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The 150-kDa oxygen-regulated protein (ORP150) regulates proteinuria in diabetic nephropathy via mediating VEGF

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ABSTRACT

The 150-kDa oxygen-regulated protein (ORP150) belongs to a family of the heat shock protein implicated in the cellular response to environmental stress. Previous data demonstrated that ORP150 regulates the secretion of vascular endothelial growth factor (VEGF) to drive progression of angiogenesis associated with proliferative diabetic retinopathy. However, the expression and biological functions of serum ORP150 levels in diabetic nephropathy (DN) remain unclear. In this study, we reported for the first time that ORP150 was up-regulated in serum of patients with DN. Moreover, we observed the dramatic increase in serum ORP150 accompanied with the elevated levels of proteinuria and serum VEGF levels in DN, indicating the possible involvement of ORP150 in regulation of albuminuria via mediating VEGF in DN. Employing the streptozotocin (STZ) to construct the DN model, we confirmed the positive correlation of ORP150 with VEGF in vivo. Monoclonal anti-ORP150 antibodies treatment significantly decreased the secretion of VEGF and albuminuria in STZ-induced DN models. Consequently, our data suggested that ORP150 levels were positively correlated with proteinuria burden via mediating VEGF in DN. It may be considered as a novel diagnostic and therapeutic target.

1. Introduction

Diabetic nephropathy (DN), a major microvascular complication occurring among approximately 30–50% of patients with type 2 diabetes mellitus (T2DM), is characterized by the progressive impairment of glomerular filtration, which is the principal factor leading to end-stage renal disease (ESRD) (Conserva et al., 2016; Fried et al., 2013). This disease results from the damage to renal tubule, glomerulus, or renal mesenchyme on account of hemodynamic factors that accompany a state of sustaining hyperglycemia and diabetes (Kim et al., 2018). During the early stage, this kidney injury is reversible but becomes irreversible once the nephropathy is overt, ultimately developing into ESRD (Nielsen et al., 2011; Park et al., 2013). The renal injury of DN is initially distinguished by the appearance of moderately increased proteinuria, followed by overt proteinuria and a gradual decline in glomerular filtration rate (GFR) (Ravindran et al., 2017; Yasuda-Yamahara et al., 2015). Accumulating evidence has demonstrated that proteinuria is a greatest predictor of renal function deterioration and DN progression (Tian et al., 2018). Recent information related to proteinuria in DN has provided the rationale for new pharmacological approaches, such as RAAS inhibitor, Huangkui capsule. However, important questions about the molecular mechanisms of proteinuria remain unanswered.

The 150-kDa oxygen-regulated protein (ORP150), a member of the heat shock protein family, is located in the ER functions as a molecular chaperone in the transport and folding of newly synthesized proteins. ORP150 was originally characterized based on its alternative expression in cultured rat astrocytes under hypoxic stress (Kuwabara et al., 1996; Tsukamoto et al., 1996). Since then, ORP150 has been reported to be induced by oxidative stress and hypoxic stress in a wide range of cells (Goswami et al., 2003; Kim et al., 2012; Ozawa et al., 2001a; Ozawa et al., 2005). Expression of ORP150 is required for cells to survive prolonged hypoxia and has been shown to provide anti-apoptotic signals (Cechowska-Pasko et al., 2006; Ozawa et al., 1999). For example, in the central nervous system, the over-expression of ORP150 was found to protect neurons from ischaemia/hypoxia by restraining apoptosis (Tamatani et al., 2001). Upregulated expression of ORP150 was demonstrated in a range of pathologic situations, such as ischaemic retina (Kim et al., 2012), ischaemic brain (Matsushita et al., 1998), wound healing (Ozawa et al., 2001a), malignant tumors (Miyagi et al., 2001; Miyagi et al., 2002; Shim et al., 2015), and atherosclerotic

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plaques (Tsukamoto et al., 1996). These data suggest that ORP150 may contribute to the cellular response suffer from environmental stress in a fundamental way.

A variety of intracellular and extracellular signaling pathways have been found to be critically involved in proteinuria progression in DN, such as the renin-angiotensin system, protein kinase C (PKC), transformation growth factor-β1 (TGF-β1), the upregulation of plasminogen activator inhibitor-1 (PAI-1), and vascular endothelial growth factor (VEGF) (Arora et al., 2013; Hakroush et al., 2009; Wang et al., 2017; Zoja et al., 2016). There is a dual role for VEGF signaling, including increasing the permeability of vascular endothelial cells and destruction the glomerular filtration barrier (Sun et al., 2014). Moreover, the landmark experiments revealed that the over-expression of VEGF to type 1 and type 2 diabetic animals increased proteinuria and glomerular hypertrophy (de Vriese et al., 2001; Flyvbjerg et al., 2002), indicating the significance of VEGF in accelerating diabetic nephropathy. Some researches have shown that ORP150 plays a critical role in the post-translational regulation of many secretory proteins, such as VEGF and TGF-β. More interestingly, optimal function of ORP150 is essential for mature VEGF transporting from ER to the Golgi compartment and secretion out of the cell (Ozawa et al., 2001a; Ozawa et al., 2001b). In addition, the upregulated levels of ORP150 in the ocular microenvironment might contribute to the initiation and progression of angiogenesis associated with proliferative diabetic retinopathy through VEGF (Abu El-Asrar et al., 2018). Based on above studies, we predicted that ORP150 may participate in the regulation of proteinuria generating via regulating the secretion of VEGF.

Given the critical roles of ORP150 in the processing and secretion of VEGF, we investigated the hypothesis that ORP150 may be involved in the pathogenesis of DN. In the present study, we found that ORP150 was significantly up-regulated in the peripheral blood of DN patients and STZ-induced DN rats, compared with healthy ones. In addition, a remarkable positive correlation was found between DN patients serum levels of ORP150 and VEGF. More importantly, monoclonal anti-ORP150 antibodies treatment significantly decreased the secretion of VEGF and albuminuria in STZ-induced DN models. These findings may provide a novel insight into mechanism underlying albuminuria progression with DN.

2. Materials and methods

2.1. Subjects

This research randomly enrolled a consecutive population of 166 patients with T2DM from the endocrine department of our hospital. These patients were diagnosed with T2DM on the basis of the American Diabetes Association (ADA) criteria. The T2DM patients were then divided into three groups according to the levels of UAE (urinary albumin excretion): those with macroalbuminuria (UAE > 300 mg/24 h; $n = 54$), those with microalbuminuria (30 ≤ UAE ≤ 300 mg/24 h, $n = 48$), and those with normoalbuminuria (UAE < 30 mg/24 h; $n = 64$) (Hameed et al., 2015). Patients with concomitant cardiomyopathy, valvular heart disease, acute renal failure, asthma, connective tissue diseases, viral or bacterial infections, tumors and T1DM were excluded from this study. The control group randomly recruited a consecutive population of 64 healthy subjects who had conventional medical check-up in our hospital. Those healthy subjects had no clinical or history symptom of diabetes , with normal fasting blood glucose (FBG) and glycosylated hemoglobin A1c (HBA1c). This study was approved by the Institutional Ethics Committee of Haian People's Hospital according to the Helsinki Declaration. Written informed consents were obtained from patients prior to inclusion.

2.2. Sample collection and patient measurements

Blood and urine samples were collected after overnight fasting and stored immediately at −20 °C until they were prepared for the corresponding analysis. Apart from that, all patient measurements were obtained at the same time.

2.3. Subject characteristics

In a period of 3 months, each of recruited patients received their usual follow-up medical examination, during which anthropometric variables, waist circumference (cm) and body mass index (BMI) (kg/m^2) were determined. Blood pressure (BP), weight, and height were measured using conventional methods. All measurements were obtained after patients giving up smoking and drinking at least 12 months, and abstaining from high-fat diet and alcohol consumption before testing. FBG, HBA1c, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL−C), and serum creatinine (Scr) were all measured by using venous blood samples. FBG was measured by using glucose oxidase. HBA1c was determined by using High Performance Liquid Chromatography (HPLC). Blood lipid and Scr were measured by an automatic biochemical analyzer (Medical Ltd., Beijing). Glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation as recommended by current guidelines. After fasting for 10–12 h, we collected the 24 h urine volume, microalbuminuria, and macroalbuminuria (mAlb, Roche, Germany) were calculated and measured. All of these measurements were obtained by our clinical laboratory.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Serum ORP150 and VEGF concentrations were measured using ELISA (R&D Systems, Inc., Minneapdis, Minnesota, USA) according to the manufacturer's protocols.

2.5. Animal experimental protocols

Thirty adult Wistar rats (male) (weigh: 200 ± 10 g, age: 3.5 months) were obtained from the Experimental Animal Center of Nantong University. The rats were kept in typical plastic cages under standard environmental conditions. Rats were divided equally into five groups and then treated as depicted in Fig. 1. Two rats in each group were killed after 4 weeks and 8 weeks. DN rats were induced by intravenous injection of streptozotocin (STZ, 45 mg/kg; Pfanstiel Europe LTD, Davenham, UK). The same dose of citric acid buffer was injected in the normal group. The 12 control rats were fed by standard laboratory diet and the 18 DN rats were fed by the high-fat diet (HFD), which purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). After 8 weeks, the level of FBG and 24 h urine albumin (24UPro) value were regarded as the DN rat model. After the establishment of DN model, DN rats were divided into 3 groups, including the model group, the isotype-matched control Ab group and the anti-ORP150 Ab group. Four weeks after administration, surplus rats were sacrificed through cardiac puncture. Blood samples, 24 h urine and kidneys were collected for detection of various indicators.

2.6. Quantitative RT-PCR

The total cellular RNA from rat venous blood was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) and RT-PCR was performed as previously described (Popivanova et al., 2008). Briefly, cDNA was synthesized by a Ready-To-Go T-Primed First Strand Kit (Fermentas, Glen Burnie, MD, USA). PCR was accomplished in the presence of 0.5 U Taq DNA polymerase (Takara, Japan). The PCR primers as follows, ORP150, forward: 5′-AGCATCACCCCTGTGTTCCACC-3′, reverse: 5′-TGGGACAGTCTCCATTCCCA-3′; VEGF, forward: 5′-GATGTGAATGC AGACCAAAGA-3′, reverse: 5′-GGAATCTCATTCGATGCATAC-3′; GAPDH, forward: 5′-TGAATACGGCTACAGCAACA-3′, reverse: 5′-AGG

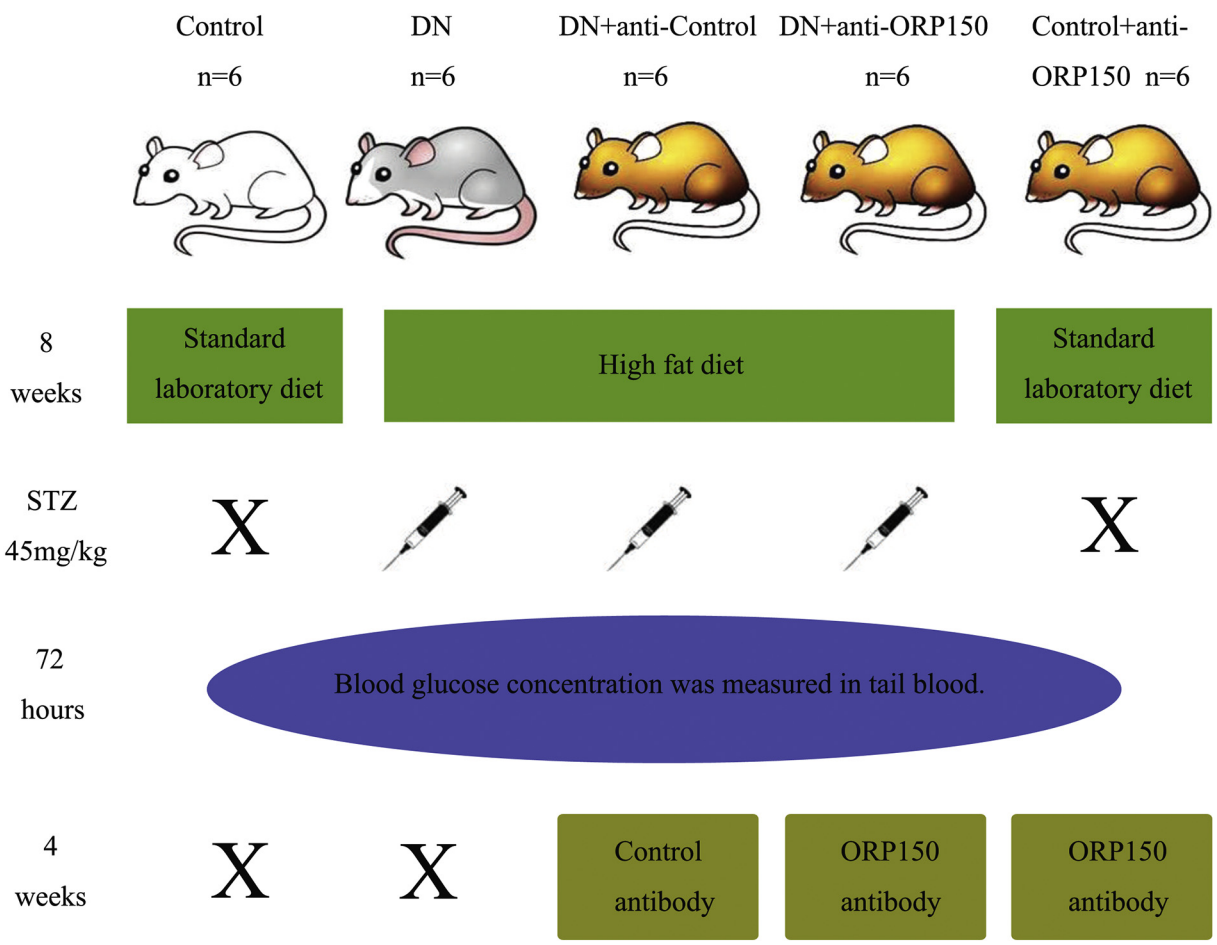

Fig. 1. Experimental scheme. Scheme of our experimental groups, number of rats we used, the treatment way, and duration. IP STZ: intraperitoneal injection of streptozotocin.

CCCCTCCTGTTATTATG-3′. RT-PCR products were separated on 1.5% agarose gels containing ethidium bromide, and then visualized and photographed by a gel documentation system (Foster City, CA, USA).

2.7. Morphological observation of kidney

The kidney tissues were fixated, dehydrating by 70, 80, 90 and 95% ethanol added in turn for treatment during dehydration, soaked via xylene and embedded via paraffin. Microtome was used to cut the sample into 4.5 μm sections. Then, the sections were stained with H&E, sealed by neutral balsam, and followed by observation of renal pathological changes under the microscope.

2.8. Anti-ORP150 antibody treatment

Monoclonal anti-ORP150 antibodies (Ab) and isotype-matched control Ab were prepared. Briefly, male Wistar rats were immunized, then accepted a booster three times, 21d apart, by subcutaneous and intraperitoneal injections of 50 mg of ORP150, emulsified with an equivalent volume of Complete Freund's Adjuvant for the primary immunization and Incomplete Freund's Adjuvant for secondary immunizations. The rats with the highest serum titer to ORP150 as measured by ELISA received an intravenous injection of additional 30 mg of immunogen in PBS, 21 days after the last immunization. Three days later, spleen cells were harvested for production of hybridomas to ORP150. Two hybridoma cell lines with highest Ab concentration and neutralizing Ab activity were cloned three to four times. The Wistar rats were received intraperitoneal injections of each cloned hybridoma, and purified IgG was prepared by Protein A chromatography (Sigma). The isotype and light chain composition of the Ab and the characterization of neutralizing activity were performed. DN rats were either untreated (n = 6) or treated with anti-ORP150 Ab (n = 6) or with isotype-matched control Ab (n = 6). One milligram of the appropriate Ab was injected intraperitoneally three times per week, starting 3d after the STZ injection until the final experiments.

2.9. Statistical analysis

Statistical analyses were completed using the Statistical Package for Social Sciences (SPSS 22.0, Illinois, USA). Measurement data are presented as mean ± SD or median (IQR). The differences of characteristics between different groups of patients and rats were compared using χ^2 tests, Kruskal-Wallis test or one-way analysis of variance (ANOVA). The correlation between serum ORP150 and VEGF were analyzed using simple and multiple stepwise linear regression analysis. The P-value < .05 was considered statistically significant. Each experiment was accomplished at least three replicates per condition.

3. Results

3.1. Subject characteristics

As presented in Table 1, higher blood pressure, FBG, HBA1c, TG, TC, LDL-C, as well as decreased levels of HDL-C were found in patients with T2DM than those in controls. Patients with abnormal albuminuria had higher Scr and UAE than the other two groups. In addition, decreased GFR was shown in the abnormal albuminuria with T2DM patients compared with the other two groups. Furthermore, increased

Table 1
The clinical and biochemical characteristics of patients in control group, type 2 diabetes mellitus with normal albuminuria group and diabetic nephropathy group.

Index	Control (n = 64)	Patients with T2DM		P value
		Normoalbuminuria (n = 64)	Abnormal albuminuria (n = 102)	
Age	63.22 ± 0.99	62.89 ± 1.06	62.26 ± 0.84	.756
Gender				
Male	30	24	47	.478
Female	34	40	55	
BMI	22.75 ± 0.37	22.67 ± 0.38	21.92 ± 0.34	.173
SBP (mmHg)	124.58 ± 1.58	128.63 ± 1.68	140.73 ± 2.05	< .001
DBP (mmHg)	80.28 ± 1.25	83.98 ± 1.20	90.80 ± 1.32	< .001
FBG (mmol/L)	5.14 ± 0.09	10.96 ± 0.76	9.80 ± 0.54	< .001
HBA1c (%)	5.27 ± 0.07	8.68 ± 0.32	8.76 ± 0.25	< .001
TG (mmol/L)	1.27 ± 0.11	2.05 ± 0.26	2.49 ± 0.15	< .001
TC (mmol/L)	3.80 ± 0.11	4.61 ± 0.12	4.96 ± 0.13	< .001
LDL-C (mmol/L)	2.11 ± 0.10	2.60 ± 0.08	3.61 ± 0.11	< .001
HDL-C (mmol/L)	1.27 ± 0.06	1.17 ± 0.05	1.00 ± 0.03	< .001
Scr (μmol/L)	70.62 ± 2.06	67.36 ± 1.90	106.67 ± 5.14	< .001
GFR (ml/min)	120.26 ± 4.63	129.51 ± 4.59	83.59 ± 2.90	< .001
ORP150 (pg/ml)	233.23 ± 19.65	546.02 ± 29.52	1408.32 ± 64.06	< .001
UAE (mg/24 h)	13.28 ± 1.16	11.52 ± 1.09	648.60 ± 57.40	< .001
VEGF (pg/ml)	355.41 ± 22.80	907.06 ± 38.27	2176.00 ± 98.93	< .001

BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; FBG - fasting blood glucose; HBA1c - glycosylated hemoglobin; TG - triglyceride; TC - total cholesterol; LDL-C - low-density lipoprotein cholesterol; HDL-C -high-density lipoprotein cholesterol; Scr - serum creatinine; GFR - glomerular filtration rate; ORP150 - oxygen-regulated protein 150-kDa; VEGF - vascular endothelial growth factor. P value < .05 represents statistical difference.

serum ORP150 and VEGF concentrations were shown in the T2DM group compared with the controls. Serum ORP150 and VEGF concentrations were further elevated in patients with T2DM with abnormal albuminuria than those in the other two groups , suggesting the pivotal role of ORP150 and VEGF in DN pathogenesis.

3.2. Baseline clinical characteristics of DN patients with microalbuminuria and macroalbuminuria groups

The characteristics of the two groups are presented in Table 2. The macroalbuminuria group had higher ORP150 levels than the microalbuminuria group (P < .001) whereas age, sex ratio, BMI, FBG, HBA1c, TG, TC, LDL-C, and HDL-C did not differ significantly between the 2 groups (P > .05). More critically, the levels of blood pressure, Scr, GFR, and VEGF were higher in the macroalbuminuria group compared with those in the microalbuminuria group (P < .001). Taken together, the serum levels of ORP150 and VEGF were positively correlated with proteinuria burden in DN pathogenesis.

Table 2
The basic characteristics patients in microalbuminuria group and macroalbuminuria group.

Index	Microalbuminuria (n = 48)	Macroalbuminuria (n = 54)	P value
Age	62.63 ± 1.19	61.94 ± 1.19	.687
Gender			
Male	19	28	.218
Female	29	26	
BMI	22.34 ± 0.47	21.54 ± 0.49	.241
SBP (mmHg)	127.12 ± 2.15	152.81 ± 2.37	< .001
DBP (mmHg)	81.21 ± 1.32	99.33 ± 1.41	< .001
FBG (mmol/L)	10.49 ± 0.85	9.18 ± 0.68	.231
HBA1c (%)	9.16 ± 0.37	9.41 ± 0.33	.136
TG (mmol/L)	2.51 ± 0.19	2.46 ± 0.22	.862
TC (mmol/L)	5.08 ± 0.17	4.84 ± 0.20	.361
LDL-C (mmol/L)	3.56 ± 0.16	3.66 ± 0.15	.641
HDL-C (mmol/L)	0.98 ± 0.03	1.02 ± 0.04	.467
Scr (μmol/L)	75.52 ± 1.69	134.37 ± 7.88	< .001
GFR (ml/min)	108.69 ± 1.97	61.28 ± 2.69	< .001
ORP150 (pg/ml)	870.96 ± 54.16	1885.98 ± 57.53	< .001
VEGF (pg/ml)	1301.46 ± 62.33	2953.37 ± 89.08	< .001

BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; FBG - fasting blood glucose; HBA1c - glycosylated hemoglobin; TG - triglyceride; TC - total cholesterol; LDL-C - low-density lipoprotein cholesterol; HDL-C -high-density lipoprotein cholesterol; Cr - serum creatinine; GFR - glomerular filtration rate; ORP150 - oxygen-regulated protein 150-kDa; VEGF - vascular endothelial growth factor. P value < .05 represents statistical difference.

3.3. Correlations between ORP150 and VEGF

A strongly positive correlation was found between DN patients serum levels of ORP150 and levels of VEGF (r = 0.74; P = .011) (Fig. 2). Thus, we supposed that ORP150 may be involved in the progression of DN and possibly had a connection with VEGF pathway. Further experiments were required to certify the specific contribution of ORP150 to the pathophysiology of DN and related molecular mechanism.

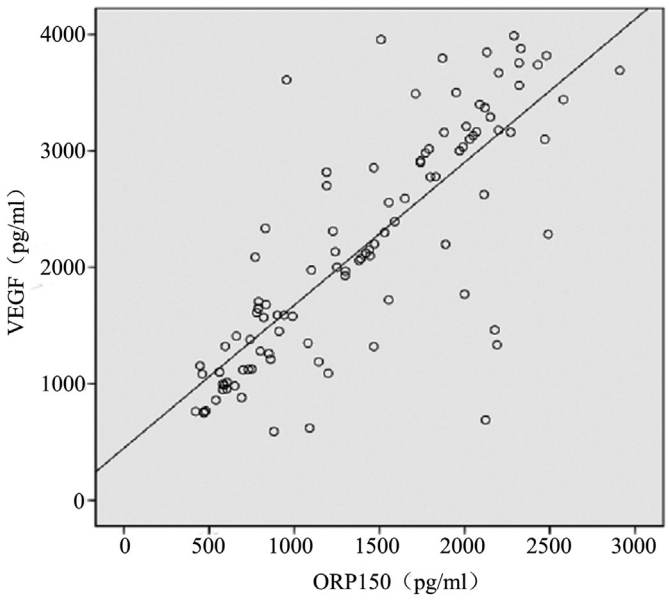

Fig. 2. The correlation between DN patients serum levels of ORP150 and VEGF.

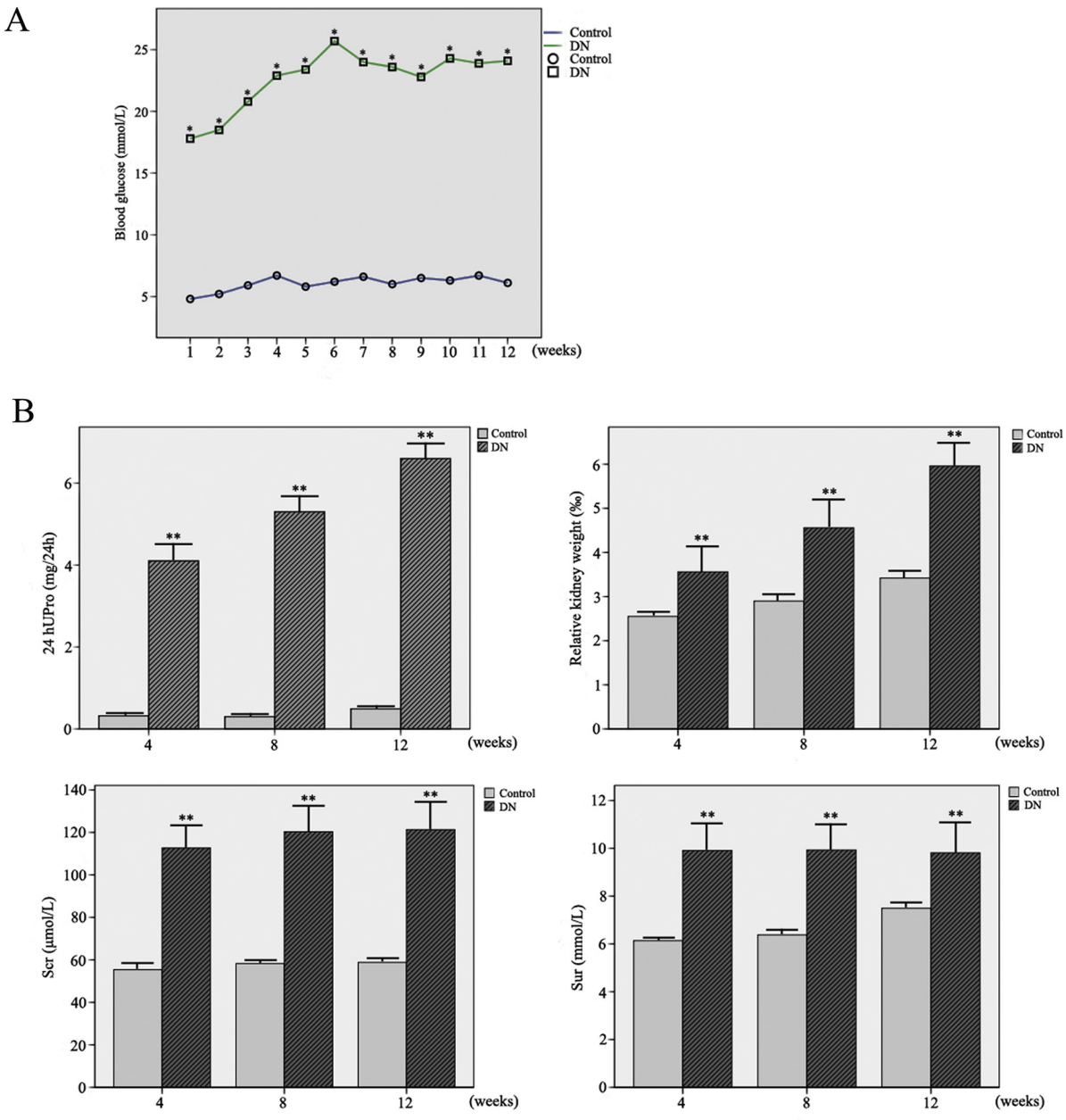

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Fig. 3. Physiological and biochemical indicators of STZ-induced DN model rats. The changes of blood glucose level (A), 24-hour urinary albumin, relatively kidney weight, Scr, Sur (B) and renal pathological change by H&E-staining (C) during the development of STZ-induced DN model rats. Each value indicates the mean ± SEM for six rats. *Significant difference at $P < 0.05$ compared with control groups. *Scale bar* column 100 μm.

3.4. Physiological and biochemical indicators of STZ-induced DN model rats

To study the role of ORP150 in DN, we established the STZ-induced DN model, which was a well-established model of DN resembling several prominent clinical and morphological features of human DN. Following STZ and HFD treatment, we performed blood glucose analysis to confirm the efficiency of the STZ-induced DN rat models. We found that the blood glucose level in diabetic nephropathy rats was increased comparable with the control (Fig. 3A). Values of 24-hour urinary albumin, relatively kidney weight, Scr, and Sur were significantly higher in the DN groups than in the control groups ($P < .05$) (Fig. 3B). Moreover, H&E staining showed that after 8 weeks of drug intervention, the mesangial cell, glomerular sclerosis and mesangial matrix hyperplasia occurred in the DN group and the degree of renal pathological change in the control group was significantly relieved compared with that in the observation group (Fig. 3C).

3.5. Serum ORP150 level in STZ-induced DN model rats

The relative serum levels of ORP150 protein in the observation group at 4, 8 and 12 weeks after intervention were significantly higher than those in the control group ($P < .05$) (Fig. 4A). Moreover, a strongly positive correlation was found between STZ-induced rats serum levels of ORP150 and VEGF ($r = 0.69$; $P = .001$) (Fig. 4B). The results of gel electrophoresis of RT-PCR products are shown in Fig. 4C. In a word, ORP150 and VEGF were increased and positive correlation significantly in DN rats serum, which were consistent with patients with DN.

3.6. Monoclonal anti-ORP150 antibody-treated model rats

DN animals had significantly higher plasma glucose levels, Scr, Sur, relatively kidney weight and VEGF as compared with the age-matched control rats ($P < .05$) (Table 3). There were no differences in the above indicators between the Ab-treated and the untreated DN rats. The levels of ORP150, Scr, Sur, and the relatively kidney weight in the DN animals that were treated with anti-ORP150 Ab tended to be lower than in the other DN groups, and the difference were effective ($P < .05$) (Table 3). More importantly, the serum levels of VEGF was approximately 50% lower in DN animals with anti-ORP150 Ab as compared with other DN groups ($P < .05$) (Table 3), indicating ORP150 exaggerating the secretion of VEGF.

Based on the above data, we studied whether the down-regulation of ORP150 can retard albuminuria via VEGF pathway. As shown in Fig. 5, untreated DN rats showed a marked elevation of the 24 h urine albumin after 12 weeks of DN, which was partially reduced by anti-ORP150 treatment but not by administration of control Ab. In short, the up-expression of ORP150 involved in proteinuria burden through VEGF secretion.

4. Discussion

The pathogenesis of diabetic nephropathy (DN) is still unclear. Currently, it is believed that the occurrence of DN is complicated, involving genetics, physics, chemistry, and environmental factors. Understanding the appearance of proteinuria is the key to reveal the etiology and pathogenic mechanisms of DN. Moreover, alleviating albuminuria could provide a new solution to reduce the complications resulting from diabetic kidney disease. Some recent studies have shown that there is an elevated serum level of VEGF within DN patients. The

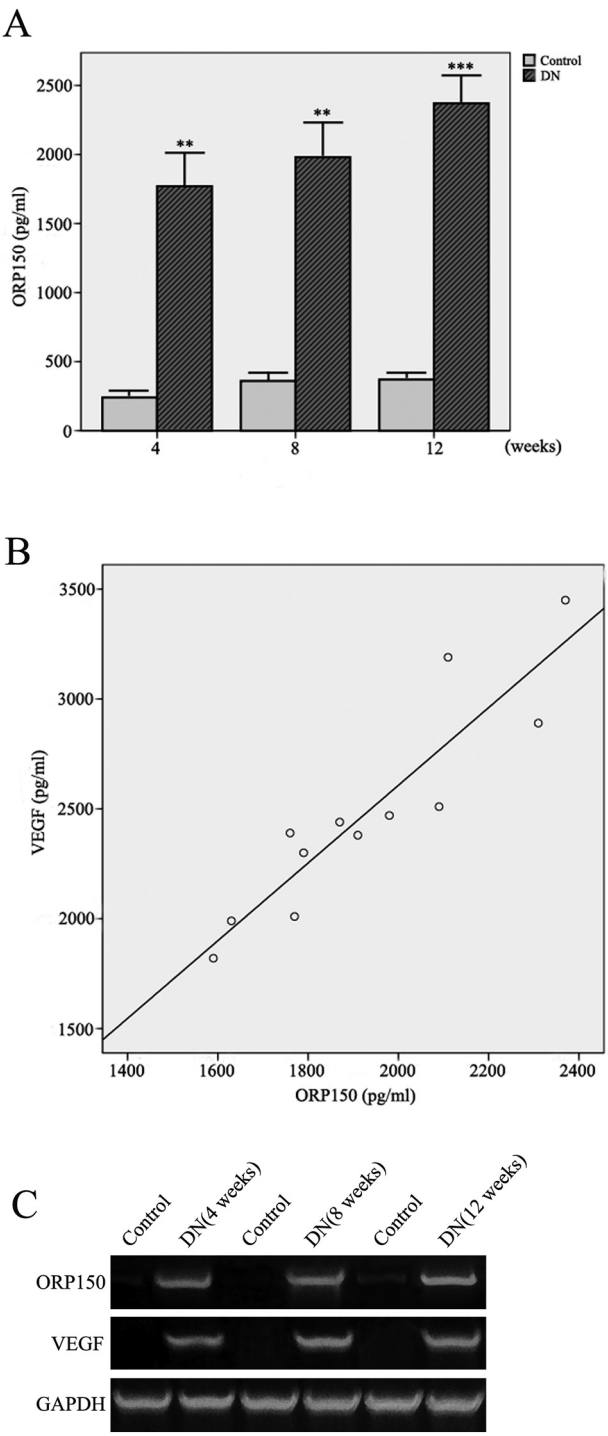

Fig. 4. Serum ORP150 level in STZ-induced DN model rats. (A) The serum levels of ORP150 protein was analysed by ELISA. (B) The correlation was analysed between STZ-induced rats serum levels of ORP150 and VEGF. (C) Gel electrophoresis results of RT-PCR amplifcation of ORP150 and VEGF mRNA in rats in both groups. *Significant difference at $P < 0.05$ compared with control groups.

correlation between DN and VEGF level is a popular research topic because VEGF raises the permeability of vascular endothelial cells and breaks the glomerular filtration barrier (Sun et al., 2014). VEGF can

Table 3
Gravimetric and biochemical characteristics of the experimental groups.

	Control (n = 6)	Control + anti-ORP150 Ab (n = 6)	DN (n = 6)	DN + anti-ORP150 Ab (n = 6)	DN + control Ab (n = 6)
ORP150 (pg/ml)	411.72 ± 14.92	398.68 ± 15.77	2350.71 ± 28.12*	854.43 ± 30.74*	2442.34 ± 31.56*
Glycemia (mmol/L)	6.07 ± 0.17	6.19 ± 0.16	22.65 ± 0.69*	19.82 ± 0.18*	21.25 ± 0.69*
Scr (μmol/L)	54.14 ± 1.50	55.12 ± 1.22	115.61 ± 2.97*	68.82 ± 1.18*	118.68 ± 2.07*
Sur (mmol/L)	6.62 ± 0.16	6.48 ± 0.14	9.71 ± 0.08*	8.02 ± 0.15*	9.90 ± 0.05*
KW/BW (‰)	2.79 ± 0.11	3.12 ± 0.14	4.25 ± 0.28*	3.16 ± 0.10*	4.66 ± 0.22*
VEGF (pg/ml)	420.83 ± 15.88	443.89 ± 14.95	2486.67 ± 39.32*	1084.44 ± 31.58*	2502.22 ± 30.74*

Scr - serum creatinine; Sur - serum urea nitrogen; KW/BW - kidney weight/body weight; VEGF - vascular endothelial growth factor; Ab - antibody.
* $P < .001$ versus controls.

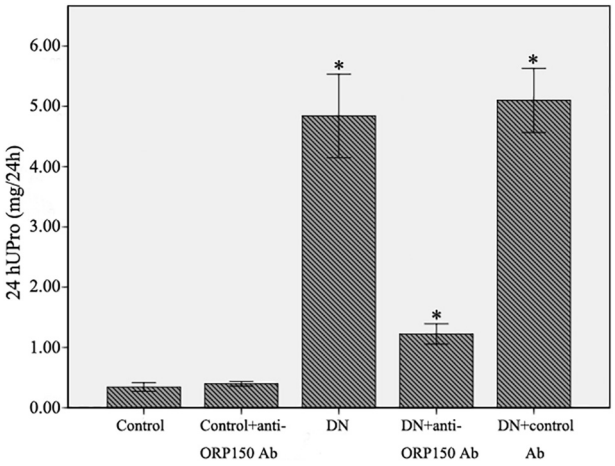

Fig. 5. The changes of 24 h urine albumin in monoclonal anti-ORP150 antibody-treated model rats and control antibody group. *Significant difference at $P < 0.05$ compared with control groups.

also aggravate renal tubular basement membrane thickening, renal interstitial fibrosis, and glomerular sclerosis, leading to renal function damage and albuminuria (de Vriese et al., 2001; Leung et al., 2016; Ma et al., 2015). There are some suggestions that VEGF is a marker of diabetic kidney disease in T2DM patients. Our research also showed significant differences in the serum level of VEGF between the control group and case group. All these researches further demonstrate that VEGF plays an important role in DN progression.

The 150-kDa oxygen-regulated protein (ORP150) is a member of the heat shock protein family located in the ER, a molecular chaperone protein, which is necessary to the folding and transport of newly synthesized proteins. ORP150 involved in regulating cell apoptosis and is required for cells to survive prolonged hypoxia (Cechowska-Pasko et al., 2006; Ozawa et al., 1999). ORP150 is upregulating expressed in a wide range of various pathologic situations, such as ischaemic retina (Kim et al., 2012), ischaemic brain (Kuwabara et al., 1996; Matsushita et al., 1998), atherosclerotic plaques (Tsukamoto et al., 1996), malignant tumors (Miyagi et al., 2002), and wound healing (Ozawa et al., 2001a). Moreover, the present study supports that up-regulation of ORP150 in DN patients serum facilitates the production of proteinuria. Using a rat DN model of STZ-induced that mimics human DN, the present study analyzed ORP150 and renal function changes in DN rats. The results confirmed that glycemia, Scr, Sur, and 24 h proteinuria expression levels were obviously elevated following renal function damage in DN rats. We also discovered that ORP150 mRNA and protein levels were significantly over-expression, companied by a concomitant increasing of proteinuria. Furthermore, treating with anti-ORP150 Ab of STZ-induced DN models could tremendously decrease the levels of serum creatinine (Scr), urea nitrogen, and 24 h urine protein, indicating that ORP150 might alleviate proteinuria in DN.

The molecular mechanism that ORP150 increases proteinuria in DN remains to be explored. ORP150 plays a critical role in the post-translational regulation of secretory proteins, such as VEGF, TGF-β (Ozawa et al., 2001a; Ozawa et al., 2001b). Optimal function of ORP150 is essential for secretion of mature VEGF. Increased levels of ORP150 promotes VEGF processing with subsequent transport from endoplasmic reticulum to the Golgi compartment, followed by export out of the cell (Ozawa et al., 2001a; Ozawa et al., 2001b). In addition, the increase of ORP150 in the ocular microenvironment might contribute to the initiation and progression of angiogenesis associated with proliferative diabetic retinopathy through VEGF (Abu El-Asrar et al., 2018). Based on the key role of VEGF in the pathogenesis of DN, we hypothesized up-regulation of ORP150 might accelerate secretion of VEGF to increase production of proteinuria. Here, we detected simultaneous expression of ORP150 and VEGF in the serum from patients with DN. Moreover, a significant positive correlation between the levels of ORP150 and the levels of VEGF was observed. Surprisingly, there was a significant decrease in the serum concentration of VEGF after the treatment of anti-ORP150 antibody in the STZ-induced DN rat model. All of our data suggested that in DN, up-regulation of ORP150 might accelerate secretion of VEGF and thus sharpen proteinuria, which should drive DN progression. Considering a relatively higher risk of developing DN, we speculated that ORP150 can become a target for therapy on different stages of the disease. Moreover, the exact functions of ORP150 might greatly depend on its specific molecular partners under certain pathological conditions.

Taken together, our study demonstrated for the first time that the serum level of ORP150 was significantly up-regulated in DN. Both in DN patients and rat models are compatible with the hypothesis that ORP150 can identify as a target of therapy via suppressing secretion of VEGF. Further research is needed to demonstrate the exact activities and detailed molecular mechanisms of ORP150 in the glomerular filtration barrier and DN development.

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Disclosure

No other potential conflicts of interest relevant to this article were reported.

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