Int J Clin Exp Pathol 2016;9(2):1276-1284
www.ijcep.com /ISSN:1936-2625/IJCEP0019190

Original Article

Endocytosis mediated by Caveolin-1 inhibits activity of matrix metalloproteinase-2 in human renal proximal tubular cells under hypoxia

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Received November 5, 2015; Accepted January 1, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: Tubulointerstitial fibrosis is characterized by tubular atrophy with basement membrane thickening and accumulation of interstitial extracellular matrix. Decreased matrix metalloproteinase-2 (MMP-2) activity may promote the process. Although human renal proximal tubular cells are sensitive to oxygen deprivation, it is unknown whether cellular endocytosis induced by hypoxia could alter the activity of MMP-2. The aim of this study was to investigate whether the endocytosis mediated by caveolin-1 has inhibitory effects on the activity of MMP-2 in human renal proximal tubular cells (HK-2 cells) under hypoxia. Our study indicated that HK-2 cells exposed to 1% O_2 hypoxic milieu for 24 hours resulted in increased level of collagen IV and decreased MMP-2 activity. After HK-2 cells were treated with filipin, the endocytosis mediated by Caveolin-1 was inhibited but the expression of MMP-2 in the cells and its activity in the cultural supernatant was respectively enhanced. Furthermore, inhibition of Caveolin-1 expression by Cav-1 shRNA led to increase of MMP-2 activity, which was similar to the results of HK-2 cells treated with an endocytotic inhibitor filipin under hypoxia. Our data suggest that hypoxia may be an important pro-fibrogenic stimulus that acts partly via endocytosis, and that Caveolin-1 is a potential target for regulating MMP-2 activity in the tubulointerstitial fibrogenesis.

Keywords: Proximal tubular cells, Caveolin-1, hypoxia, endocytosis, matrix metalloproteinase-2

Introduction

Chronic kidney disease (CKD) is increasingly recognized as a worldwide public health issue [1, 2]. It is known that tubular atrophy is a typical characteristic of tubulointerstitial fibrosis in kidney diseases. When the interstitial cell number increases with the appearance of myofibroblastic cells and extracellular matrix (ECM) accumulation, tubulointerstitial fibrosis occurs, leading to increased thickness of the tubular basement membrane (TBM) and accumulation of interstitial ECM [3-5]. MMP-2 plays a major role in the degradation of the ECM macromolecules [6]. In particular, MMP-2 is involved in the breakdown of ECM, degrading type IV collagen (Col-IV), a major component of the TMB [4, 7-11]. MMP-2 is synthesized as a secreted proenzyme, the removal of an amino-terminal propeptide on the MMP converts it into an active form [12].

Chronic hypoxia often occurs in the kidney tissues of many patients with CKD. *In vitro* data suggest that hypoxia can induce functional and phenotypic changes in renal epithelial cells and fibroblasts, which is consistent with the changes observed in these cells in fibrosis [13]. Adaptation to hypoxia at the cellular level is regulated by a dual mechanism. On one hand, hypoxia leads to an increase in the efficiency of energy-producing pathways, and on the other hand, it decreases energy-consuming processes such as the activity of Na- and K-ATPases [14]. As a cellular adaptive response, hypoxia decreases Na- and K-ATPase activity by triggering the endocytosis of the α1 subunit in alveolar epithelia [15], suggesting that hypoxia induces endocytosis.

Some studies found that matrix metalloproteinase-2 (MMP-2) activity was decreased in human renal proximal tubular cells (HK-2 cells) under hypoxic condition, but the mechanism is still

unclear [16]. We previously reported that altered expression and activity of MMP-2 in hepatic stellate cells under hypoxic condition [17]. Recent studies have shown that MMP-2 activity is inhibited in the heart by Caveolin-1 (Cav-1), which is the principal component of the caveolae membranes and is involved in receptor-independent endocytosis [18]. Breakdown of Cav-1 activates MMP-2 in HT1080 cells [19], while the overexpression of Cav-1 in some cells causes decreased MMP-2 activity [20]. However, the effect of Cav-1 on MMP-2 activity in kidney disease is unknown.

In this study, we present evidence that a sensitive inhibitor of caveolae enhanced the activity of MMP-2 in HK-2 cells under hypoxia and that suppressing the expression of Cav-1 by interference techniques also increased MMP-2 activity. Considering that HK-2 cells play a key role in the development of renal fibrosis [21], our study provides a useful clue for controlling the progression of renal fibrosis.

Materials and methods

Cell culture and hypoxic treatment

Human renal proximal tubular epithelial cell line (HK-2 cells) was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The HK-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific, USA). The cells were seeded on 6-well plates at a density of 5×10^5 cells/well. To mimic hypoxic conditions, the cells in culture medium were then subjected to low-oxygen condition. The oxygen concentrations were maintained at 1%-3% using three gas incubator of Thermo Fisher, which was held under a positive pressure in an atmosphere of 94%-92% N_2/5% CO_2/1%-3% O_2. There were four treatment groups in triplicate wells, including normoxic group (control cells), hypoxic group, hypoxic+filipin (filipin III, Sigma) group and hypoxic+Cav-1 shRNA group for 24 hours. The control cells were cultured under the normoxic condition.

Stable transfection of cells

The EGFP-Rab7 (Rab7 gene No. HSU44104) lentiviral vector (Shanghai GenePharma. China) was infected into the cells following the manufacturer's instructions. Short hairpin RNAs (shRNA) directed towards Cav-1 (ACGAGCTGAGCGAGAAGCAAGTGTATTCAAGAGATACACTTGCTTCTCGCTCAGCTCGT) were delivered to HK-2 cells (creating stable Cav-1 shRNA expressing cells) using lentiviral vector (Shanghai Hanbio. China) following the manufacturer's instructions. The dosage of G418 used for selection was determined by challenging HK-2 cells with gradually increased concentrations of the drug. The optimal dose was 500 μg/mL.

Transmission electron microscopy (TEM)

TEM was used to observe and analyze the morphology of the endocytosis in HK-2 cells post hypoxic treatment. The cells were exposed in an atmosphere of 94%-92 N_2/5% CO_2/1%-3% O_2 for 24 hours. The control cells were cultured under the normoxic condition. Fixed in 2.5% glutaraldehyde for 2 hours. Then the post-fixation with 1% osmium tetroxide was performed. Finally, the specimens were cut into ultra-thin sections (5070 nm), which were placed onto copper grids and stained with uranyl acetate and lead citrate. The ultrastructural analysis was performed using a JEM-1011EX TEM instrument (JEOL, Japan).

Confocal microscopy

Cells were plated on cover slips in a 24-well plate and fixed with 4% paraformaldehyde for 15 minutes at room temperature. After were extensively washed and treated with nuclear stain (4,6-Diamidino-2-phenylindole, DAPI) for 5 minutes at room temperature. Finally, the images were taken using a Confocal Microscope (FV1000, Olympus Corporation, Japan).

Western blotting

The cells were lysed with immunoprecipitation assay buffer containing protease inhibitors. The protein concentrations were examined using the BCA Protein Assay kit (Vazyme Biotech, China). 40 μg of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a piece of polyvinylidene diluoride membrane. The membranes were blocked with 5% non-fat milk in TBST (Tris-buffered saline with 0.5% of Triton X-100) for 1 hour at room temperature, and then incubated with the appropri-

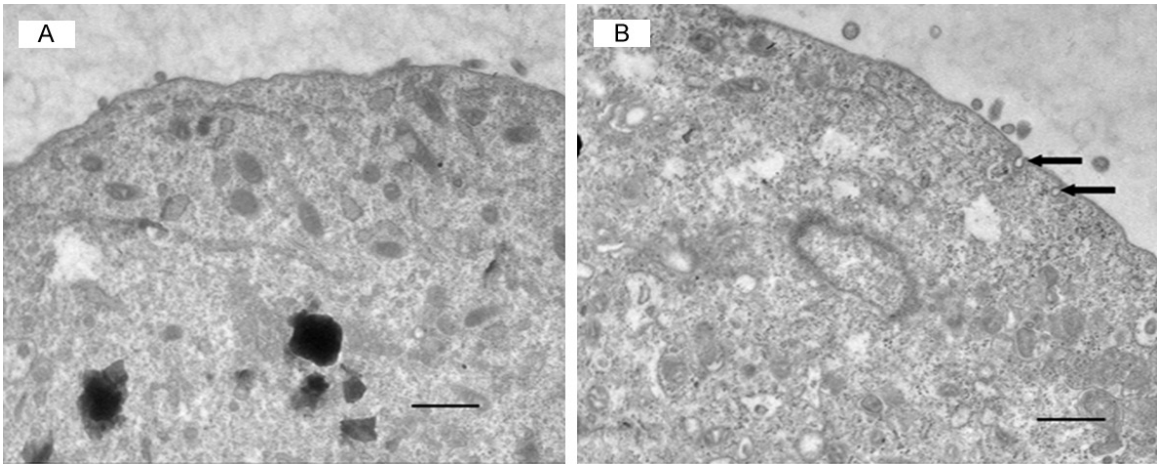

Figure 1. Endocytosis induced by hypoxia in HK-2 cells. TEM images of HK-2 cells under normoxic (A) and hypoxic (B) conditions for 24 hours. Endosome: black arrows in (B). Abbreviations: TEM, transmission electron microscopy.

ate primary antibody against Cav-1 (Abcam, UK) or GAPDH (Protech, China) overnight at 4°C. The membranes were washed three times in TBST, followed by incubation with the appropriate horseradish peroxidase-linked secondary antibodies (Protech, China) for 1 hour at room temperature. The specific proteins on the blots were developed with enhanced chemiluminescence (Vazyme Biotech, China) and visualized as the bands on the CL-XPosure Film (Thermo Fisher Scientific, USA).

Real-time PCR

Total RNA from HK-2 cells was isolated and reverse transcription was performed using the RNeasy Mini kit (Vazyme Biotech, China). Real-Time PCR was performed to determine the expression of human MMP-2 in a SYBR Green PCR Master mix (Vazyme Biotech, China) using the StepOnePlus™ Real-Time PCR Detection systems (Step One Plus 2.1 software) with universal thermal cycling parameters. The following primers were used: MMP-2 Forward primer: 5'-GAGAACCAAAGTCTGAAGAG-3', Reverse primer: 5'-GGAGTGAGAATGCTGATTAG-3', GAPDH Forward primer: 5'-GGAAGGTGAAGGTCGGAGTCA-3', Reverse primer: 5'-GCAACAATATCCACTTTACCAG-3'.

ELISA to quantify MMP-2 and Col-IV in culture media

ELISA kits were used to detect the contents of MMP-2 and Col-IV protein in culture media following the manufacturer's instructions (Shanghai Hengyuan Biological Technology, China). Purified MMP-2 or Col-IV antibody were applied to coat the microtiter plate wells, then culture supernatants were added to the wells and incubated at 37°C for 2 hours. After the plates were washed, another MMP-2 antibody or Col-IV antibody labeled with HRP was added to the plate. Then the plates were washed thoroughly, the 3,3',5,5'-Tetramethylbenzidine substrate solution was added. The reaction was terminated by the addition of a sulphuric acid solution, and the color was measured by a spectrophotometry at a wavelength 450 nm. The level of MMP-2 or Col-IV protein in the samples was then calculated according to the standard curve.

Detection of MMP-2 activity by zymography

The activity of MMP-2 was determined by gelatin zymography using MMP Zymography Assay Kit (Applygen Technologies, China) according to the manufacturer's protocol. MMP-2 was separated by SDS-PAGE. Subsequently, the SDS was extracted with Triton X-100 from the gels, then incubated for 48 hours at 37°C. The gels were stained with Coomassie brilliant blue G250 and decolorized. The clear band against a blue background represented the activity of MMP-2 and was measured by using a Gel Image System (Image master 1D analysis software, Pharmacia) and recorded with the total A (area of clear band times mean A) [22].

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., USA). The data were expressed as mean ±

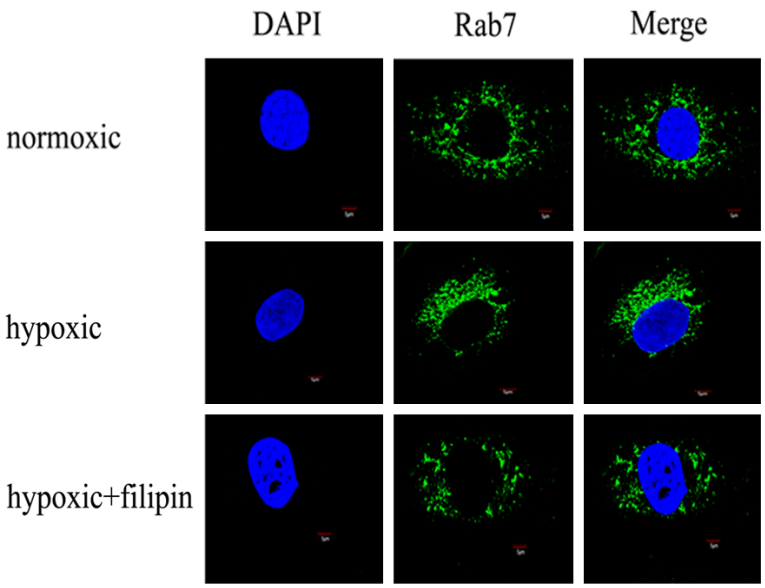

Figure 2. Endosome formation inhibited by filipin in HK-2 cells infected with expressing EGFP-Rab7 protein under hypoxia. HK-2 cells stably expressing EGFP-Rab7 were exposed to filipin for 24 hours in hypoxia, then fixed. Nuclear staining was performed with DAPI (shown in blue in each panel). Pictures were taken using a confocal microscope. The change of EGFP-Rab7 was shown in hypoxic HK-2 cells.

standard deviation (SD). Differences between groups were analyzed with Student's *t*-test. $P<0.05$ was considered to be statistically significant.

Results

Endocytosis in HK-2 cells subjected to hypoxia

To observe the ultrastructure of HK-2 cells in 1% O_2 hypoxic milieu for 24 hours, we used the TEM to observe the changes. The images revealed in **Figure 1B** that there were endosomes (black arrows) in the cytoplasm of HK-2 cells subjected to hypoxia. In contrast, endocytosis was rarely observed in control cells (**Figure 1A**).

Filipin inhibited endocytosis in HK-2 cells with EGFP-Rab7 under hypoxia

Rab7 is a Ras-related protein involved in endocytosis, vesicular transport and maturation of endosomes [23]. To verify whether filipin inhibits endocytosis induced by hypoxia, we generated stably infected HK-2 cells with EGFP-Rab7 overexpression. At first, we observed by fluorescence microscopy that the EGFP signal was associated with round vesicles labeled with Rab7, indicating that Rab7 was related to the membrane of endocytosis vesicles under both normoxic and hypoxic conditions. By inducing endocytosis in cells with hypoxia, it was found that the dense puncta formation of Rab7 represented aggregates of Rab7 protein. Rab7 fine puncta were diffusely distributed in the cytoplasm of normoxic cells (**Figure 2**). In contrast, there were more Rab7 aggregates in hypoxia-treated cells, and the protein was located near the nucleus compared to normoxic cells (**Figure 2**). Furthermore, the cells treated with inhibitors for endocytosis showed reduced Rab7 aggregates under hypoxic conditions. In other words, Rab7 indicates the level of endocytosis in this study. These data suggest that filipin is an effective inhibitor for endocytosis in hypoxia.

Inhibition of endocytosis increased the MMP-2 activity in culture media of hypoxia-treated HK-2 cells

Gel zymography of MMP-2 generally showed two major bands, one at 62 kDa, representing the active form of MMP-2, and another at 72 kDa, representing pro-MMP-2. The activity of MMP-2 in the hypoxic group (0.36±0.06) was found to be lower than that in the normoxic group (1.00±0.00) (**Figure 3A**). The relative activity of MMP-2 in HK-2 cells treated with filipin (0.60±0.08) was significantly elevated in the group treated with filipin and hypoxia compared with the hypoxic group ($P<0.01$) (**Figure 3A**, **3B**).

Inhibition of endocytosis enhanced MMP-2 expression at the mRNA level in HK-2 cells treated with hypoxia

To understand MMP-2 expression and its correlation with endocytosis, we investigated the expression of MMP-2 in HK-2 cells by real-time PCR. Compared with the normoxic group, the level of MMP-2 mRNA was not affected in the

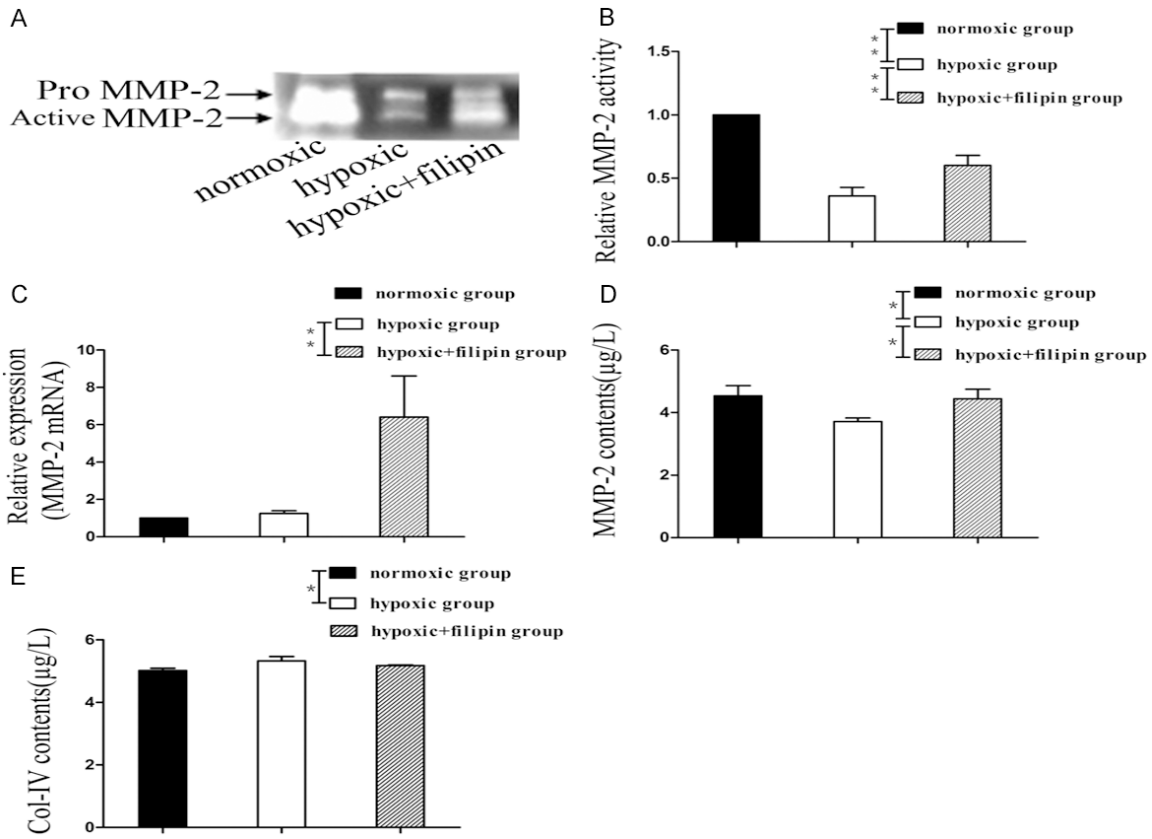

Figure 3. Effects of filipin on HK-2 cells under hypoxia. (A) Relative MMP-2 activity in the normoxic group, hypoxic group, and hypoxic+filipin group for 24 hours. (B) Gels were scanned and quantified by densitometry, and relative MMP-2 activity was calculated as a percentage of the relevant control values (assigned values of 1) from arbitrary densitometry units. Data is the mean of three separate experiments. (C) Relative mRNA levels in the normoxic group, hypoxic group, and hypoxic+filipin group for 24 hours as a percentage of the relevant control values. The content of MMP-2 protein (D) and Col-IV protein (E) in the normoxic group, hypoxic group, and hypoxic+filipin group for 24 hours, respectively. Results were analyzed by unpaired Student's t-test, **P*<0.05, ***P*<0.01.

cells treated with hypoxia. However, filipin dramatically increased the level of MMP-2 mRNA in hypoxic cells (*P*<0.01). The relative amount of MMP-2 in hypoxic group was 1.24±0.16 and 6.40±4.22 in hypoxic+filipin group (**Figure 3C**).

Inhibition of endocytosis altered the levels of MMP-2 protein and Col-IV protein in culture media of hypoxia-treated cells

MMP-2 protein in cell culture media was determined by ELISA. The MMP-2 protein in culture media included pro-MMP-2 protein and active MMP-2 protein. As shown in **Figure 3D**, MMP-2 protein (3.71±0.12 μg/L) was significantly diminished in hypoxia-treated cells compared with normoxic group (4.54±0.33 μg/L) (*P*<0.05). When the cells were incubated with the inhibitor filipin under hypoxia for 24 hours, the level of MMP-2 protein in the culture media was increased (4.44±0.31 μg/L) (**Figure 3D**) (*P*<0.05) compared with hypoxic group. Because the level of Col-IV protein in cell culture media is opposite to the MMP-2 activity, we examined Col-IV protein by ELISA to assess the enzymatic activity of MMP-2. **Figure 3E** showed that the level of Col-IV protein in the hypoxic group (5.33±0.14 μg/L) was increased compared with the normoxic group (5.02±0.07 μg/L), suggesting decreased activity of MMP-2. The level of Col-IV protein was also decreased in the hypoxic+filipin group (5.18±0.02 μg/L) compared to the hypoxic group (**Figure 3E**). These data indicate increased MMP-2 activity in the presence of filipin.

Suppression of Cav-1 by shRNA in hypoxia-treated cells

To investigate the role of Cav-1 in HK-2 cells, we used the Cav-1 shRNA lentiviral vector to infect the cells. We generated a stable HK-2 cell line

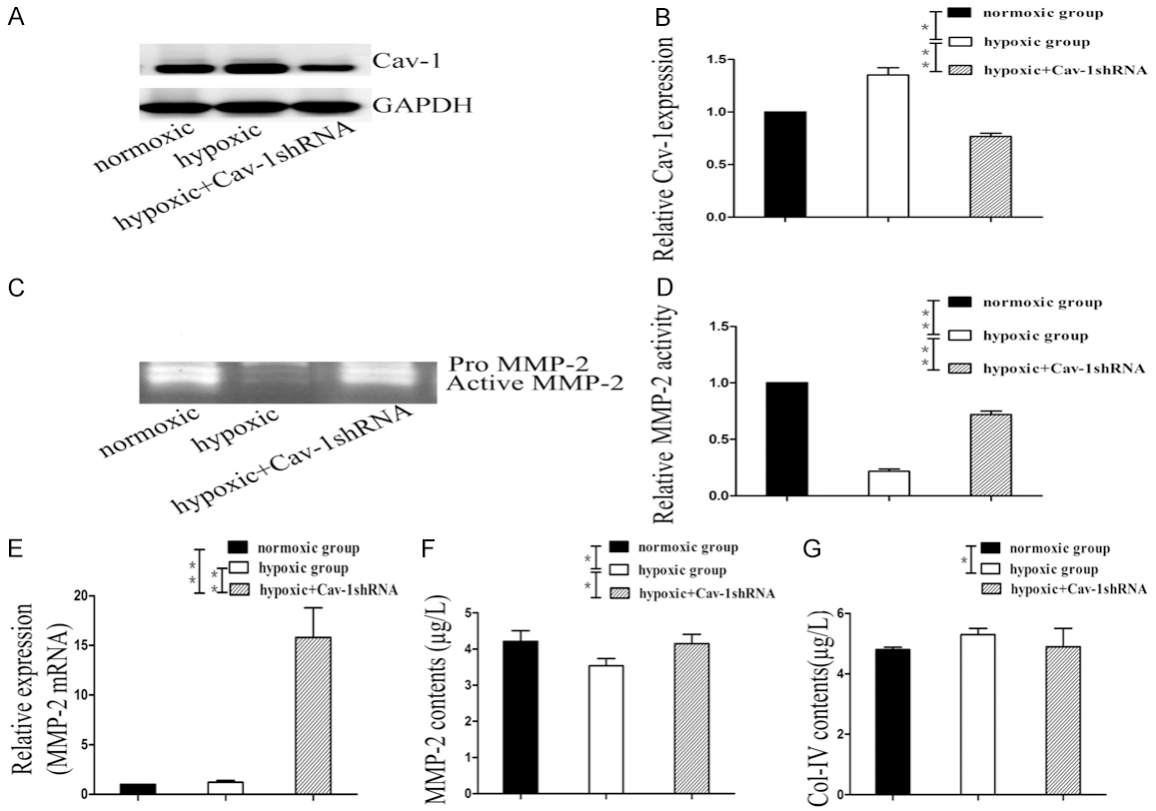

Figure 4. Effects of shRNA on HK-2 cells under hypoxia. (A) Western blotting analysis of Cav-1 proteins. (B) Expression of Cav-1 relative to GAPDH. (C) Relative MMP-2 activity in the normoxic group, hypoxic group, and hypoxic+Cav-1 shRNA group for 24 hours. (D) Gels were scanned and quantified by densitometry, and relative MMP-2 activity was calculated as a percentage of the relevant control values (assigned values of 1) from arbitrary densitometry units. Data is the mean of three separate experiments. (E) Relative mRNA levels in the normoxic group, hypoxic group, and hypoxic+Cav-1 shRNA group for 24 hours. The contents of MMP-2 protein (F) and Col-IV protein (G) in the normoxic group, hypoxic group, and hypoxic+Cav-1 shRNA group for 24 hours, respectively. Results were analyzed by unpaired Student's t-test, **P*<0.05, ***P*<0.01.

(Cav-1 shRNA) in which Cav-1 expression was suppressed by approximately 50% in protein levels compared with the non-shRNA group in hypoxia (**Figure 4A**, **4B**).

Effect on MMP-2 activity in culture media by Cav-1 shRNA in hypoxia-treated cells

In the effect of Cav-1 suppression on MMP-2 activity, gel zymography of MMP-2 was performed. After 24 hours of hypoxia, there was a significant increase in MMP-2 activity by shRNA in the hypoxic+Cav-1 shRNA group (**Figure 4C**), which was still suppressed in the hypoxic group under hypoxia. When the HK-2 cells were treated with shRNA of Cav-1, the MMP-2 activity of the hypoxic+Cav-1 shRNA group was 0.72±0.03 (**Figure 4A**), the activity of the hypoxic group was 0.22±0.02. The difference in the data from the two groups is significant (*P*<0.01) (**Figure 4C**, **4D**).

Effect on MMP-2 expression at the mRNA level by Cav-1 shRNA in hypoxia-treated cells

To evaluate the effect of Cav-1 suppression on MMP-2 mRNA level, we tested the mRNA level of MMP-2 by real-time PCR. The mRNA level of MMP-2 in HK-2 cells interfered with Cav-1 shRNA was higher than that of in hypoxic group. TherelativeamountofMMP-2was1.24±0.16(hypoxic group) and 15.80±3.11 (hypoxic+shRNA group). These results demonstrate that down-regulation of the Cav-1 gene not only enhances MMP-2 activity but also increases the expression of MMP-2 at the mRNA level (**Figure 4E**).

Effect on the levels of MMP-2 protein and Col-IV protein in culture media by Cav-1 shRNA

The culture media was analyzed using an ELISA assay to measure the levels of MMP-2 and Col-IV proteins. As shown in **Figure 4F** and **4G**, the

level of MMP-2 in the hypoxic group was 3.54±0.20 μg/L, and a marked increase appeared in the hypoxic+Cav-1 shRNA group (4.15±0.26 μg/L). The level trend of Col-IV protein in the cell culture media is opposite to the level trend of MMP-2 protein in culture media. As shown in **Figure 4F** and **4G**, displaying the changes in MMP-2 protein and Col-IV protein, the level of MMP-2 in the hypoxic+Cav-1 shRNA group was higher than that of hypoxic group. In contrast, the level of Col-IV protein (5.30±0.20 μg/L) in the hypoxic group was higher than that of in the hypoxic+Cav-1 shRNA group (4.90±0.60 μg/L). These data suggest that MMP-2 activity was increased with the silencing of Cav-1 by Cav-1 shRNA (**Figure 4F**, **4G**).

Discussion

Renal hypoxia is a major factor in the pathophysiology of acute kidney injury to CKD transition [24]. To maintain normal metabolism, HK-2 cells require a large amount of oxygen to function properly, and renal proximal tubular cells may be the primary target of a hypoxic insult [25]. Hypoxia induces endocytosis. There are many endocytic receptors on the renal proximal tubule [26], which may affect the endocytotic activity of the cells. While the thickening of TBM and decreasing activity of MMP-2 in patients of tubulointerstitial fibrosis are well known [7], however, it is unclear whether hypoxia-induced endocytosis would affect the activity of MMP-2 in the proximal tubular cells. In this study, we first observed an increase of endocytosis under hypoxia in HK-2 cells by TEM analysis. Then, we examined the change in Rab7 in hypoxia-treated HK-2 cells with EGFP-Rab7 using filipin, a sensitive inhibitor of endocytosis involving Cav-1 [27]. When the HK-2 cells with EGFP-Rab7 were treated with filipin under hypoxia, decreased endocytosis was observed in the cells, suggesting that filipin effectively inhibits endocytosis in hypoxia.

Next, we found that the specific endocytosis inhibitor altered the activity of MMP-2 in hypoxia-treated HK-2 cells. ELISA results for Col-IV protein and MMP-2 protein in culture media confirmed the observations. The endocytosis inhibitor also increased the MMP-2 mRNA levels in cells subjected to hypoxia. These data imply that endocytosis altered the expression and activity of MMP-2 under hypoxic conditions via Cav-1. Although the underlying mechanism remains unclear, this information may provide new insight into understanding the mechanism.

To further investigate the possible role of Cav-1 in HK-2 cells under hypoxia, we employed shRNA technology to suppress the expression of the Cav-1 gene in HK-2 cells under hypoxia. In this study, we found that the Cav-1 protein was increased in cells in hypoxia compared with HK-2 cells in the normoxic group. The expression level of the Cav-1 gene in hypoxia was suppressed by approximately 1-fold in Cav-1 protein level compared with HK-2 cells in hypoxia (hypoxic group). Next, we analyzed the MMP-2 activity by detecting the level of Cav-1 suppressed by shRNA. The Cav-1 shRNA group exhibited a 3.4-fold increase in MMP-2 activity with respect to the non-shRNA group (hypoxic group) under hypoxia. In addition, the significant suppression of Cav-1 up-regulated the expression of MMP-2 at the mRNA level under hypoxia.

We found that endocytosis mediated by Cav-1 inhibits the activity of MMP-2 in hypoxic HK-2 cells. The ELISA assay results on the MMP-2 protein and Col-IV protein in the culture media were consistent with the results of gel zymography. Previous reports have shown that Cav-1 is responsible for maintaining MMP-2 in a membrane-associated and inhibited configuration [18], and that the overexpression of Cav-1 in some cells causes decreased MMP-2 activity [19, 20]. Our results showed that Cav-1 also inhibits the mRNA level of MMP-2 in addition to inhibiting MMP-2 activity. Studies showed that the binding of caveolin scaffolding domain to the motifs of MMP-2 may inhibit MMP-2 activity by preventing substrate binding [28]. More Cav-1 protein anchors MMP-2 to the membrane, preventing activation of the enzyme [29, 30]. Our results from the presented study proved this point that means the expression of Cav-1 influences MMP-2 activity in HK-2 cells under hypoxia. However, further investigation of interactions between MMP-2 and Cav-1 is warranted.

In summary, we have firstly demonstrated that endocytosis mediated by Cav-1 inhibits the activity of MMP-2 in HK-2 cells under hypoxia using inhibitor and Cav-1 shRNA. Our study provides the novel insight that Cav-1 may be a potential pharmacological target to inhibit renal

fibrogenesis and prevent TBM thickness and accumulation of interstitial ECM in renal diseases.

Acknowledgements

We thank Professor Wandong Zhang at the National Research Council of Canada for critically reading. This work was financially supported by the National Nature Science Foundation of the People's Republic of China (No. 81370868), the Fundamental Research Funds for the Central Universities and Jiangsu Province Scientific Research Innovation Project for Graduate Students (No. KYLX_0197).

Disclosure of conflict of interest

None.

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References

[1] Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt KU, Nahas ME, Jaber BL, Jadoul M, Levin A, Powe NR, Rossert J, Wheeler DC, Lameire N, Eknoyan G. Chronic kidney disease as a global public health problem: approaches and initiatives-position statement from Kidney Disease Improving Global Outcomes. Kidney Int 2007; 72: 247-59.

[2] Schieppati A, Remuzzi G. Chronic renal diseases as a public health problem: epidemiology, social, and economic implications. Kidney Int Suppl 2005; S7-S10.

[3] Jacobson HR. Chronic renal failure: Pathophysiology. Lancet 1991; 338: 419-23.

[4] Luo X, Deng L, Lamsal LP, Xu W, Xiang C, Cheng L. AMP-activated protein kinase alleviates extracellular matrix accumulation in high glucose-induced renalfibroblasts through mTOR signaling pathway. Cell Physiol Biochem 2015; 35: 191-200.

[5] Zhou X, Zhang J, Xu C, Wang W. Curcumin ameliorates renal fibrosis by inhibiting local fibroblast proliferation and extracellular matrix deposition. J Pharmacol Sci 2014; 126: 344-50.

[6] Catania JM, Chen G, Parrish AR. Role of matrix metalloproteinases in renal pathophysiologies. Am J Physiol Renal Physiol 2007; 292: F905-11.

[7] Ronco P, Chatziantoniou C. Matrix metalloproteinases and matrix receptors in progression and reversal of kidney disease: therapeuticperspectives. Kidney Int 2008; 74: 873-8.

[8] Racca MA, Novoa PA, Rodríguez I, Della Vedova AB, Pellizas CG, Demarchi M, Donadio AC. Renal dysfunction and intragraft proMMP9 activity in renal transplant recipients with interstitial fibrosis andtubular atrophy. Transpl Int 2015; 28: 71-8.

[9] Zhou TB, Qin YH, Lei FY, Huang WF, Drummen GP. Prohibitin attenuates oxidative stress and extracellular matrix accumulation in renal interstitial fibrosis disease. PLoS One 2013; 8: e77187.

[10] DeCoux A, Lindsey ML, Villarreal F, Garcia RA, Schulz R. Myocardial matrix metalloproteinase-2: inside out and upside down. J Mol Cell Cardiol 2014; 77: 64-72.

[11] Alexius-Lindgren M, Andersson E, Lindstedt I, Engström W. The RECK ENE and biological malignancy-its significance in angiogenesis and inhibition of matrix metalloproteinases. Anticancer Res 2014; 34: 3867-73.

[12] Cheng S, Pollock AS, Mahimkar R, Olson JL, Lovett DH. Matrix metalloproteinase 2 and basement membrane integrity: unifying mechanism for progressive renal injury. FASEB J 2006; 20: 1898-900.

[13] Norman JT, Fine LG. Chronic hypoxia as a mechanism for progressive renal fibrosis. Drug Discovery Today: Disease Mechanisms 2007; 4: 29-36.

[14] Michiels C. Physiological and pathological responses to hypoxia. Am J Pathol 2004; 164: 1875-82.

[15] Dada LA, Welch LC, Zhou G, Ben-Saadon R, Ciechanover A, Sznajder JI. Phosphorylation and ubiquitination are necessary for Na, K-ATPase endocytosis during hypoxia. Cell Signal 2007; 19: 1893-8.

[16] Orphanides C, Fine LG, Norman JT. Hypoxia stimulates proximal tubular cell matrix production via a TGF-beta1-independent mechanism. Kidney Int 1997; 52: 637-47.

[17] Li J, Fan R, Zhao S, Liu L, Guo S, Wu N, Zhang W, Chen P. Reactive oxygen species released from hypoxic hepatocytes regulates MMP-2 expression in hepatic stellate cells. Int J Mol Sci 2011; 12: 2434-47.

[18] Chow AK, Cena J, El-Yazbi AF, Crawford BD, Holt A, Cho WJ, Daniel EE, Schulz R. Caveolin-1 inhibits matrix metalloproteinase-2 activity in the heart. J Mol Cell Cardiol 2007; 42: 896-901.

[19] Atkinson SJ, English JL, Holway N, Murphy G. Cellular cholesterol regulates MT1 MMP dependent activation of MMP 2 via MEK-1 in HT1080 fibrosarcoma cells. FEBS Lett 2004; 566: 65-70.

[20] Fiucci G, Ravid D, Reich R, Liscovitch M. Caveolin-1 inhibits anchorage-independent

growth, anoikis and invasiveness in MCF-7 human breast cancer cells. Oncogene 2002; 21: 2365-75.
[21] D'Amico G. Tubulo-interstitial damage in glomerular diseases: its role in the progression of the renal damage. Nephrol Dial Transplant 1998; 13 Suppl 1: 80-5.
[22] Takahara T, Furui K, Funaki J, Nakayama Y, Itoh H, Miyabayashi C, Sato H, Seiki M, Ooshima A, Watanabe A. Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats. Hepatology 1995; 21: 787-95.
[23] Hyttinen JM, Niittykoski M, Salminen A, Kaarniranta K. Maturation of autophagosomes and endosomes: a key role for Rab7. Biochim Biophys Acta 2013; 1833: 503-10.
[24] Tanaka S, Tanaka T, Nangaku M. Hypoxia as a key player in the AKI-to-CKD transition. Am J Physiol Renal Physiol 2014; 307: F1187-95.
[25] Norman JT, Orphanides C, Garcia P, Fine LG. Hypoxia-induced changes in extracellular matrix metabolism in renal cells. Exp Nephrol 1999; 7: 463-9.
[26] Christensen EI, Birn H, Storm T, Weyer K, Nielsen R. Endocytic receptors in the renal proximal tubule. Physiology (Bethesda) 2012; 27: 223-36.
[27] Schnitzer JE, Oh P, Pinney E, Allard J. Filipin-sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules. J Cell Biol 1994; 127: 1217-32.
[28] Wallon UM, Overall CM. The hemopexin-like domain (C domain) of human gelatinase A (matrix metalloproteinase-2) requires Ca2+ forfibronectin and heparin binding. Binding properties of recombinant gelatinase A C domain to extracellular matrix and basement membrane components. J Biol Chem 1997; 272: 7473-81.
[29] Fridman R, Fuerst TR, Bird RE, Hoyhtya M, Oelkuct M, Kraus S, Komarek D, Liotta LA, Berman ML, Stetler-Stevenson WG. Domain structure of human 72-kDa gelatinase/type IV collagenase. Characterization of proteolytic activity and identification of the tissue inhibitor of metalloproteinase-2 (TIMP-2) binding regions. J Biol Chem 1992; 267: 15398-405.
[30] Feron O, Balligand JL. Caveolins and the regulation of endothelial nitric oxide synthase in the heart. Cardiovasc Res 2006; 69: 788-97.