upregulated relative to control at 1, 3 and 6 weeks after IR injury (Figure 2). These results demonstrate that SCUBE1 expression peaks during the remodelling period of IR injury with decreased expression evident with resolving renal injury at later time points. In comparison, SCUBE1 expression was absent or only just detectable in control kidneys at all time points.

Immunofluorescence microscopy of SCUBE1
The localization of SCUBE1 in IR mice was investigated using immunofluorescence microscopy. As SCUBE1 mRNA expression was abundant at 3 weeks after IR injury, immunofluorescence confocal microscopy was examined at this time point. Histopathology at 3 weeks after IR suggests endogenous kidney recovery from injury (Figure 1). Confocal microscopy demonstrated that SCUBE1 protein expression was co-localized to the CD31-positive endothelial cells of the glomerulus and the peritubular capillary network surrounding the cortical and corticomedullary tubules (Figure 3). SCUBE1 staining was not detected in the right contralateral control kidney.

Quantification of PCNA-positive cells was used to assess the degree of tubular epithelial cell proliferation occurring during the initial injury phase (1 week) and resolution phase (3 and 6 weeks) of IR injury. Quantification of the number of PCNA-positive cells showed that, at 3 weeks, there was a significant increase in the number of proximal tubular epithelial cells with PCNA localization (23.25 ± 1.7 vs 5.75 ± 2.36; P<0.0001) compared to control kidneys (Figure 4G).

SCUBE1 expression in serum-starved and hypoxia-conditioned endothelial cells
To further determine the regulation of SCUBE1 under the hypoxic conditions that are a feature of renal injury and disease, MMECs were cultured under conditions of cell stress in vitro and assessed for SCUBE1 expression. Immunolocalization of SCUBE1 protein expression was evaluated in endothelial cells cultured in either serum-free conditions or in media with the addition of 200 µM CoCl2 as a method to stimulate hypoxia. In addition, serum starving was performed to induce cell stress in MMEC cultures. Immunofluorescence staining for SCUBE1 protein expression in serum-starved cells showed that, 12 and 24 h after the addition of CoCl2, MMEC cells expressed SCUBE1...
SCUBE1 expression was performed at the mRNA level in cell cultures under stress conditions for 3, 6, 12 and 24 h. Total RNA was obtained from cells cultured in serum-free media or with the addition of CoCl₂ in comparison to control conditions. qPCR showed no significant difference between the two experimental groups after either 3 or 6 h in culture (Figure 5C). However, SCUBE1 mRNA expression was significantly elevated at 12 h (12 h vs control: $3.26 \times 10^{-6} \pm 9.52 \times 10^{-7}$ vs $2.22 \times 10^{-6} \pm 4.54 \times 10^{-7}, P<0.05$) and 24 h (24 h vs control: $5.49 \times 10^{-6} \pm 4.96 \times 10^{-7}$ vs $2.70 \times 10^{-6} \pm 1.22 \times 10^{-7}, P<0.05$) after serum starving compared to controls. The addition of CoCl₂ was also found to significantly elevate SCUBE1 mRNA expression at 24 h (24 h vs control: $6.86 \times 10^{-6} \pm 1.33 \times 10^{-6}$ vs $2.52 \times 10^{-6} \pm 1.22 \times 10^{-6}, P<0.05$) in comparison to control cultures (Figure 5C).

(Figure 5). In comparison, there was no SCUBE1 expression in control cell cultures at the same time points.

SCUBE1 expression was found to co-localize with CD31, a marker of the endothelial vascular cell network, present in the interstitium surrounding the tubules and the glomerulus (arrows); in the control kidney (merge image A), there was no SCUBE1 expression evident in the tubulointerstitium [C and D represents SCUBE1 (red), E and F represents CD31 (green) and G and H represents DAPI (blue); magnification ×600].

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Fig. 3. Localization of SCUBE1 in mouse kidneys with IR injury or contralateral control kidneys: confocal microscopy demonstrating SCUBE1 protein localization at 3 weeks in control (A, C, E, G) and post-IR kidneys (B, D, F, H); at 3 weeks after IR (merge image B), SCUBE1 was found to co-localize with CD31, a marker of the endothelial vascular cell network, present in the interstitium surrounding the tubules and the glomerulus (arrows); in the control kidney (merge image A), there was no SCUBE1 expression evident in the tubulointerstitium [C and D represents SCUBE1 (red), E and F represents CD31 (green) and G and H represents DAPI (blue); magnification ×600].

Fig. 4. Immunofluorescence microscopy of PCNA expression in IR kidneys: immunofluorescence staining of PCNA in kidneys at 3 weeks post-IR injury (merge image A) and a corresponding control kidney (merge image B) showing PCNA (red; C and D) and DAPI (blue; E and F), magnification ×600; G quantification of the number of PCNA-positive proximal tubular epithelial cells in the cortex and corticomedullary region of IR kidneys compared to corresponding control kidneys; *$P<0.001$. 

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SCUBE1 silencing in endothelial cells and epithelial cell proliferation

The present study assessed the effect of silencing SCUBE1 expression on tubular cell proliferation. Using siRNA transfection, immunofluorescence demonstrated that SCUBE1 siRNA-transfected endothelial cells had a significantly lower expression of SCUBE1 protein in comparison to controls (Figure 6). To assess whether SCUBE1 secreted from endothelial cells affected tubular cell proliferation, the media from post-serum starvation siRNA-transfected MMEC cells was added to cultured MCT cells and a proliferation assay after 24 h of culture was performed. Figure 6 demonstrates a significant difference ($P<0.001$) in cell proliferation between MCT cells cultured with the addition of media obtained from MMEC SCUBE1 gene-silencing cultures compared to MCT cells in media from control siRNA-transfected MMEC cells. These results suggest that SCUBE1, secreted from endothelial cells, following injury may participate in endogenous renal remodelling and cellular replacement by increasing tubular cell proliferation.

SCUBE1 protein expression in supernatants following cell transfection

*In vitro* cell culture was used to further confirm the protein expression of SCUBE1 in the media from MMECs following SCUBE1 siRNA transfection. SCUBE1 siRNA and control siRNA were transfected into a MMEC endothelial cell line post-serum starvation. Twenty-four hours post-culture, the supernatants were concentrated 20-fold and analysed by Western blot analysis for SCUBE1 protein expression in supernatants from SCUBE1 siRNA-transfected and control siRNA-transfected cells at 24 h after culture; in contrast, an upregulated expression of SCUBE1 protein was detected in control siRNA-treated cell supernatants at the same time point after transfection.
Discussion

The present study demonstrates that SCUBE1 is localized to CD31-positive endothelial cells in damaged kidneys following IR injury. The peak expression of SCUBE1 mRNA expression was evident at 3 weeks after IR when endogenous remodelling, particularly extracellular matrix reorganization and replacement of damaged tubular epithelial cells, were evident. In addition, SCUBE1 was expressed in cultured endothelial cells (MMEC) under conditions simulating cell stress. The in vitro analysis of MCT cells cultured in media from MMEC cells following SCUBE1 gene silencing provide insight into the direct actions of SCUBE1 on tubular epithelial cell proliferation. In these studies, the MCT cultured following siRNA transfection to silence SCUBE1 expression showed a marked decrease in epithelial cell proliferation compared with control cultures grown in media from untreated cells, suggesting that SCUBE1 secreted by endothelial cells may promote epithelial cell proliferation.

The name SCUBE1 was originally termed signal peptide, CUB (complement proteins C1r/C1s, Uegf and Bmp1) and epidermal growth factor (EGF)-like domain containing protein 1 [11,12]. Mouse and human Scube1 are homologous genes and strictly conserved amino acids have been observed in the CUB domain of both SCUBE1 proteins [3]. The SCUBE1 gene is highly expressed in the developing gonad, nervous system, somites, surface ectoderm and limb buds in mouse embryos from day 8.5 to 13.5 [11]. It is a secreted and cell-associated protein that localizes to human vascular endothelium [42]. Tu et al. [37] reported that SCUBE1 functions as a novel adhesive molecule in human platelets. Moreover, targeted disruption of the Scube1 gene indicates that SCUBE1 is required during the early stages of central nervous system development, possibly through modulating the activity of members of the bone morphogenetic protein family [38]. SCUBE2 and SCUBE3 are the other two members in the SCUBE family. SCUBE2, also known as Cegp1, is important in neural development in the embryo [11] and is expressed in adult tissue including the heart, lung and testis [12,42]. Hollway et al. [15] reported that the Scube2 zebrafish mutant is associated with disruption of the HH signal transduction pathway, resulting in developmental defects. Recently, Yang and colleagues [41] demonstrated that SCUBE3 expression may regulate cardiac growth and the remodelling response acting via the TGF-β1 signalling pathway.

In addition to the expression of SCUBE1 in the endothelium [38,42] and platelets [37,38], there is evidence that SCUBE1 is expressed in the urogenital system and during branching morphogenesis suggestive of an important role in the differentiation of the metanephric mesenchyme during kidney development [11,12,40]. There is growing consensus that tissue regeneration and repair in adult organs involves the re-expression of a number of genes and represents a recapitulation of developmental pathways that are normally switched off at birth [28,30,32,36]. The control of kidney branching morphogenesis is regulated by an array of growth factors that include inductive and inhibitory signals. Genome-wide expression studies of the kidney have identified many common developmental genes, such as Wnt-4 and sonic hedgehog (Shh) that show changes in expression as a function of developmental time [6,40]. Wnt-4 proteins are expressed early during the formation of the metanephric mesenchyme, are critical for kidney tubulogenesis and can act as auto-inducers of the mesenchymal-to-epithelial transition that underlies nephron development [34]. Scube2 is involved in Shh signal transduction [15] and is essential for mesenchymal patterning [44].

In contrast to the early induction of the SCUBE genes during organogenesis, less is known about the role of SCUBE1 in post-embryonic processes [38]. The in vitro studies enabled the direct assessment of SCUBE1 protein expression on tubular cell proliferation and following hypoxic injury under culture conditions. In contrast to the kinetics of an in vitro setting, the peak expression of SCUBE1 protein at 3 weeks after IR injury provides evidence that SCUBE1 may be an indirect consequence of the initial ischemia, and therefore, form part of the downstream trophic milieu following reperfusion that regulates the promotion of cellular replacement leading to subsequent endogenous repair. We report that SCUBE1 was expressed during the resolution phase following blood reperfusion after ischaemic injury, a time when vascular remodelling is evident. At this time point, SCUBE1 was localized to endothelial cells of the peritubular and glomerular capillary network that are essential components contributing to the promotion of tubular epithelial cell integrity and re-vascularization.

There has been extensive interest in the origin of tubular epithelial cells that contribute to re-epithelialization of damaged nephron tubules. It is now generally accepted that the majority of tubular epithelial cells that regenerate following injury are derived from an intra-renal source [2,16,24]. Proximal and distal tubular epithelial cells and cells of the collecting tubule can respond to stress and cell death of large portions of the nephron through a process of self-regeneration via local proliferation [16]. Closely associated with structural integrity and functional recovery of the kidney following acute damage is the interaction between the renal tubular epithelium and the peritubular capillary endothelial cells of the surrounding vasculature network. Endothelial progenitor cells (EPCs) originating from the bone marrow may infiltrate damaged kidneys and replace damaged endothelial cells [18,33,39]. Therefore, both EPCs and the surviving endothelial cells incorporate and contribute to the replenishment of the damaged endothelium, leading to endogenous cellular recovery following acute ischaemia [1]. In a rat model of anti-Thy1.1 glomerulonephritis, Uchimura et al. [39] demonstrated that bone marrow-derived EPCs incorporated into the glomerular endothelium enhanced cellular repair and protected the kidney against further glomerular injury. Endothelial cell swelling as a result of dysfunction of the cell junctions of the renal capillaries [35] is a pivotal feature post-IR injury that can determine the extent of damage and reversibility or irreversibility of tissue damage [8]. During IR injury, endothelial cell dysfunction may also influence epithelial cell integrity and the ability of the kidney to respond to damage [4]. Hypoxic insult to endothelial cells
can lead to the production of a variety of inflammatory mediators and angiogenic factors, including intracellular adhesion molecule-1 (ICAM-1), endothelin [9,17,31] and nitric oxide [21,43]. Kelly et al. [20] demonstrated that increased ICAM-1 expression in the vascular endothelium plays a major pathophysiological role in the pathogenesis of IR injury, leading to renal dysfunction. In addition, vascular endothelial cell growth factor (VEGF) is an angiogenic factor secreted by podocytes, tubular epithelial cells and endothelial cells in the kidney [5,7,13] that plays an important role in maintaining kidney integrity [7,19,27,29]. VEGF production by endothelial cells supports a close relationship with tubular cell proliferation thereby stabilizing renal function, as has previously been reported in a rat remnant kidney model [19].

Conclusion

In summary, the present study explored the relationship between endothelial cell SCUBE1 expression and the promotion of tubular epithelial cell proliferation. SCUBE1 protein was localized to the endothelial cells of glomeruli and peritubular capillaries during the resolution of IR injury and cellular replacement. The in vitro analysis of MCT epithelial cells cultured in media from endothelial cells following SCUBE1 siRNA transfection provides insight into the direct actions of SCUBE1 on tubular epithelial proliferation correlating to the in vivo findings. The promotion of vascular integrity following hypoxic and acute injury is of utmost importance for the accelerated repair response that is closely associated with tubular epithelial and glomerular cell replacement.

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Conflict of interest statement. None declared.

References

Calcitonin-stimulated renal Ca\(^{2+}\) reabsorption occurs independently of TRPV5

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Abstract

**Background.** Calcitonin (CT) is known to affect renal Ca\(^{2+}\) handling. However, it remains unclear how CT affects Ca\(^{2+}\) transport in the distal convolutions. The aim of this study was to investigate the contribution of the renal epithelial Ca\(^{2+}\) channel, transient receptor potential vanilloid 5 (TRPV5), to renal Ca\(^{2+}\) handling in response to CT.

**Methods.** C57BL/6 mice received a single overnight (16 hr) injection of CT. In addition, TRPV5 knockout (TRPV5\(^{-/-}\)) mice and their wild type (TRPV5\(^{+/+}\)) controls, received three bolus injections of CT over a 40-hr study period. All experimental groups were placed in metabolic cages.

**Results.** C57BL/6 mice received a single bolus injection of CT, which significantly reduced the urinary Ca\(^{2+}\) excretion. In addition, urinary Na\(^{+}\) and K\(^{+}\) excretion also decreased after CT administration. No apparent changes in renal expression of TRPV5, calbindin-D\(_{28K}\) (CaBP28K) or TRPV6 could be detected between CT- and vehicle-treated mice. To evaluate whether TRPV5 activity is needed for the CT-induced increase in Ca\(^{2+}\) reabsorption, mice with genetic ablation of TRPV5 (TRPV5\(^{-/-}\)) were employed. TRPV5\(^{-/-}\) mice as well as their wild-type (TRPV5\(^{+/+}\)) controls received three bolus injections of CT over a 40-hr study period. Overnight (16 hrs) as well as the subsequent 24-hr urine was collected. Overnight urinary Ca\(^{2+}\) excretion was reduced in both TRPV5\(^{-/-}\) and TRPV5\(^{+/+}\) mice after a bolus injection of CT. The subsequent 24-hr urinary excretion of Ca\(^{2+}\) which was collected after the third bolus injection showed no effect of CT on renal Ca\(^{2+}\) handling in either mice group. Accordingly, CT did not alter the intrarenal protein abundance of TRPV5 and CaBP28K after three bolus injections of CT.

**Conclusion.** CT augments the renal reabsorptive capacity for Ca\(^{2+}\). This increase is likely to occur independently of TRPV5.

**Keywords:** calbindin; calcitonin; calcium; distal convoluted tubule; TRPV5

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