

Research Article

TMEM63C, a Potential Novel Target for Albuminuria Development, Is Regulated by MicroRNA-564 and Transforming Growth Factor beta in Human Renal Cells

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Keywords

Transmembrane protein 63C · MicroRNA · Podocyte · Transforming growth factor beta · Epithelial-mesenchymal transition

Abstract

Introduction: Transmembrane protein (TMEM) 63C is a member of the TMEM gene family and was recently linked to glomerular filtration barrier function and albuminuria. Its molecular function and expression regulation are largely unknown. **Objective:** In this study, we set out to characterize the regulating impact of microRNAs (miRNAs) such as miRNA-564 (miR-564) on TMEM63C expression in renal cells. Also, we examined the influence of transforming growth factor beta (TGF- β) on TMEM63C expression and the potential impact of TMEM63C inhibition on epithelial-mesenchymal transition (EMT) in renal cells and on cell viability in human embryonic kidney 293 cells (HEK 293). **Methods:** Expression analyses were done using real-time PCR and Western blot. Dual luciferase assay was performed to determine the miRNA-mediated expression control. Cell viability was assessed via trypan blue exclusion staining. **Results and Conclusions:** MiR-564 reduced TMEM63C expression in HEK 293 and human podocytes (hPC). The treatment of renal cells with TGF- β led to an increased expression of TMEM63C. Moreover, a reduced TMEM63C expression was associated with a changed ratio of EMT marker proteins such as α -smooth muscle actin versus E-cadherin in HEK 293 and

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decreased nephrin expression in hPC. In addition, cell viability was reduced upon inhibition of TMEM63C expression in HEK 293. This study demonstrates first mechanisms involved in TMEM63C expression regulation and a link to EMT in renal cells.

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Published by S. Karger AG, Basel

Introduction

Podocytes are terminally differentiated and highly specialized glomerular cells with a great importance for the glomerular filtration barrier (GFB) [1]. In this context, nephrin is known as an essential structural protein of the slit diaphragm and is considerably involved in the preservation of glomerular function as well as podocyte viability [2]. The injury or loss of podocytes leads to albuminuria and plays a pivotal role in several glomerular diseases including diabetic and non-diabetic kidney diseases [3, 4]. While albuminuria itself represents a marker of renal damage, it also has a direct pathogenic effect on renal tissue [5, 6]. Therefore, albuminuria is discussed as suitable therapeutic target for intervention to slow the progression of CKD [5].

Recently, a study identified transmembrane protein (TMEM) 63C as a novel candidate for albuminuria development [7]. TMEM63C, a member of the TMEM gene family, is expressed in the kidney, but also in many other tissues such as the cerebral cortex and endocrine tissues [7]. Schulz et al. [7] showed a loss of TMEM63C expression in podocytes of patients with focal segmental glomerulosclerosis. In addition, functional studies in zebra fish models suggested that TMEM63C could play an important role in GFB function [7]. Nevertheless, the biological function of TMEM63C and its expression regulation are widely unknown.

MicroRNAs (miRNAs) are short, non-coding RNAs consisting of 20–22 nucleotides, which are known to be influential regulators of post-transcriptional gene expression [8]. The regulation and function of miRNAs are subject of current research. In this context, previous studies described a major influence of miRNAs on kidney diseases [8–10]. For example, miRNA-21 (miR-21), miR-29, and miR-192 were found to play a pathophysiological role in renal fibrosis induced by transforming growth factor beta (TGF- β) [10]. Furthermore, the regulating effect of miRNAs becomes more important in cancer research. In this regard, the impact of several miRNAs, such as miR-21, miR-22, and miR-566, were described in the pathogenesis of renal cell cancer [11–13].

This study sets out to characterize the post-transcriptional regulation of TMEM63C expression by miRNAs including miR-30b and miR-564 in renal cells. Moreover, we analyzed the impact of small interfering RNA (siRNA)-mediated TMEM63C inhibition on cell viability. In addition, the effect of TGF- β on TMEM63C regulation and the impact of TMEM63C deficiency on epithelial-mesenchymal transition (EMT) were analyzed in cell culture studies.

Methods

Cell Culture

Human podocytes (hPC; supplied by Dr. M. Saleem, University of Bristol, Bristol, UK) were cultured and differentiated as described previously [14]. For our investigations, we not only used hPC, but also human embryonic kidney 293 cells (HEK 293) due to its high transfection efficiency and as suitable model to study epithelial characteristics in kidney cells. HEK 293 (a kind gift of Prof. Dr. H. Fechner, Technical University of Berlin, Berlin, Germany) were cultured in DMEM enriched with 10% fetal bovine serum and 1% penicillin/streptomycin – all provided by Biochrom GmbH, Berlin, Germany. HEK 293 were grown in a humidified incu-

Table 1. The primer sequences of real-time PCR

Gene	Primer sequences (5'→3')
<i>TMEM63C</i>	
Forward	CAAGCGTGTCCGTAAGGATT
Reverse	ACAATTGGGTTCTGCAGCTT
<i>GAPDH</i>	
Forward	GAGTCAACGGATTTGGTTCGT
Reverse	GATCTCGCTCCTGGAAGATG
<i>Nephrin</i>	
Forward	ATCCTCTCCATCCTGGTTCC
Reverse	GTCCTGGAGGGAACAGAAACA
<i>E-cadherin</i>	
Forward	ACATTTCCCAACTCCTCTCC
Reverse	TCTGTCACCTTCAGCCATC
α -SMA	
Forward	CGAAGCACAGAGCAAAAGAG
Reverse	AGGCATAGAGAGACAGCACCG

bator at 37°C and with 5% CO₂. Before transfection, HEK 293 and hPC were starved in fetal bovine serum-free DMEM or RPMI 1640, respectively. 200 nM of miR-30b, miR-564, or negative control (miRControl) miRNA mimic was used for miRNA transfection. For siRNA transfection, 200 nM of TMEM63C siRNAs (siTMEM63C) or scrambled control siRNAs (siControl) were deployed. Transfection was done by using Lipofectamine™ 2000 (Invitrogen GmbH, Karlsruhe, Germany). The inhibitory effect of siTMEM63C was examined on mRNA and protein levels. For stimulation, hPC and HEK 293 were treated with 5 and 10 ng/mL TGF- β (eBioscience; Thermo Fisher Scientific GmbH, Waltham, MA, USA), respectively, for 48 h. Transfection efficiency, determined by Dy547 transfection control (200 nmol/L; Fisher Scientific – Germany GmbH, Schwerte, Germany), was determined to be 25% in hPC [14] and 62% in HEK 293.

Real-Time PCR

Analyses of mRNA expression were performed as described previously [14]. Specific TaqMan® Gene Expression Assays (Life Technologies GmbH, Darmstadt, Germany) were utilized for determination of TMEM63C, nephrin, α -smooth muscle actin (α -SMA), E-cadherin, and glyceraldehyde-3-phosphate dehydrogenase expression, following the manufacturer's instructions. Real-time PCR was accomplished by means of 7500 Fast Real-time PCR System (Applied Biosystems, Carlsbad, CA, USA) using the following conditions: 50°C, 2 min; 95°C, 20 s; 45 cycles 95°C, 3 s; 60°C, 30 s. The PCR primers used are shown in Table 1.

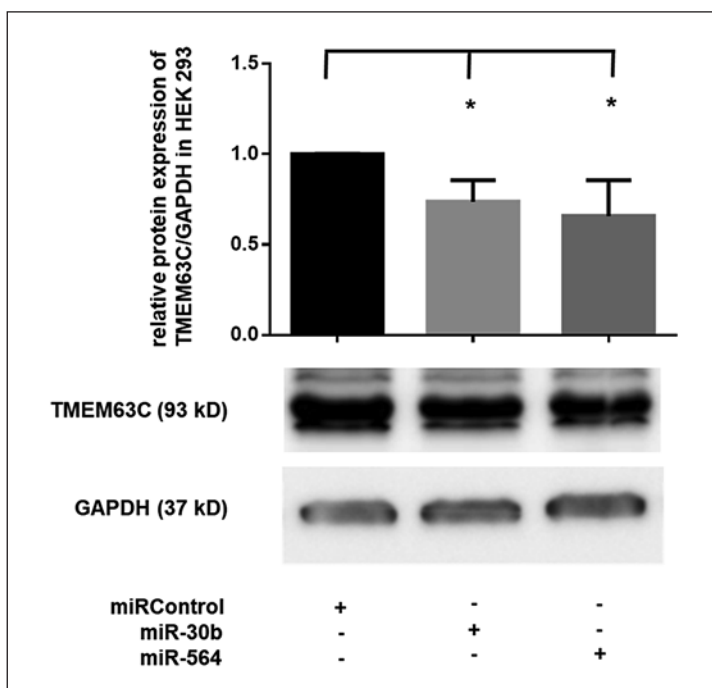
Western Blotting

Western blot analyses were done as described previously [15]. Specific antibodies against TMEM63C (Sigma-Aldrich Chemie GmbH, Munich, Germany) and glyceraldehyde-3-phosphate dehydrogenase (Calbiochem, Darmstadt, Germany) were used for detection. Blots were visualized and quantified by using FUSION FX7 (Peqlab Biotechnologie GmbH, Erlangen, Deutschland) and Gel-Pro Analyser software version 4.0.00.001 (Media Cybernetics, Bethesda, MD, USA).

Dual Luciferase Activity Assay

For determination of dual luciferase activity, 2×10^4 HEK 293 were treated with miR-30b, miR-564, or miRControl in 96-well plates. Cells were co-transfected with aforementioned

Fig. 1. The impact of miR-30b and miR-564 on TMEM63C protein expression in HEK 293. The protein expression of TMEM63C in HEK 293 transfected with miR-30b, miR-564, or a negative control miRNA mimic (miRControl) is depicted. The expression was analyzed after 48 h. GAPDH was used for normalization. Shown is the mean \pm SEM of at least 3 independent experiments. (*) $p < 0.05$. miR, miRNA; TMEM63C, transmembrane protein 63C; HEK 293, human embryonic kidney cells 293; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



miRNAs and 200 ng/mL dual luciferase reporter vector (3'UTR-TMEM63C vec) containing the 3'-untranslated region (3'UTR) of TMEM63C, a reporter vector (3'UTR-TMEM63C-mut vec) containing a mutated miRNA binding site in the 3'UTR of TMEM63C or a negative control vector (control vec), respectively (provided by GeneCopoeia, Inc., Rockville, MD, USA). After 24 h, dual luciferase activity was analyzed by using a luciferase reporter assay (Promega GmbH, Mannheim, Germany) following the manufacturer's protocol.

Cell Viability Assay

Cell viability was determined by a trypan blue staining assay. In brief, HEK 293 were seeded in 12-well plates and transfected with 200 nM siTMEM63C or siControl, as described previously. After 48 h, cells were removed from the wells using trypsin-EDTA and subsequently resuspended in fresh DMEM. 10 μ L of cell suspension was mixed with 90 μ L of trypan blue solution (Biochrom GmbH, Berlin, Germany). Eventually, living and trypan blue-stained dead cells were counted using a C-Chip Neubauer improved hemocytometer chamber (Carl Roth GmbH & Co. KG., Karlsruhe, Germany).

Statistical Analysis

All results were represented as mean \pm standard error the mean (SEM) and analyzed by Student's *t* test or one-way ANOVA. A probability value (p) < 0.05 was deemed as significant.

Results

Impact of Different miRNAs on TMEM63C Expression in HEK 293

First, we performed in silico analysis to identify potential miRNAs modulating TMEM63C expression on post-transcriptional level. In our analysis, we searched relevant databases, such as TargetScanHuman 7.1 (www.targetscan.org), miRDB (www.mirdb.org), and Diana

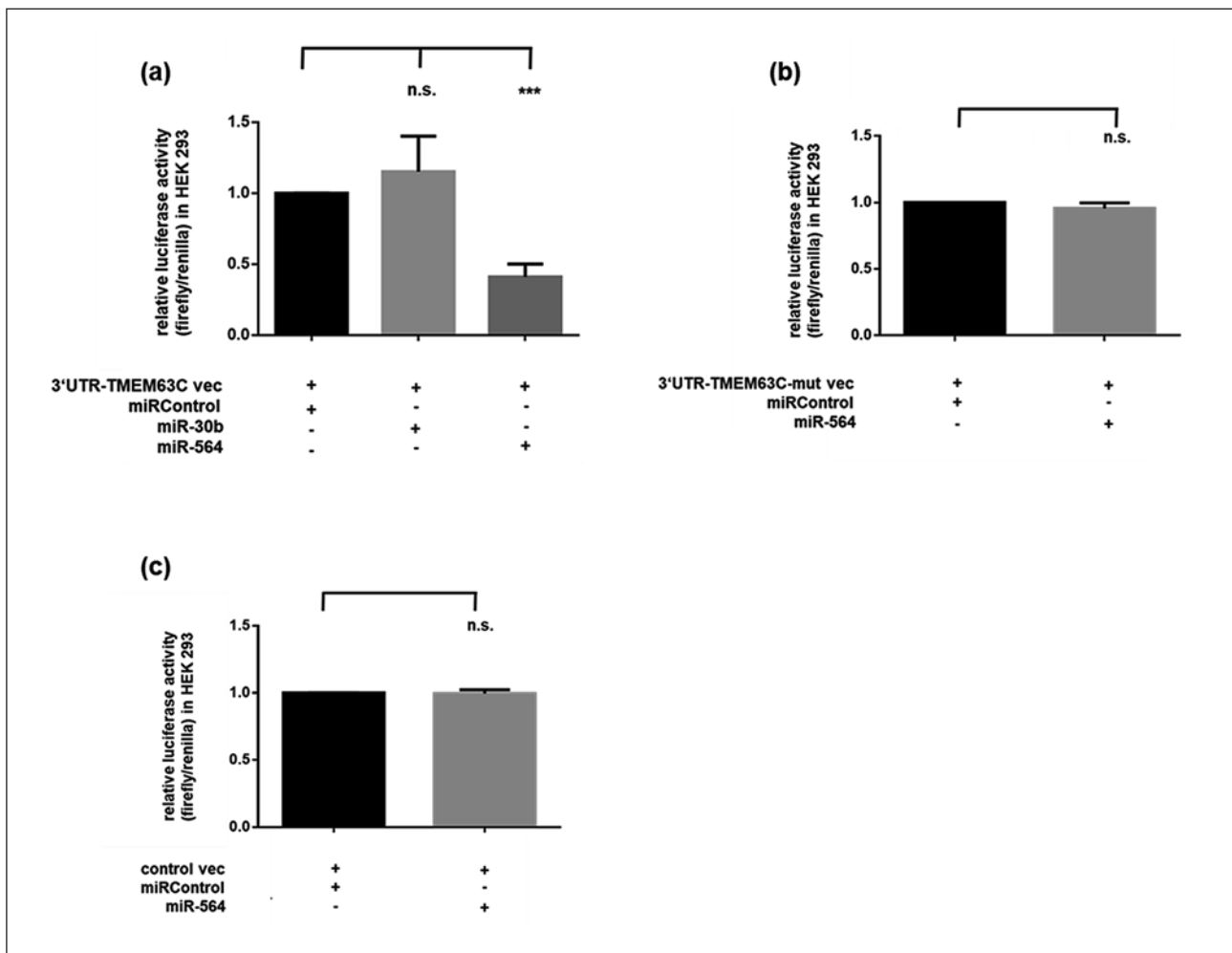


Fig. 2. The influence of miR-30b and miR-564 on luciferase activity in HEK 293. Shown is the luciferase activity in HEK 293 transfected with (a) luciferase reporter construct containing 3'UTR of TMEM63C (3'UTR-TMEM63C vec) and co-transfected with miR-30b, miR-564, or a negative control miRNA mimic (miRControl). For control, HEK 293 were co-transfected with (b) control vector (3'UTR-TMEM63C-mut vec), containing a mutated miRNA binding site in the 3'UTR of TMEM63C, and (c) a non-functional control vector (control vec), respectively. Firefly luciferase activity was determined 24 h after transfection and normalized against renilla luciferase activity. In a, b, c, the mean \pm SEM of at least 3 independent experiments is shown. (***) $p < 0.001$. n.s., no significant difference; miR, miRNA; HEK 293, human embryonic kidney cells 293; 3'UTR, 3'-untranslated region; TMEM63C, transmembrane protein 63C.

Tools (www.diana.imis.athena-innovation.gr). All of the abovementioned prediction tools revealed miR-30b and miR-564 as potential candidates for TMEM63C expression regulation via binding to the 3'UTR of TMEM63C mRNA. To examine the effect of the potential miRNA candidates on TMEM63C expression, we used respective miRNA mimics in our experiments. Next, we determined the influence of the aforementioned miRNAs on protein expression of TMEM63C in HEK 293. In our experiments, miR-30b and miR-564 led to a decreased protein generation of TMEM63C after 48 h (Fig. 1).

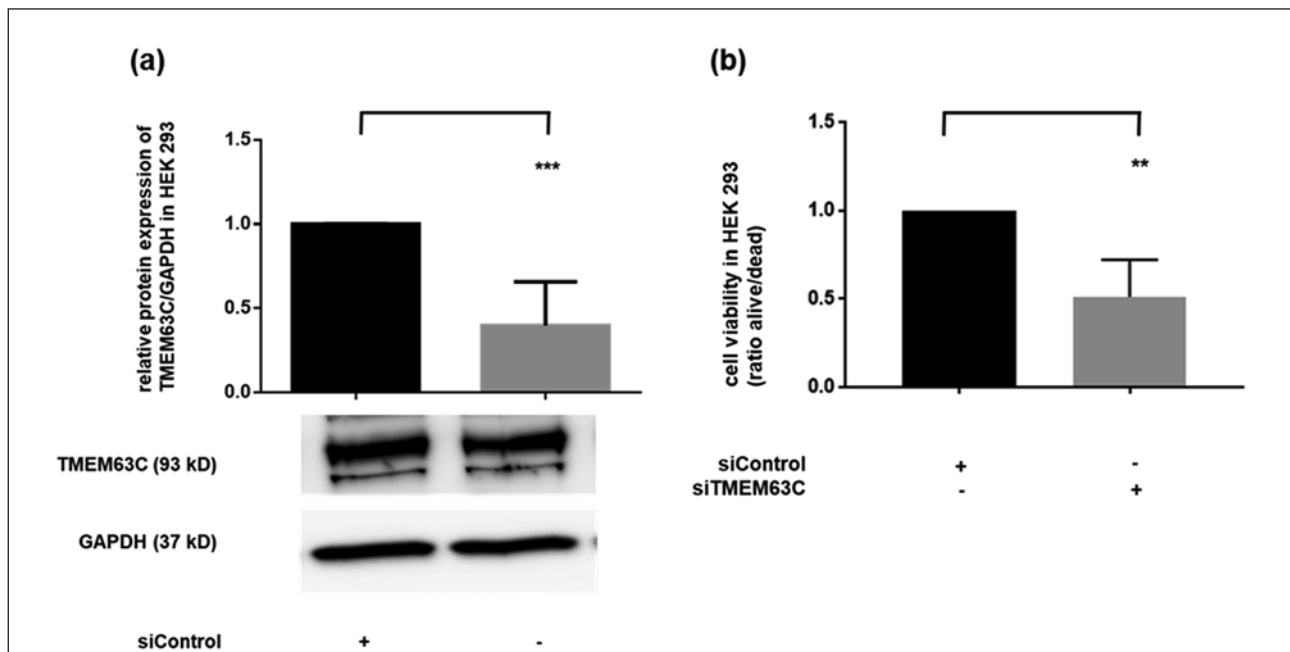


Fig. 3. The impact of TMEM63C inhibition on cell viability in HEK 293. HEK 293 were treated with specific siRNAs against TMEM63C (siTMEM63C) or scrambled control siRNAs (siControl) for 48 h. Shown is the (a) protein expression of TMEM63C and the (b) cell viability of HEK 293. The mean \pm SEM of at least 3 independent experiments is represented. (***) $p < 0.001$; (**) $p < 0.01$. TMEM63C, transmembrane protein 63C; HEK 293, human embryonic kidney cells 293; siRNA, small interfering RNA.

Relative Luciferase Activity Measurement in HEK 293

Compared to controls, co-transfection of cells with the TMEM63C reporter vector and miR-564 led to a reduced luciferase activity, whereas co-transfection with a TMEM63C reporter vector including a mutated 3'UTR binding site and miR-564 exhibited no significant difference in luciferase activity (Fig. 2a, b). In contrast, co-treatment of HEK 293 with the reporter construct and miR-30b had no significant impact on luciferase activity in relation to controls (Fig. 2a).

Cell Viability of HEK 293 Was Decreased by siRNA-Mediated Inhibition of TMEM63C

After 48 h, we observed a significant reduction of TMEM63C protein expression in siTMEM63C-treated cells (Fig. 3a). Compared to controls, cell viability was also significantly reduced in siTMEM63C-transfected HEK 293 after 48 h (Fig. 3b).

TGF- β Stimulation Increased TMEM63C Protein Expression in HEK 293

We analyzed the impact of TGF- β stimulation on TMEM63C expression in HEK 293 after 48 h. Compared to controls, treatment of the cells with TGF- β increased TMEM63C expression on protein level in a concentration-dependent manner (Fig. 4).

The Ratio of α -SMA versus E-Cadherin Was Affected by TMEM63C Expression

To investigate the impact of TMEM63C on EMT, we transfected HEK 293 with specific siRNAs against TMEM63C for 48 h and analyzed the ratio of α -SMA versus E-cadherin mRNA expression. Compared to controls, the ratio of α -SMA versus E-cadherin increased significantly in siTMEM63C-treated cells (Fig. 5).

Fig. 4. The effect of TGF- β stimulation on TMEM63C protein expression in HEK 293. HEK 293 were stimulated with 5 or 10 ng/mL TGF- β for 48 h. TMEM63C was normalized against GAPDH. The mean \pm SEM of at least 3 independent experiments is shown. (*) $p < 0.05$. n.s., no significant difference; TGF- β , transforming growth factor beta; TMEM63C, transmembrane protein 63C; HEK 293, human embryonic kidney cells 293; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

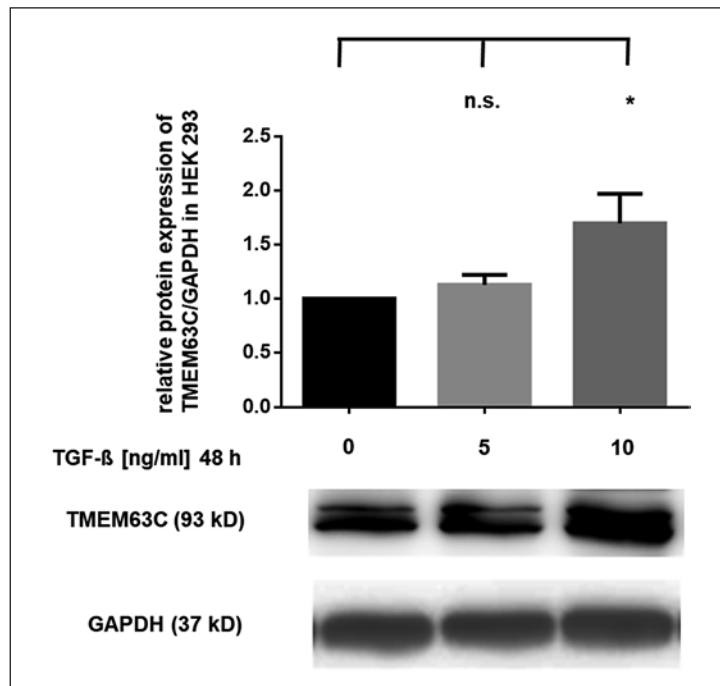
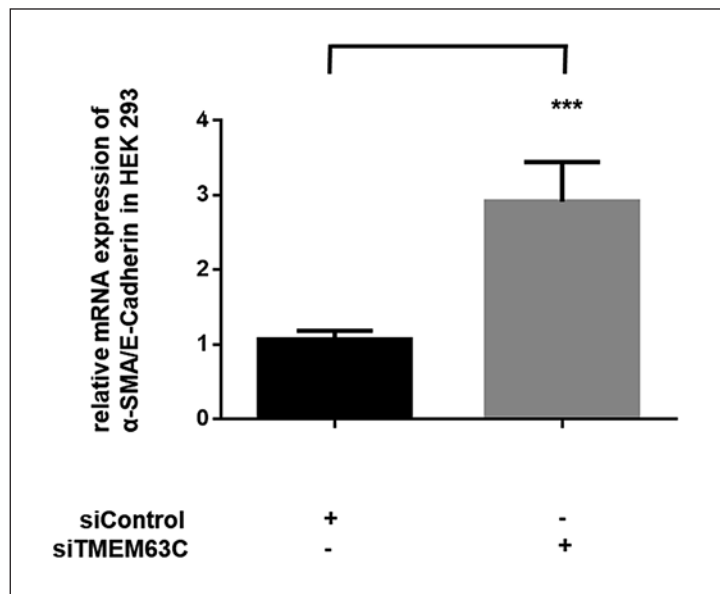


Fig. 5. The effect of TMEM63C inhibition on α -SMA versus E-cadherin. HEK 293 were transfected with inhibitory siRNAs against TMEM63C (siTMEM63C) or non-sense control siRNAs (siControl) for 48 h. Shown is the ratio of mRNA expression of α -SMA versus E-cadherin normalized against GAPDH. The mean \pm SEM of at least 3 independent experiments is represented. (***) $p < 0.001$. TMEM63C, transmembrane protein 63C; α -SMA, α -smooth muscle actin; HEK 293, human embryonic kidney cells 293; siRNA, small interfering RNA.



MiR-564 Decreased TMEM63C Expression in hPC

In addition to the experiments in HEK 293, we analyzed the impact of miR-564 on TMEM63C expression in hPC. Compared to controls, TMEM63C mRNA expression was significantly reduced in miR-564-transfected cells after 48 h (Fig. 6).

TGF- β Stimulation Increased TMEM63C Protein Expression in hPC

Subsequently, we investigated the impact of TGF- β on TMEM63C expression in hPC. Compared to controls, the treatment of the cells with 5 or 10 ng/mL TGF- β for 48 h increased TMEM63C expression on protein level in a concentration-dependent manner (Fig. 7).

Fig. 6. The effect of miR-564 on TMEM63C mRNA expression in hPC. Shown is the expression of TMEM63C mRNA in hPC. Cells were transfected with miR-564 or a negative control miRNA mimic (miRControl). The expression was analyzed after 48 h. GAPDH was used for normalization. The mean ± SEM of at least 3 independent experiments is shown. (***) $p < 0.001$. miR, miRNA; TMEM63C, transmembrane protein 63C; hPC, human podocytes; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

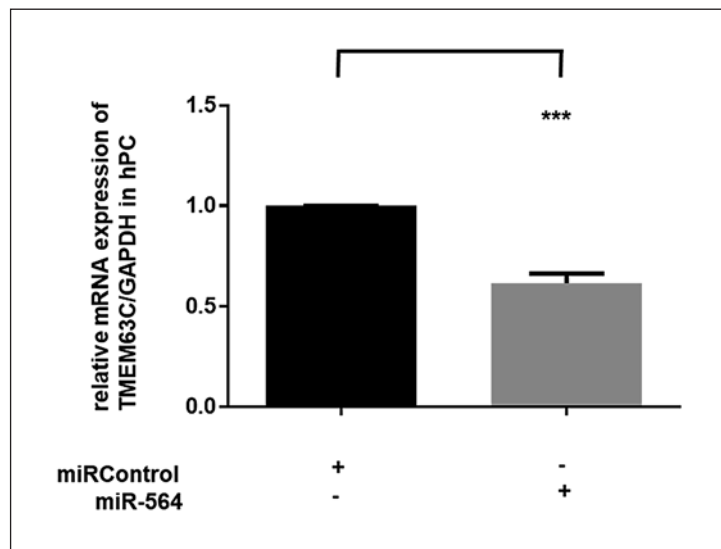
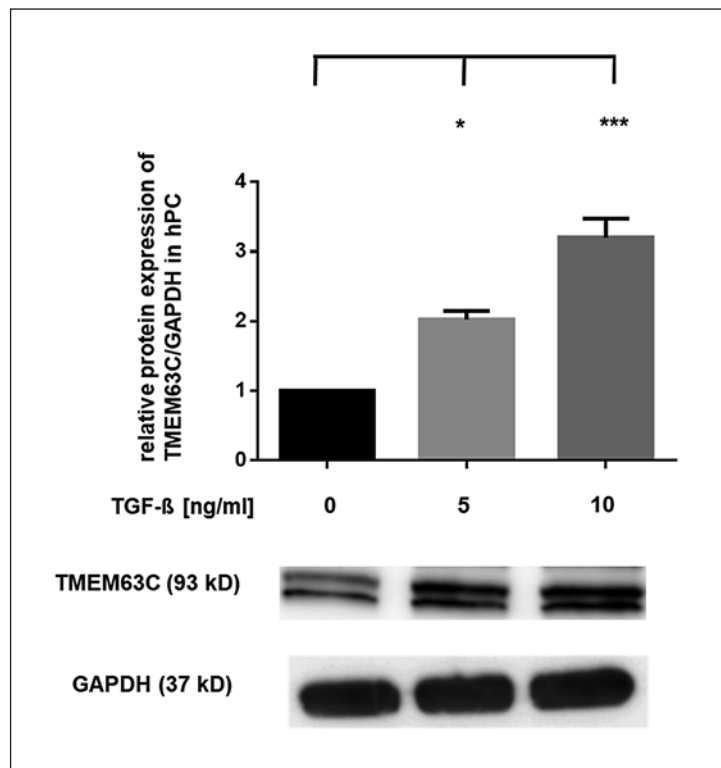


Fig. 7. The effect of TGF-β stimulation on TMEM63C protein expression in hPC. hPC were stimulated with 5 and 10 ng/mL TGF-β for 48 h, respectively. TMEM63C was normalized against GAPDH. The mean ± SEM of at least 3 independent experiments is shown. (*) $p < 0.05$; (***) $p < 0.001$. TGF-β, transforming growth factor beta; TMEM63C, transmembrane protein 63C; hPC, human podocytes; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



Expression of Nephrin Was Reduced by siRNA-Mediated Inhibition of TMEM63C in hPC

Moreover, we studied the impact of TMEM63C expression on nephrin generation in hPC. Transfection of cells with TMEM63C-specific siRNAs led to a significant reduction of TMEM63C mRNA expression in hPC (Fig. 8a). Compared to controls, siRNA-mediated downregulation of TMEM63C was additionally associated with a significant decrease in nephrin expression on mRNA level after 48 h (Fig. 8b).

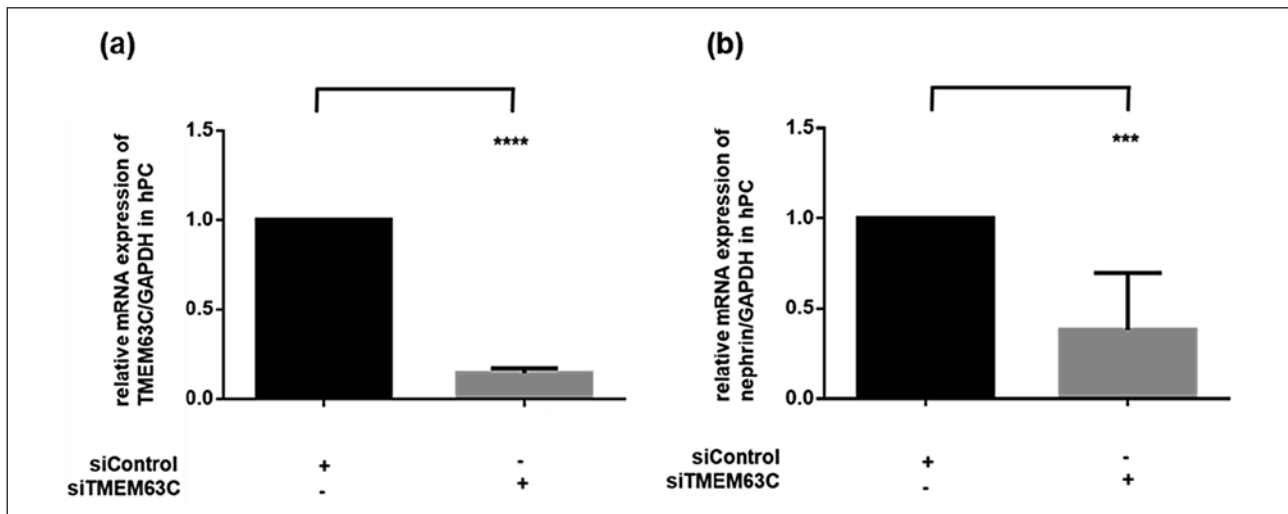


Fig. 8. TMEM63C and nephrin expression in siTMEM63C-treated hPC. hPC were transfected with inhibitory siRNAs against TMEM63C (siTMEM63C) or nonsense control siRNAs (siControl) for 48 h. Shown is the mRNA expression of (a) TMEM63C and (b) nephrin. The mean \pm SEM of at least 3 independent experiments is represented. (***) $p < 0.001$; (****) $p < 0.0001$. TMEM63C, transmembrane protein 63C; hPC, human podocytes; siRNA, small interfering RNA.

Discussion

In this study, we identified TMEM63C as a direct target of miR-564. Furthermore, we showed an association between TMEM63C deficiency and reduced cell viability. Moreover, TMEM63C expression was found to be modulated by TGF- β stimulation in kidney cells. In addition to previous data [7], our data indicate that TMEM63C could be involved not only in GFB function but also in EMT.

Post-transcriptional Regulation of TMEM63C Expression by miR-564

We investigated the role of miRNAs in the regulation of TMEM63C expression. In a first step, we performed in silico analysis to identify potential miRNAs modulating TMEM63C expression on post-transcriptional level. In silico analysis is based on different computational algorithms, which are using different parameters to predict the probability of a functional miRNA binding site within a given mRNA target [16]. However, the characterization of miRNA-mRNA interactions is challenging and difficult to predict [17]. For this reason, several miRNAs were predicted to regulate TMEM63C expression. For our in vitro experiments, we selected miR-30b and miR-564 as very likely candidates for TMEM63C regulation. Both were predicted by at least 3 different prediction tools. In this study, we showed a direct reduction of TMEM63C expression by miR-564 treatment in renal cells, while miR-30b inhibited TMEM63C possibly rather indirectly. Both miRNAs were shown to be expressed in kidney cells [18].

Some miRNAs are already known to be significant participants in renal pathologies, such as miR-21 or miR-192 in kidney fibrosis [10]. In addition, previous studies described a modulating influence of miRNAs, such as miR-21, miR-22, or miR-566, in the genesis or prevention of renal cell cancer [11–13]. So far, miR-564 has not been identified in the context of renal pathologies, but various studies reported on the relevance of miR-564 and its function in different cancer biology. In this regard, miR-564 was demonstrated to induce cell apoptosis and to suppress cell proliferation in osteosarcoma cells by targeting protein kinase B [19].

Mutlu et al. [20] showed an inhibitory effect of miR-564 on cell proliferation in breast cancer cells due to suppression of phosphoinositide 3-kinase and mitogen-activated protein kinase. In addition, ectopic expression of miR-564 was described to stop EMT and reduce the migration and invasion of breast cancer cells [20]. In further studies, miR-564 was found to modulate TGF- β in different cell types [21, 22]. Jiang et al. [21] showed that miR-564 reduced TGF- β expression in glioblastoma cells. In another experimental setting, Xiao and Colleagues [22] presented an upregulation of miR-564 to be associated with an increased TGF- β expression in hypertrophic scars. So far, the role of miR-564 in the kidney, and especially in the context of renal pathologies, is unknown. However, miR-564 and its regulative function on TGF- β and EMT may not only be important in cancer, but could also be relevant in the modulation of renal fibrosis. In our study, miR-564 suppressed the expression of TMEM63C. For this reason, we investigated the effects of reduced TMEM63C expression on renal cells and studied the impact of TGF- β on TMEM63C expression.

Expression Regulation and Functional Effects of TMEM63C in HEK 293

Schulz et al. [7] showed the importance of TMEM63C expression for the maintenance of GFB in zebra fish models and demonstrated its potential relevance in patients with focal segmental glomerulosclerosis in translational studies. However, until now, the expression regulation of TMEM63C is widely unexplored. In our experiments, we used HEK 293 – due to its high transfection efficiency – to examine the expression regulation and impact of TMEM63C. The recent report already showed impaired cell viability due to suppression of TMEM63C in hPC [7]. In our study, we found downregulation of TMEM63C to be associated with reduced viability also in other renal cells (HEK 293). These results suggest that TMEM63C may have a beneficial effect on renal cell survival. Next, we analyzed the impact of TGF- β on TMEM63C expression. TGF- β is known as regulator and initiator of EMT and has been described as key mediator of glomerular and tubulointerstitial pathobiology in CKD [23, 24]. In CKD, TGF- β led to tubulointerstitial fibrosis and dysfunction of podocytes [25]. Previous studies showed that miR-564 is involved in the regulation of TGF- β [21, 22]. In this study, we found TGF- β to affect TMEM63C expression in a concentration-dependent manner. Further, we observed an inhibiting effect of miR-564 on TMEM63C generation. For this reason, we also studied the potential role of TMEM63C in EMT. In EMT, cells lose progressively their epithelial characteristics and acquire markers of mesenchymal phenotype [26]. Therefore, the ratio of epithelial and mesenchymal marker proteins is changed [27]. Typical epithelial markers are E-cadherin and fibronectin, whereas typical mesenchymal proteins are N-cadherin, α -SMA, and vimentin [27]. HEK 293 were shown to express both epithelial and mesenchymal marker proteins [28]. Therefore, we used these cells as suitable model to study the change of epithelial characteristics by altered TMEM63C expression. We found reduced TMEM63C expression to be associated with an increased ratio of α -SMA versus E-cadherin in HEK 293. This changing ratio of α -SMA versus E-cadherin is discussed as an indicator for tubular EMT [29]. However, the precise role of TMEM63C in EMT of renal tubular epithelial cells remains uncertain and should be investigated in further experiments. In conclusion, we found siTMEM63C to reduce cell viability in HEK 293. In addition, we showed an association between reduced TMEM63C expression and a changing expression of proteins that are involved in EMT. Therefore, the increased expression of TMEM63C by TGF- β might reflect a potential protective mechanism of TMEM63C in renal cells under pathophysiological conditions, such as the development of renal fibrosis.

Expression Regulation and Functional Effects of TMEM63C in hPC

Similar to HEK 293, TGF- β treatment increased TMEM63C expression in a concentration-dependent manner in hPC. Enhanced TGF- β concentration is associated with podocyte apoptosis and detachment from the glomerular basement membrane, EMT, and the development

of glomerulosclerosis [30]. For EMT studies in podocytes, it is important to consider that these cells have been described as atypical epithelial cells with a different expression of epithelial and mesenchymal proteins [31]. EMT in podocytes, also referred to as “podocyte disease transformation,” is associated with a decrease in proteins such as podocin, nephrin, and P-cadherin and an increase in α -SMA and N-cadherin [31, 32]. Moreover, Ghiggeri et al. [33] indicated that nephrin elimination may trigger EMT in hPC. We showed that reduced TMEM63C expression is associated with decreased generation of nephrin in hPC. These data support our suspicion that TMEM63C may play a potential role in modulation of EMT in renal cells. Further, nephrin is known as an essential structural protein of the slit diaphragm and is pivotal for the preservation of GFB function as well as podocyte viability [2]. Langham et al. [34] also showed reduced nephrin expression to be associated with proteinuria in relevant kidney diseases such as diabetic nephropathy. We found reduced nephrin expression by inhibiting TMEM63C. For this reason, the current data support our previous observations linking TMEM63C to the development of albuminuria [7]. Altogether, our results demonstrated that TMEM63C is regulated by miR-564 and TGF- β in hPC and affects the expression of nephrin and could therefore influence the preservation of renal filter function.

Conclusion

In this study, we identified TMEM63C as a direct target of miR-564 in human renal cells. Moreover, the current study demonstrated that TMEM63C is regulated by TGF- β and could be involved in EMT. However, the effects of TMEM63C on EMT have not yet been clarified and should be investigated in further experiments. These experiments may include *in vivo* studies or at least studies in primary kidney cells, since experiments in immortalized cell lines, although important for mechanistic studies, should be viewed with some caution due to a potential permissive oncogenic phenotype for EMT studies [35]. Moreover, the role of EMT *per se* in the context of renal fibrosis has been questioned [36] and has been a matter of controversy [37]. Nevertheless, more recent studies indicated that at least partial EMT may be sufficient to promote renal fibrosis [38, 39] although evidence for human CKD is scant [37].

Our current results also support recently reported findings linking TMEM63C-expression to GFB function and albuminuria [7]. In the latter study, downregulation of TMEM63C resulted in albuminuria in zebra fish models and reduced cell viability of hPC, indicating a potential protective effect of TMEM63C for renal filter function [7]. In line with these findings, the current study showed siTMEM63C to reduce nephrin, a pivotal protein for the preservation of GFB function as well as podocyte viability [2]. Moreover, siRNA-mediated downregulation of TMEM63C led to a reduced cell viability of HEK 293. On the other hand, downregulation of TMEM63C in HEK 293 was linked to EMT by increasing the α -SMA/E-cadherin ratio and was thus associated with the activation of pro-fibrotic mechanisms [37]. In this regard, our finding demonstrating an upregulation of TMEM63C by TGF- β , which is a major driver of renal fibrosis, may reflect a counter-balancing mechanistic link between TMEM63C and TGF- β . Taken together, our data indicate that TMEM63C is not only linked to GFB function but may also be involved in EMT control. Therefore, TMEM63C is a novel functional candidate with potential impact for the development and progression of CKD.

Acknowledgements

We thank Claudia Plum, Petra Karsten, Sarah Podlech, Dr. Anja Brehm, and Marie Käbel for experimental and technical support.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by the DFG (German Research Foundation) – Project number 394046635-SFG 1365 and the Open Access Publication Funds of Charité – Universitätsmedizin Berlin.

Author Contributions

M.O., A.G., J.S., K.B., and A.E. carried out the cell culture studies. K.B., M.O., and A.E. participated in the PCR tests. M.O. and A.E. performed the statistical analysis. M.O., A.E., and R.K. conceived the study, participated in its design and coordination, and drafted the manuscript. A.G., J.S., and K.B. revised the manuscript critically. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work and to ensure that questions relating to the accuracy or integrity of any part of the work have been appropriately investigated and resolved.

TMEM63C, transmembrane protein 63C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; α -SMA, α -smooth muscle actin.

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