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Expression levels of serum vasohibin-1 and other biomarkers in type 2 diabetes mellitus patients with different urinary albumin to creatinine ratios

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ABSTRACT

Aim: To determine the serum levels of vasohibin (VASH)-1 and other biomarkers in type 2 diabetes mellitus (T2DM) patients with different urinary albumin to creatinine ratios (UACR), and correlate VASH-1 expression with the inflammation and fibrosis in diabetic kidney disease (DKD).
Methods: A total of 697 T2DM patients were stratified into four groups: N-UAlb (UACR <30 mg/g with normal blood pressure, $n = 144$), M-UAlb (UACR 30–300 mg/g with normal blood pressure, $n = 143$), L-UAlb (UACR >300 mg/g with normal blood pressure, $n = 126$), and L-UAlb+HP (UACR >300 mg/g with hypertension, $n = 134$). In addition, 150 healthy subjects were included as normal controls (NC). In addition to recording the age and duration of diabetes, the serum levels of VASH-1, silent information regulator factor 2-related enzyme 1 (sirtuin-1, SIRT1), hypoxia inducible factor 1α (HIF1α), vascular endothelial growth factor (VEGF), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), and the erythrocyte sedimentation rate (ESR) were measured. Clinical parameters related to UACR and VASH-1 were analyzed by one-way ANOVA, Pearson correlation and ridge regression analysis.
Results: The UACR, VASH-1, glycosylated hemoglobin (HbA1c), ESR, CRP, VEGF, HIF1α, TNF-α and TGF-β1 levels in all patient groups were significantly higher, and SIRT1 levels were lower compared to the NC group. Pearson correlation analysis showed that UACR and VASH-1 levels were positively correlated with HbA1c, ESR, CRP, VEGF, HIF1α, TNF-α and TGF-β1, and negatively with SIRT1. Ridge regression analysis showed that every serological marker was an independent factor affecting UACR.
Conclusion: Serum VASH-1 may be associated with the expression of renal inflammation and fibrosis-related factors, and have a potential connection with DKD.

1. Introduction

The prevalence of diabetic kidney disease (DKD) has increased in recent years due to considerable lifestyle changes, and has become the most common cause of end-stage renal disease (ESRD) in Japan and Western countries. It is also one of the major microvascular complications of type 2 diabetes mellitus (T2DM), and is characterized by glomerular hyper-filtration and hypertrophy, thickening of the glomerular and tubular basement membranes, and mesangial expansion.[1–4] These changes finally lead to glomerular sclerosis and tubule-interstitial fibrosis, and even ESRD, which is responsible for >40% of renal dialysis cases. Therefore, early diagnosis and treatment of DKD is vital.[5]

Recent studies show that vasohibin-1 (VASH-1), a novel endothelial cell (EC)-derived factor, inhibits the pro-angiogenic effects of the vascular endothelial growth factor (VEGF) in DKD progression via a negative feedback mechanism.[6] Changes in VASH-1 concentration may therefore be closely associated with DKD. However, very few studies have explored the relationship between VASH-1 and the pathogenesis of DKD. In this study, we analyzed serum VASH-1 levels in T2DM patients with different urinary albumin to creatinine ratios (UACR), and determined its significance in the early diagnosis of DKD.

2. Methods

2.1. Subjects

A total of 697 newly diagnosed T2DM patients who visited the outpatient or inpatient clinic at the Department of Endocrine and Metabolism

Conflicts of interest: The authors declare that they have no conflicts of interest.

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of the First Affiliated Hospital of China Medical University from January 2017 to August 2018 were enrolled in the study. T2DM diagnosis was based on the 1999 World Health Organization criteria, and that of hypertension on the International Society of Hypertension (WHO/ISH) and 1999 World Health Organization criteria.[7,8] Patients with cardiac, hepatic, rheumatic, hematological and neoplastic disorders, infections, systemic and metabolic diseases other than T2DM, renal diseases other than DKD, chronic inflammation, urinary tract disorders, prostatic diseases (in males), pregnancy, and those receiving medication that might interfere with either glucose homeostasis or urinary albumin excretion (such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), were excluded. The remaining patients were classified into four groups according to UACR and blood pressure: N-UAlb (UACR <30 mg/g with normal blood pressure, $n = 144$), M-UAlb (UACR 30–300 mg/g with normal blood pressure, $n = 143$), L-UAlb (UACR >300 mg/g with normal blood pressure, $n = 126$), and L-UAlb+HP (UACR >300 mg/g with hypertension, $n = 134$). In addition, 150 healthy volunteers (NC) were recruited as controls. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of China Medical University (Approval no-AF-SOP-07-1.1-01), and written informed consent was obtained from all subjects.

2.2. Data and sample collection

All patients provided information on age, sex, and disease duration. A regularly calibrated scale and stadiometer were used to measure the height and weight of all participants while they were wearing light clothing and no shoes. The body mass index (BMI) was calculated as height $(m)^2$/weight (kg), and certified technicians measured the systolic and diastolic blood pressure of participants in seated resting positions, according to the standard protocol.[9] Morning urine and fasting blood samples were also obtained from the patients. The blood was centrifuged in a Beckman J-6M Induction Drive Centrifuge (Beckman Coulter, Inc., Brea, CA, USA) to separate the serum. All urine, serum and plasma samples were stored at −80 °C until further analysis.

2.3. Anthropometric and analytical measurements

Fasting plasma glucose (FPG) and fasting plasma insulin (FINS) levels were evaluated in terms of glucose oxidase, measured by radio immunoassay (RIA) using insulin detection kit (IRMA, DSL-6000, Inner-variation rate < 8%, inter-variation rate < 10%, Webster, TX, USA) at the Laboratory of Endocrine and Metabolism of the First Hospital of China Medical University. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin resistance as per the following formula: insulin (mU/L) × glucose (mM/L)/22.5. Glycosylated hemoglobin (HbA1c) levels were detected using an automated HbA1c analyzer (Bio-Rad, Hercules, CA, USA). Automatic biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA) was used to measure urinary protein and creatinine (uCr) levels, serum levels of total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid (UA) and C-reactive protein (CRP). Erythrocyte sedimentation rates (ESR) was measured by a Microtest1 ESR analyzer. Commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to measure levels of VASH-1 (#CSB-L025794HU; CUSABIO, Wuhan, P.R. China), silencing factor 2-related enzyme 1 (Sirtuin 1/SIRT1; #SEE912Hu, Cloud-Clone Corp, Wuhan, P.R. China), hypoxia inducible factor 1α (HIF1α; #CSB-E12112h, CUSABIO, Wuhan, P.R. China), tumor necrosis factor-α (TNF-α; #CSB-E04740h, CUSABIO, Wuhan, P.R. China), VEGF (#CSB-E11718h, CUSABIO, Wuhan, P.R. China), and transforming growth factor-β1 (TGF-β1; #CSB-E04725H; CUSABIO, Wuhan, P.R. China). Inter- and intra-assay coefficients of variation using the ELISA kits were <8% and <10% respectively. UACR was calculated to estimate urinary protein.[10]

2.4. Statistical analysis

IBM SPSS Statistics (V.19.0, IBM Corp., Armonk, NY, USA) was used for data analysis. The results are expressed as the mean ± SD for normally distributed values and as the median (interquartile range) for non-parametric values. Differences between groups were analyzed by one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test for normally distributed values and non-parametric values after logarithmic transformation. Clinical parameters related to Ln UACR and VASH-1 were analyzed by Pearson correlation. Ridge regression analysis was also performed to determine the association between the clinical parameters and Ln UACR. All *P*-values reported are two-tailed, and $P < 0.05$ was considered statistically significant.

Table 1
Clinical characteristic of patient and control groups.

	NC	N-UAlb	M-UAlb	L-UAlb	L-UAlb+HP
N (M/F)	150 (78/72)	144 (74/70)	143 (75/68)	126 (65/61)	134 (72/62)
Age (years)	56.23 ± 10.98	55.79 ± 10.91	54.60 ± 10.33	55.54 ± 10.52	57.31 ± 10.22
Duration (years)	0.00 ± 0.00	5.76 ± 7.47[a]	4.03 ± 6.37[a]	3.26 ± 5.38[a]	4.87 ± 6.99[a]
BMI (kg/m^2)	25.86 ± 3.27	25.84 ± 2.87	26.43 ± 3.38	25.32 ± 3.18	25.83 ± 2.39
SBP (mmHg)	114.79 ± 8.34	115.79 ± 8.68	115.81 ± 8.79	114.26 ± 8.52	174.25 ± 14.56[d]
DBP (mmHg)	68.61 ± 5.86	70.73 ± 5.68	70.40 ± 5.66	69.74 ± 5.90	111.22 ± 8.58[d]
UACR (mg/g)	9.55 (4.23,14.01)	10.87 (6.69,14.96)	95.34 (61.33,142.51)[a,b]	579.05 (364.57,1497.56)[a,b,c]	1535.10 (642.36,3822.83)[a,b,c,d]
HbA1c (%)	5.06 ± 1.24	8.13 ± 2.23[a]	8.85 ± 1.64[a,b]	9.85 ± 1.91[a,b,c]	10.59 ± 2.60[a,b,c,d]
HOMA-IR	5.87 ± 7.53	5.38 ± 5.61	5.15 ± 5.99	5.08 ± 5.59	6.25 ± 5.32
HDL-C (mM)	1.34 ± 0.36	1.11 ± 0.27[a]	1.07 ± 0.40[a]	1.14 ± 0.52[a]	1.02 ± 0.31[a]
LDL-C (mM)	3.22 ± 1.02	3.02 ± 0.80	3.29 ± 1.27	3.04 ± 0.99	4.36 ± 1.59[d]
TC (mM)	4.97 ± 1.42	4.95 ± 1.41	4.73 ± 1.27	5.14 ± 1.21	6.14 ± 1.68[d]
TG (mM)	2.55 ± 3.24	2.32 ± 2.26	2.17 ± 1.89	2.42 ± 2.49	2.61 ± 1.09
sUA (mM)	301.97 ± 87.03	315.04 ± 96.83	296.52 ± 86.84	293.74 ± 90.58	383.63 ± 73.38[d]

Data are expressed as the mean ± SD or the median (interquartile range).
M: Male; F: female; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; UACR: urinary albumin to creatinine ratio; HbA1c: glycated hemoglobin; HOMA-IR: homeostatic model assessment of insulin resistance (HOMA-IR = FPG × FINS/22.5); TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; sUA: serum uric acid.
Patients with T2DM vs. NC.
[a] $P << 0.05$, Patients with T2DM vs. NC.
[b] $P < 0.05$, M-UAlb, L-UAlb, L-UAlb+HP vs. N-UAlb.
[c] $P < 0.05$, L-UAlb, L-UAlb+HP vs. M-UAlb.
[d] $P < 0.05$, L-UAlb+HP vs. L-UAlb.

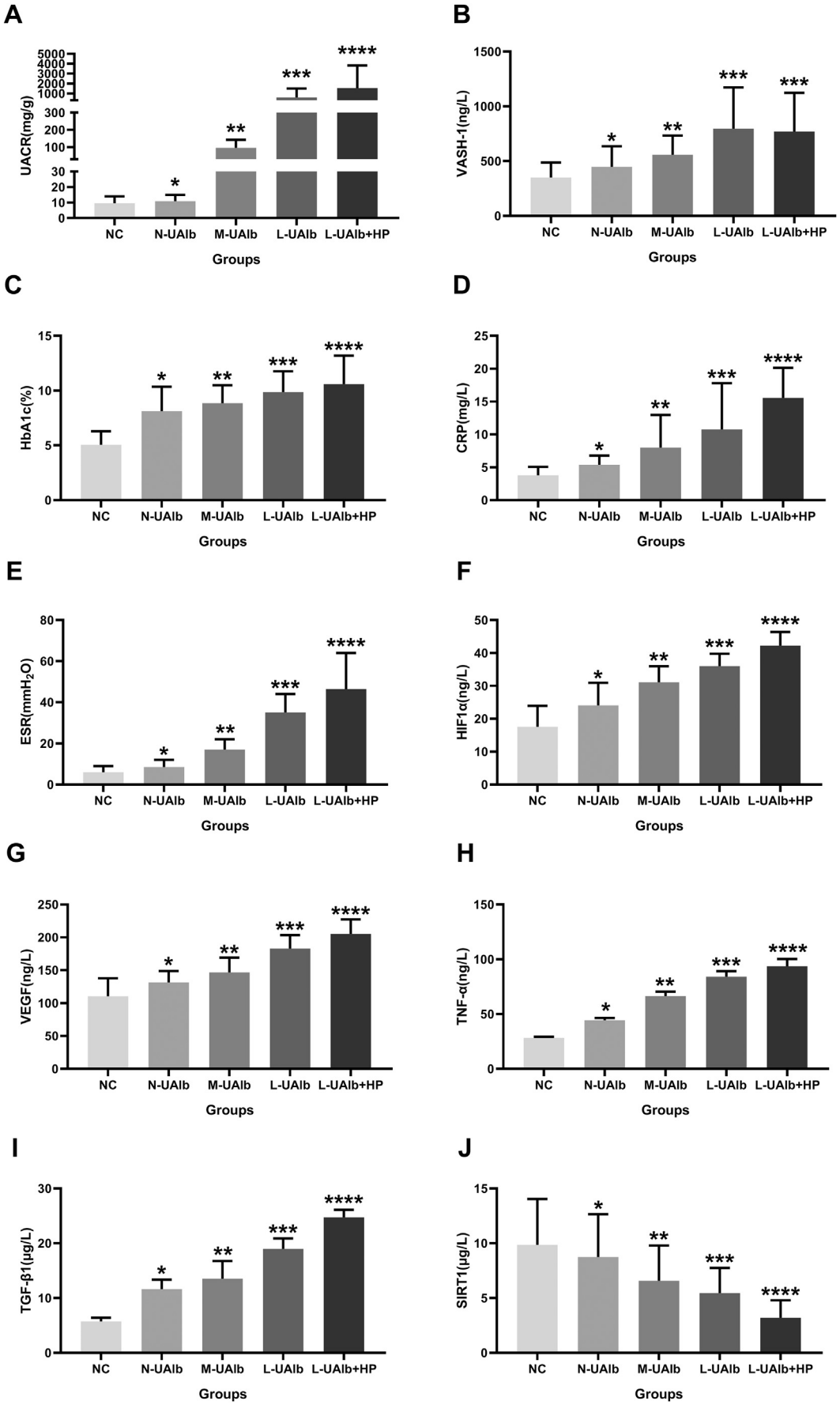

Fig. 1. Expression of A UACR, B VASH-1, C HbA1c, D CRP, E ESR, F HIF1α, G VEGF, H TNF-α, I TGF-β1, J SIRT1 levels in healthy controls and T2DM patients with N-UAlb, M-UAlb, L-UAlb, and L-UAlb+HP groups. Notes: $^{*}P < 0.05$ vs. NC; $^{**}P < 0.05$ vs. N-UAlb; $^{***}P < 0.05$ vs. M-UAlb; $^{****}P < 0.05$ vs. L-UAlb.

Table 2
Serum CRP, ESR, VASH-1, SIRT1, HIF1α, VEGF, TNF-α and TGF-β1 levels in the studied groups.

	NC	N-UAlb	M-UAlb	L-UAlb	L-UAlb+HP
CRP (mg/L)	3.78 ± 1.30	5.38 ± 1.40[a]	8.02 ± 4.96[ab]	10.78 ± 7.02[abc]	15.54 ± 4.60[a,b,c,d]
ESR (mmH_2O)[*]	6.00 (4.00, 9.00)	8.50 (6.25, 12.00)[a]	17.00 (13.00, 22.00)[a,b]	35.00 (26.00, 44.00)[a,b,c]	46.50 (23.50, 64.00)[a,b,c,d]
VASH-1 (ng/L)	350.45 ± 136.65	448.23 ± 187.87[a]	558.38 ± 174.83[a,b]	796.68 ± 376.90[a,b,c]	770.85 ± 351.91[a,b,c]
SIRT1 (μg/L)	9.86 ± 4.18	8.74 ± 3.91[a]	6.58 ± 3.22[a,b]	5.45 ± 2.30[a,b,c]	3.20 ± 1.59[a,b,c,d]
HIF1α (ng/L)	17.55 ± 6.40	24.06 ± 6.88[a]	31.05 ± 4.92[a,b]	36.03 ± 3.72[a,b,c]	42.24 ± 4.14[a,b,c,d]
VEGF (ng/L)	110.28 ± 27.59	131.36 ± 17.59[a]	146.70 ± 22.65[a,b]	182.93 ± 20.54[a,b,c]	205.11 ± 22.38[a,b,c,d]
TNF-α (ng/L)	28.36 ± 0.99	44.34 ± 2.10[a]	66.36 ± 4.08[a,b]	84.10 ± 5.05[a,b,c]	93.68 ± 6.59[a,b,c,d]
TGF-β1 (μg/L)	5.72 ± 0.69	11.61 ± 1.76[a]	13.51 ± 3.24[a,b]	18.98 ± 1.92[a,b,c]	24.73 ± 1.38[a,b,c,d]

Data are expressed as the mean ± SD or the median (interquartile range).
VASH-1: vasohibin-1; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SIRT1: silent information regulator1; TGF-β1: transforming growth factor-β1; HIF1α: hypoxia inducible factor 1α; VEGF: vascular endothelial growth factor; TNF-α: tumor necrosis factor-α; TGF-β1: transforming growth factor-β1.
Patients with T2DM vs. NC.
[*] The normal range of ESR in males was 0–15 mm/H_2O and 0–20 mm/H_2O in females.
[a] $P < 0.05$, Patients with T2DM vs. NC.
[b] $P < 0.05$, M-UAlb, L-UAlb, L-UAlb+HP vs. N-UAlb.
[c] $P < 0.05$, L-UAlb, L-UAlb+HP vs. M-UAlb.
[d] $P < 0.05$, L-UAlb+HP vs. L-UAlb.

3. Results

3.1. Clinical characteristics of patients and controls

The baseline anthropometric and biochemical characteristics of the cohort are shown in Table 1 and Fig. 1. There were no significant differences in the age, sex, BMI, or duration of diabetes between the patient's groups stratified on the basis of urinary albumin and blood pressure ($P > 0.05$), while the UACR levels were significantly different across the different groups ($P < 0.05$). Compared to the NC group, HbA1c levels were significantly increased in T2DM patients regardless of the urinary albumin levels ($P < 0.05$). The blood pressure (SBP and DBP) and UA in the L-UAlb+HP group was significantly higher compared to that in L-UAlb group ($P < 0.05$). However, only subtle differences were observed in the levels of HOMA-IR, HDL-C, LDL-C, TC and TG across all groups ($P < 0.05$).

3.2. Serum marker levels in the different groups

The serum marker levels in the NC, N-UAlb, M-UAlb, L-UAlb and L-UAlb+HP are summarized in Table 2 and Fig. 1. Compared to the healthy controls, the T2DM patients had significantly higher CRP, ESR, VASH-1, VEGF, HIF1α, TNF-α and TGF-β1 levels, but lower SIRT1 levels ($P < 0.05$), which were all further exacerbated by hypertension ($P < 0.05$). However, VASH-1 levels were similar between the L-UAlb and L-UAlb+HP groups ($P > 0.05$).

Table 3
Correlation between UACR levels and clinical parameters in patients with T2DM.

	Ln UACR	
	r	*P*
VASH-1	0.532	<0.001
HbA1c	0.526	<0.001
Ln ESR	0.694	<0.001
CRP	0.499	<0.001
SIRT1	−0.386	<0.001
HIF1α	0.663	<0.001
VEGF	0.654	<0.001
TNF-α	0.869	<0.001
TGF-β1	0.765	<0.001

VASH-1: vasohibin-1; UACR: urinary albumin to creatinine ratio; HbA1c: glycated hemoglobin; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SIRT1: silent information regulator1; TGF-β1: transforming growth factor-β1; HIF1α: hypoxia inducible factor 1α; VEGF: vascular endothelial growth factor; TNF-α: tumor necrosis factor-α; TGF-β1: transforming growth factor-β1.

3.3. Correlation between the clinical/serological parameters and serum UACR in patients

The correlation of UACR levels with other clinical parameters is summarized in Table 3 and Fig. 2. UACR of different T2DM patient groups with normal blood pressure were positively correlated to VASH-1, HbA1c, CRP, ESR, VEGF, HIF1α, TNF-α and TGF-β1 ($r = 0.532$, 0.526, 0.499, 0.694, 0.654, 0.663, 0.869, 0.765, respectively, $P < 0.001$), and negatively correlated to SIRT1 ($r = 0.386$, $P < 0.001$). Exploratory multiple regression analysis showed that there was a significant correlation between the respective variables. As common linear regression analysis revealed severe collinearity (the collinearity diagnosis found that the maximum conditional index >30, variance expansion factor >10, variance component >0.5), we adopted a ridge regression analysis using Ln UACR levels as the dependent variable, and VASH-1, Ln ESR, TGF-β1, CRP, HbA1c, HIF1α, SIRT1, VEGF and TNF-α levels as the independent variables (X1-X9), in T2DM patients with normal blood pressure (Fig. 3). The parameters were estimated with $k = 0.4$, when the standardized regression coefficients of each independent variable are stable. The results indicated that VASH-1, HbA1c, CRP, ESR, SIRT1, VEGF, HIF1α, TNF-α and TGF-β1 were independent factors associated with UACR levels ($B = 0.001$, 0.029, 0.037, 0.335, −0.035, 0.004, 0.013, 0.029 and 0.062; *wald* = 0.115, 0.033, 0.085, 0.128, −0.063, 0.064, 0.052, 0.282 and 0.147, $P < 0.001$; Table 4).

3.4. Correlation between VASH-1 levels and the clinical/serological parameters

VASH-1 levels were positively correlated with Ln UACR, HbA1c, CRP, Ln ESR, VEGF, HIF1α, TNF-α and TGF-β1 levels ($r = 0.532$, 0.320, 0.274, 0.429, 0.382, 0.377, 0.539, 0.507, respectively, $P < 0.001$), and negatively with serum SIRT1 levels ($r = -0.202$, $P < 0.001$) in individuals with normal blood pressure (Table 5 and Fig. 4).

4. Discussion

VASH-1 is an angiogenesis inhibitor produced by the ECs,[11] and blocks EC migration and proliferation via a negative feedback mechanism. Mechanistically, it inhibits VEGFs and fibroblast growth factors, thereby retarding vascular sprouting and stabilizing the number of mature blood vessels.[12,13] Furthermore, VASH-1 also reduces the microvascular density, likely via an anti-angiogenic effect, in carcinoma, oculopathy, bronchitis obliterans, hepatic cirrhosis, DKD and rheumatoid arthritis lesions.[14–22] A recent study showed that VASH-1 also played a renal-protective role by modifying glomerular crescent formation, inhibiting inflammation, and decreasing VEGF receptor 2 levels.[23]

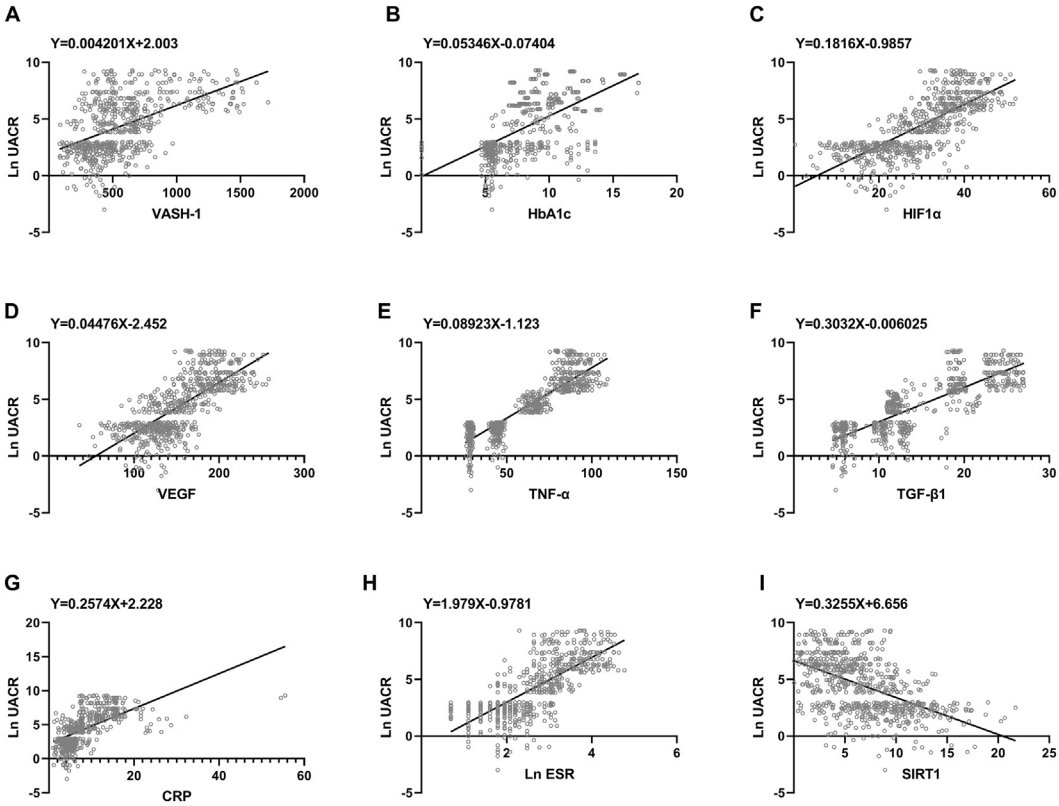

Fig. 2. Correlation between Ln UACR and other physiological indicators. A VASH-1, B HbA1c, C HIF1α, D VEGF, E TNF-α, F TGF-β1, G CRP, H Ln ESR, I SIRT1.

Our previous studies have found a potential relationship between VASH-1 and UACR.[24] Here, we further analyzed the changes in serum VASH-1 levels in T2DM patients harboring different levels of urinary albumin, and adopted newly diagnosed T2DM patients to exclude cross reactivity of the assay with all therapeutic drugs. The UACR and serum VASH-1 levels in the N-UAlb, M-UAlb, and L-UAlb patients were significantly higher compared to the healthy controls, indicating that elevated UACR increases VASH-1 levels in T2DM patients. Furthermore, VASH-1 levels also differed significantly among the patients, and increased with higher urinary albumin excretion rate. This could be a protective mechanism in response to DKD. Previous studies have implicated VEGF in the abnormal angiogenesis seen during the early stages of DKD, as well as glomerular hypertrophy, renal tubule-interstitial lesions and increased urinary albumin secretion.[25] In addition, VEGF-A was up-regulated in the podocytes of the renal cortex of VASH-$1^{+/-}$ and VASH-$1^{-/-}$ mice fed with high glucose, and exacerbation of DKD.[23] Based on

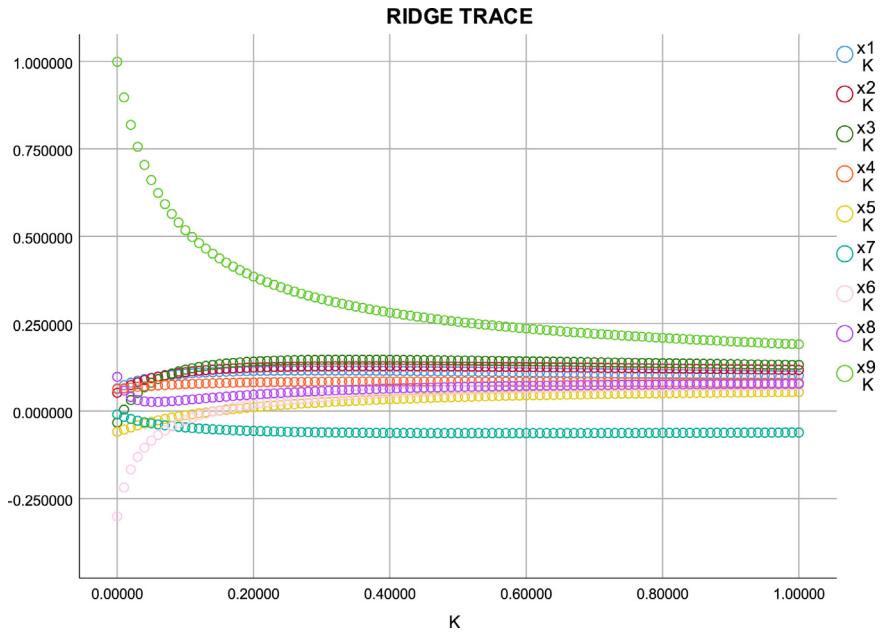

Fig. 3. Ridge trace curve of the association between the clinical parameters and Ln UACR. Y = Ln UACR as dependent variables, and X1 = VASH-1, X2 = Ln ESR, X3 = TGF-β1, X4 = CRP, X5 = HbA1c, X6 = HIF1α, X7 = SIRT1, X8 = VEGF and X9 = TNF-α as the independent variables.

Table 4
Ridge regression analysis of UACR levels and clinical parameters in patients with T2DM.

	Ln UACR					
	B	*SE*	*wald*	*t*	*P*	95%CI
Constant	−1.044	0.203	–	−5.147	<0.001	(−1.441, −0.646)
VASH-1	0.001	0.000	0.115	6.997	<0.001	(0.001, 0.001)
HbA1c	0.029	0.014	0.033	2.025	0.043	(0.001, 0.057)
Ln ESR	0.335	0.043	0.128	7.744	<0.001	(0.250, 0.420)
CRP	0.037	0.007	0.085	5.143	<0.001	(0.023, 0.051)
SIRT1	−0.035	0.009	−0.063	−3.86	<0.001	(−0.053, −0.017)
HIF1α	0.013	0.003	0.052	4.325	<0.001	(0.007, 0.018)
VEGF	0.004	0.001	0.064	5.11	<0.001	(0.003, 0.006)
TNF-α	0.029	0.001	0.282	22.519	<0.001	(0.026, 0.031)
TGF-β1	0.062	0.007	0.147	9.465	<0.001	(0.049, 0.075)

VASH-1: vasohibin-1; UACR: urinary albumin to creatinine ratio; HbA1c: glycated hemoglobin; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SIRT1: silent information regulator1; TGF-β1: transforming growth factor-β1; HIF1α: hypoxia inducible factor 1α; VEGF: vascular endothelial growth factor; TNF-α: tumor necrosis factor-α; TGF-β1: transforming growth factor-β1.

the above evidence, we hypothesized that VASH-1 inhibits angiogenesis in DKD. The serum VEGF levels were significantly higher in the N-UAlb, M-UAlb, and L-UAlb patients compared to healthy controls, indicating that elevated UACR also increased the VEGF levels in T2DM patients. Furthermore, the UACR and serum VASH-1 levels were positively correlated to VEGF levels, which likely affects angiogenesis in DKD. In addition, serum VEGF levels were also affected by SBP and DBP, indicating a potential impact of blood pressure on angiogenesis.

Renal fibrosis is one of the important pathological changes occurring in the later stages of DKD, and involves biochemical disorders and microcirculatory disturbances associated with hyperglycemia, such as protein kinase C activation, non-enzymatic protein glycation, oxidative stress and increased renin–angiotensin system activity. These pathways increase TGF-β1 levels, resulting in extracellular matrix accumulation and glomerular sclerosis. Multiple studies have confirmed TGF-β1 as a predictive biomarker of DKD fibrosis.[26] VASH-1 has shown therapeutic effects against renal fibrosis in a murine model of type I DM with early DKD.[14] Administration of the human VASH-1 gene in obese mice with T2DM significantly inhibited glomerular hypertrophy, glomerular ultrafiltration and proteinuria, as well as type IV collagen accumulation in the mesangial matrix caused by DKD. In addition, TGF-β1 levels are significantly increased in renal tissues of T2DM patients, and VASH-1 inhibits renal interstitial fibrosis by downregulating TGF-β1,[27] Smad3 phosphorylation, and NF-κB signaling.[25,28,29] We reported a positive correlation between serum VASH-1 and TGF-β1 levels in T2DM patients with different levels of urinary albumin for the first time. Our findings indicate that VASH-1 regulates urinary albumin excretion in T2DM patients by antagonizing the fibrotic effects of TGF-β1, and therefore is a potential new biomarker for DKD fibrosis.

Table 5
Correlation between serum VASH-1 levels and clinical parameters in patients with T2DM.

	VASH-1	
	r	*P*
Ln UACR	0.532	<0.001
HbA1c	0.32	<0.001
Ln ESR	0.429	<0.001
CRP	0.274	<0.001
SIRT1	−0.202	<0.001
HIF1α	0.377	<0.001
VEGF	0.382	<0.001
TNF-α	0.539	<0.001
TGF-β1	0.507	<0.001

VASH-1: vasohibin-1; UACR: urinary albumin to creatinine ratio; HbA1c: glycated hemoglobin; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SIRT1: silent information regulator1; TGF-β1: transforming growth factor-β1; HIF1α: hypoxia inducible factor 1α; VEGF: vascular endothelial growth factor; TNF-α: tumor necrosis factor-α; TGF-β1: transforming growth factor-β1.

SIRT1, a member of the NAD+ terminal dependent deacetylase family, protects blood vessels against atherosclerosis, diabetic vascular complications and other physiological diseases.[30–32] HIF1α is a hypoxia-induced response element which can specifically bind to the erythropoietin gene, and is associated with multiple pathological and physiological processes.[33] We previously observed that the levels of SIRT1 and HIF1α were significantly altered in glomerular mesangial cells cultured with high glucose, indicating their potential roles in the pathogenesis of DKD.[28] In this study, we found that UACR and serum VASH-1 levels were positively correlated to SIRT1 and HIF1α levels in T2DM patients with different levels of urinary albumin.

Renal inflammation and hemodynamic disorder are the mechanisms underlying DKD development. CRP increases oxidative stress in glomerular endothelial cells by triggering the inflammatory response, which promotes glycation end product formation that modify vascular wall proteins and accelerate glomerular hardening.[23] ESR is an inflammatory and immunological marker[33] which is upregulated along with CRP in DKD patients, indicating its utility as a biomarker for the inflammatory response seen in DKD.[34] TNF-α is produced by mononuclear macrophages, and not only has cytolytic effects on tumor cells but is also is a potent inflammatory factor that drives immune nephropathies like lupus nephritis and anti-glomerular basement membrane (GBM) diseases.[35,36] Patients with DKD have higher levels of TNF-α compared to non-DKD patients, and increased levels of TNF-α are seen in the renal interstitium of diabetic rats with high urinary protein clearance. Taken together, TNF-α is also an inflammatory marker of DKD.[37]

UACR and serum VASH-1 levels were positively correlated to HbA1c, ESR, CRP and serum TNF-α levels, indicating that VASH-1 is associated with glucose metabolism and inflammation in DKD. The top highest levels of standardized coefficient of ridge regression adjusting UACR were TNF-α, TGF-β1, Ln ESR and VASH1 (0.282, 0.147, 0.128 and 0.115, respectively), which indicated the influence affecting albuminuria. However, the VASH-1 levels in the L-UAlb and L-UAlb+HP groups were similar, indicating that blood pressure had little effect on the serum VASH-1 levels in T2DM patients. Furthermore, both UACR and serum VASH-1 were not significantly correlated to age, disease duration or BMI, and the levels of HOMA-IR and blood lipids (TC, TG, HDL-C, and LDL-C) were only slightly different across the groups. Finally, UA levels were significantly different between the L-UAlb and L-UAlb+HP groups, indicating its potentially correlation with hypertension and DKD.

Interestingly, previous basic experiments have suggested the potential protective roles of VASH-1 on DKD and renal fibrosis.[14,23,27,28] Moreover, the expression of VASH-1, despite its anti-cancer effect, has been proved to increase as cancer progression in human patients, suggesting the compensatory up-regulation of VASH-1 against tumor angiogenesis.[17,19,21,22] Therefore, the elevated serum VASH-1 levels in T2DM patients with different UACR might reflect a compensatory up-regulation in order to prevent the progression of angiogenesis, inflammation and fibrosis mediated by VEGF, TNF-α and TGF-β1.

In summary, this study demonstrates that serum VASH-1 levels gradually increase with the urinary albumin excretion rate and HbA1c in T2DM patients, indicating that VASH-1 may have a potential association with T2DM and DKD. UACR and serum VASH-1 levels were significantly correlated with that of VEGF, TGFβ1, SIRT1, HIF1α, TNF-α, ESR and CRP, indicating a compensatory up-regulative role of VASH-1 in preventing angiogenesis, inflammation and fibrosis. This has potential implications on the treatment of patients with DKD. However, our study has several limitations that should be considered. As a cross-sectional study, the serological tests were not followed-up, which raises ambiguity on the mechanisms regulating urinary albumin clearance, angiogenesis, inflammation and fibrosis in DKD. Further studies are therefore required to validate our findings.

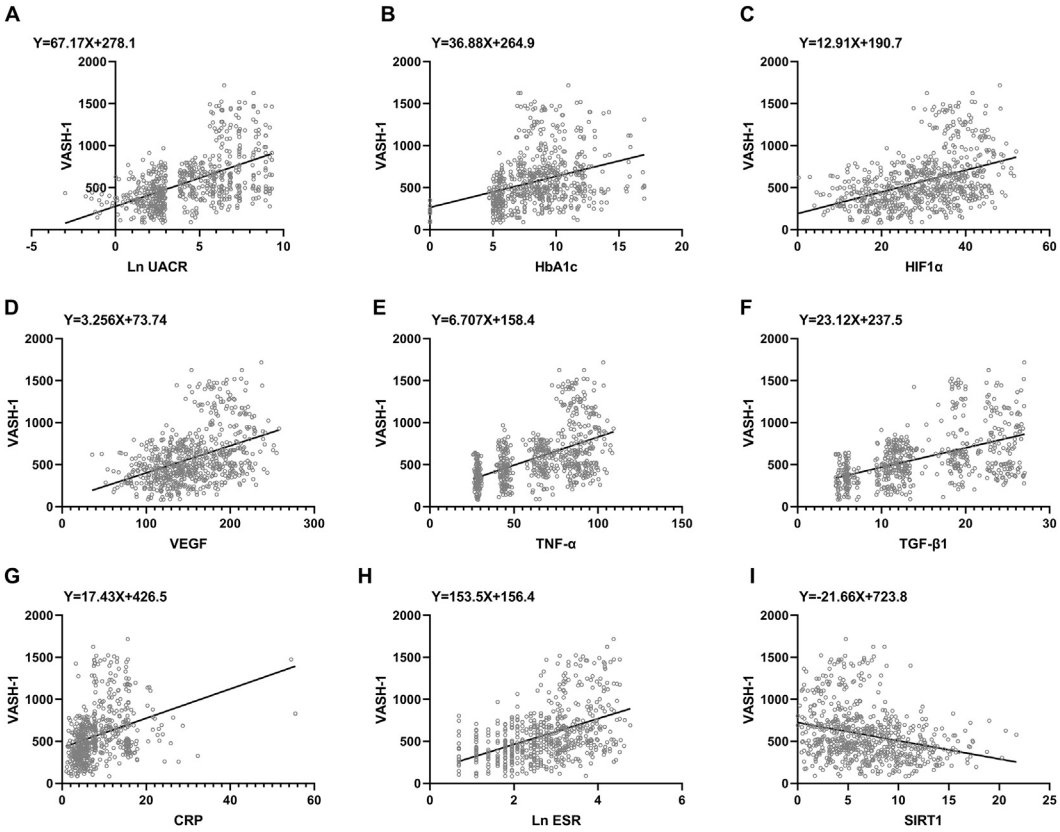

Fig. 4. Correlation between VASH-1 and other physiological indicators. A Ln UACR, B HbA1c, C HIF1α, D VEGF, E TNF-α, F TGF-β1, G CRP, H Ln ESR, I SIRT1.

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Statement of human rights

This study was approved by the Medical Ethics Committee at the First Affiliated Hospital of China Medical University (Approval no-AF-SOP-07-1.1-01). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Sandeep V. Type 2 diabetes. Ann Intern Med 2015;162:231-42.
2. Quiroga B, Arroyo D, De AG. Present and future in the treatment of diabetic kidney disease. J Diabetes Res 2015;2015:1-13.
3. Tothmanikowski S, Atta MG. Diabetic kidney disease: pathophysiology and therapeutic targets. J Diabetes Res 2015;2015:1-16.
4. Chamberlain JJ, Herman WH, Leal S, Rhinehart AS, Shubrook JH, Skolnik N, et al. Pharmacologic therapy for type 2 diabetes: synopsis of the 2017 American Diabetes Association Standards of Medical Care in Diabetes. Ann Intern Med 2017;164:542.
5. Brownlee M, Aiello LP, Cooper ME, Vinik AI, Plutzky J, Boulton AJM. *Chapter 33 - complications of diabetes mellitus[M]//Williams textbook of endocrinology*. Elsevier Inc. 2016.
6. Watanabe K, Hasegawa Y, Yamashita H, Shimizu K, Ding Y, Abe M, et al. Vasohibin as an endothelium-derived negative feedback regulator of angiogenesis. Transplant Proc 2004;114:898-907.
7. Association A D. Standards of medical care in diabetes—2007. Diabetes Care 2007;30: S13-61.
8. Chalmers J. The 1999 WHO-ISH guidelines for the management of hypertension. Med J Aust 1999;171:458-9.
9. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JLJ, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA 2003;289: 2560-72.
10. Sato Y. The vasohibin family: a novel family for angiogenesis regulation. J Biochem 2013;153:5-11.
11. Affara M, Sanders D, Araki H, Tamada Y, Dunmore BJ, Humphreys S, et al. Vasohibin-1 is identified as a master-regulator of endothelial cell apoptosis using gene network analysis. BMC Genomics 2013;14:23.
12. Shimizu K, Watanabe K, Yamashita H, Abe M, Yoshimatsu H, Ohta H, et al. Gene regulation of a novel angiogenesis inhibitor, vasohibin, in endothelial cells. Biochem Biophys Res Commun 2005;327:700-6.
13. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592-603.
14. Nasu T, Maeshima Y, Kinomura M, Hirokoshi-Kawahara K, Tanabe K, Sugiyama H, et al. Vasohibin-1, a negative feedback regulator of angiogenesis, ameliorates renal alterations in a mouse model of diabetic nephropathy. Diabetes 2009;58: 2365-75.
15. Onami H, Nagai N, Kaji H, Nishizawa M, Sato Y, Osumi N, et al. Transscleral sustained vasohibin-1 delivery by a novel device suppressed experimentally-induced choroidal neovascularization. PLoS One 2013;8, e58580.
16. Watanabe T, Okada Y, Hoshikawa Y, Eba S, Notsuda H, Watanabe Y, et al. A potent anti-angiogenic factor, vasohibin-1, ameliorates experimental bronchiolitis obliterans. Transplant Proc 2012;44:1155-7.
17. Murakami K, Kasajima A, Kawagishi N, Sekiguchi S, Fujishima F, Watanabe M, et al. The prognostic significance of vasohibin 1-associated angiogenesis in patients with hepatocellular carcinoma. Hum Pathol 2014;45:589-97.
18. Miyake K, Nishida K, Kadota Y, Yamasaki H, Nasu T, Saitou D, et al. Inflammatory cytokine-induced expression of vasohibin-1 by rheumatoid synovial fibroblasts. Acta Med Okayama 2009;63:349-58.
19. Hosaka T, Kimura H, Heishi T, Suzuki Y, Miyashita H, Ohta H, et al. Vasohibin-1 expression in endothelium of tumor blood vessels regulates angiogenesis. Am J Pathol 2009;175:430-9.
20. Naito H, Kidoya H, Sato Y, Takakura N. Induction and expression of anti-angiogenic vasohibins in the hematopoietic stem/progenitor cell population. J Biochem 2009;145:653-9.

21. Kanomata N, Sato Y, Miyaji Y, Nagai A, Moriya T. Vasohibin-1 is a new predictor of disease-free survival in operated patients with renal cell carcinoma. J Clin Pathol 2013;66:613-9.
22. Kosaka T, Miyazaki Y, Miyajima A, Mikami S, Hayashi Y, Tanaka N, et al. The prognostic significance of vasohibin-1 expression in patients with prostate cancer. Br J Cancer 2013;108:2123-9.
23. Hinamoto N, Maeshima Y, Yamasaki H, Nasu T, Saito D, Watatani H, et al. Exacerbation of diabetic renal alterations in mice lacking vasohibin-1. PLoS One 2014;9, e107934.
24. Ren H, Lv C, Shao Y, Wu C, Wang X, An L, et al. Expression levels of serum vasohibin-1 in type 2 diabetes mellitus patients with different excretion rates of urinary albumin and its clinical significance. Chin J Endocrinol Metab 2016;32:647-51.
25. Dei Cas A, Gnudi L. VEGF and angiopoietins in diabetic glomerulopathy: how far for a new treatment? Metabolism 2012;61:1666-73.
26. Cao Z, Cooper ME. Pathogenesis of diabetic nephropathy. J Diabetes Investig 2011;2:243-7.
27. Saito D, Maeshima Y, Nasu T, Yamasaki H, Tanabe K, Sugiyama H, et al. Amelioration of renal alterations in obese type 2 diabetic mice by vasohibin-1, a negative feedback regulator of angiogenesis. Am J Physiol Ren Physiol 2011;300:F873-86.
28. Watatani H, Maeshima Y, Hinamoto N, Yamasaki H, Ujike H, Tanabe K, et al. Vasohibin-1 deficiency enhances renal fibrosis and inflammation after unilateral ureteral obstruction. Physiol Rep 2014;2.
29. Lan HY, Chung AC. Transforming growth factor-beta and Smads. Contrib Nephrol 2011;170:75-82.
30. Sivaskandarajah GA, Jeansson M, Maezawa Y, Eremina V, Baelde HJ, Quaggin SE. Vegfa protects the glomerular microvasculature in diabetes. Diabetes 2012;61:2958-66.
31. García-García MP. Inflammation in diabetic kidney disease. World J Diabetes 2014;5:431.
32. Yang J, Lin SC, Chen G, et al. Adiponectin promotes monocyte-to-fibroblast transition in renal fibrosis[J]. J Am Soc Nephrol 2013;24:1644-59.
33. You H, Gao T, Cooper TK, et al. Macrophages directly mediate diabetic renal injury. Am J Physiol Ren Physiol 2013;305:1719-27.
34. Lu C, Han HD, Mangala LS, et al. Regulation of tumor angiogenesis by EZH2. Cancer Cell 2010;18:185-97.
35. Morris CJ, Purvis TE, Hu K, et al. Circadian misalignment increases cardiovascular disease risk factors in humans. Proc Natl Acad Sci U S A 2016;113:E1402.
36. Ionescu L, Byrne RN, Van HT, et al. Stem cell conditioned medium improves acute lung injury in mice: in vivo evidence for stem cell paracrine action. Am J Physiol Lung Cell Mol Physiol 2016;303:L967.
37. Fathy SA, Mohamed MR, Ali MAM, et al. Influence of IL-6, IL-10, IFN-γ and TNF-α genetic variants on susceptibility to diabetic kidney disease in type 2 diabetes mellitus patients. Biomarkers 2018:1-38.