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Protective effect of umbilical cord mesenchymal stem cells combined with resveratrol against renal podocyte damage in NOD mice

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ABSTRACT

Background: The role of chronic inflammation initiated by persistent hyperglycemia in podocyte injury has attracted increasing attention. The advanced glycation end products (RAGE) receptor- nuclear factor-kappa B (NF-κB) signaling pathway is involved in the occurrence of inflammation. We speculate that treatment with human umbilical cord mesenchymal stem cells (hUCMSCs) combined with resveratrol can block this signaling pathway and protect podocyte function.

Methods: Non obesity diabetes(NOD) mice were randomly divided into 5 groups: NOD-T1DM, Res, hUCMSCs, hUCMSCs + Res and insulin (INS)groups. Mice without diabetes were classified as NOD control group(NOD group). Blood glucose(BG), blood urea nitrogen(BUN), serum creatinine(SCr), 24-h urine albumin excretion rate (UAER) were measured. The expression of nephrin, WT1 and RAGE, MCP-1 in renal tissues were detected by Western blot, expression of NF-κB protein(P65) was determined by immunohistochemistry.

Results: The combined treatment of hUCMSCs and Resveratrol can reduce BG, BUN, SCr, 24-h UAER, and the expression of the inflammatory factors MCP-1, RAGE and NF-κB; increase the number of podocytes and the expression of the podocyte-related proteins nephrin and WT1 in type 1 diabetes mellitus, and improve renal pathological structure.

Conclusions: Combining of hUCMSCs and resveratrol can better protect renal podocyte function, and the effects on the reduction of blood glucose and renal injury are better than those obtained by insulin treatment. This indicated that the combination of Res and hUCMSCs may be a novel therapeutic method for the treatment of DN.

1. Introduction

Diabetic nephropathy (DN) is the most common fatal complication of diabetes and the most important cause of both glomerulosclerosis and end-stage renal disease (ESRD) [1]. Approximately 30–40% of patients with diabetes develop DN, and the main clinical manifestation is proteinuria [2]. DN has become a chronic disease that not only seriously

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threatens the health of individuals but also imposes enormous medical and socioeconomic burdens.

The pathogenesis of DN is still unclear. Recent studies have shown that changes in podocyte structure and a decrease in podocyte number are important causes of DN-induced proteinuria [3]. The role of chronic inflammation initiated by persistent hyperglycemia in podocyte injury has attracted increasing attention. Podocytes are the main component of the glomerular filtration barrier (GFB) [4]. Podocyte injury leads to impaired GFB integrity and is closely associated with diffuse glomerular sclerosis and ESRD [5,6]. Persistent hyperglycemia can lead to an increase in advanced glycation end products (AGEs). The advanced glycation end product receptor (RAGE) is a key receptor for AGE signal transduction. It can produce oxidative stress by binding AGEs, activate downstream inflammatory response signaling pathways, and cause changes in kidney structure and impairment of renal function [7,8].

Therefore, blocking the inflammatory response pathway induced by AGEs-RAGE is a promising method to treat DN. Aminoguanidine is a well-studied AGE inhibitor, but serious adverse reactions have been found in phase III clinical trials; thus it cannot be applied in clinical practice. At present, clinical treatment cannot prevent the progression of DN. Therefore, a regeneration strategy is urgently needed. Treatment aimed at preventing or limiting podocyte damage and/or promoting podocyte repair or regeneration has significant clinical and economic potentia [9,10].

Studies have shown that human umbilical cord mesenchymal stem cells (hUCMSCs) can be induced to differentiate in vitro into islet-like cells, tubular cells, and vascular endothelial cells, among others [11]. Therefore, mesenchymal stem cell transplantation has been the focus of research for the treatment of diabetes and its complications.

Resveratrol (Res) is a common polyphenolic substance that is widely present in grape skins, grape seeds, nuts and other plants. Res has many functions, such as cardiovascular protection, protection against antioxidant stress, anti-inflammatory effects, anti-tumor effects, and regulation of blood glucose metabolism [12]. Studies have shown that in rats with streptozotocin (STZ)-induced diabetes and db/db mice, Res intervention can reduce proteinuria and improve renal function, but the target and mechanism of Res are not yet completely clear [13,14].

In this study, we used hUCMSCs combined with Res to treat mice with nonobese diabetes(NOD mice) and then observed subsequent changes in the blood glucose and urine albumin levels, determined the expression of inflammatory factors, such as monocyte chemoattractant factor-1 (MCP-1), RAGE and nuclear factor-kappa B (NF-κB), and examined the expression of the podocyte-associated molecules nephrin and Wilms' Tumor gene-1 (WT1). This study was performed to explore whether combined treatment with hUCMSCs and Res can reduce podocyte damage by inhibiting the RAGE-NF-κB signaling pathway, which may provide a new target for the treatment of DN.

2. Materials and methods

2.1. hUCMSCs preparation

hUCMSCs were derived from the Stem Cell and Transformation Medicine Laboratory of the Affiliated Hospital of Qingdao University. The application of hUCMSCs was approved by the hospital's Medical Ethics Council (2010KEYANSHEN No. GAN-5).

The hUCMSCs cultured to P3 generation were digested with trypsin, centrifuged at 3000 rpm for 10 min, discarded the supernatant, washed with PBS and mixed, the cells were counted on the cell counting board to ensure the number of cells in each tube was no less than 1×10^5, and then placed the tubes into centrifuge, repeated the procedure for 2 times. After the last mixing, 100 μl of PBS containing 1% bovine serum albumin was added, then anti-humanCD34-PEantibody, anti-human CD45-PE antibody, anti-human CD14-PE antibody, anti-human CD73-PE antibody, anti-human CD90-PE antibody, anti-human CD105-PE antibody were added and was mixed with anti-human HLA-DR-PE antibody. The tubes was incubated at 4 °C for 20–30 min in the dark and mixed the contents every 5 min. After removing the centrifuge tubes, added 1 ml PBS, centrifuged at 4500 rpm for 5 min and discarded the supernatant. After adding 400 μl of PBS, each tube was re-suspended and filtered into the flow tube, then detected by the becton dickinson calibur. The results were analyzed by BD Accuri C6 Software.

2.2. NOD mice

This study was carried out in strict accordance with the relevant standards of NIH publication No. 85-23 proposed by the National Institutes of Health of the United States (Laboratory Animal License Number G20160318). A total of 100 healthy 6- to 8-week-old female NOD mice weighing from 20 to 24 g were purchased. The experimental animals were raised in the specific pathogen-free (SPF) mouse feeder room of the animal laboratory of the Affiliated Hospital of Qingdao University at a temperature of approximately 25 °C and humidity of 60% under a 12-h light cycle (6:00 a.m. to 6:00 p.m.). The mice were housed with 4–5 mice per cage and were tagged using picric acid. The mice were allowed ad libitum access to SPF-grade (irradiated with cobalt-60) food and autoclaved water during the experimental maintenance period.

2.3. Experimental design

Starting after one week of adaptive feeding, the mice were weighed at a regular time (2:00 p.m.) once a week. Blood was collected from the tail vein to obtain random blood glucose values using the Johnson & Johnson One Touch Ultra system (Johnson and Johnson Ltd., Milpitas, CA, USA). When two consecutive tests showed a blood glucose level $\geq$16.6 mmol/L, the mouse was diagnosed with type 1 diabetes (T1DM). A total of 63 T1DM mice were then randomly divided into the following groups:

(1) NOD-T1DM group (15 mice): no interventions after diabetes onset.
(2) INS group (12 mice): As an insulin control group, insulin glargine (Lantus; sanofi-aventis, Paris, France) was injected subcutaneously once a day (0.5 U/d, the dosage was adjusted according to the blood glucose level) after diabetes onset.
(3) Res group (12 mice): From the 3rd day after the onset of diabetes mellitus, mice were administered 200 mg/kg * d Res by gavage once a day until the end of the experiment.
(4) hUCMSCs group (12 mice): 1×10^6 hUCMSCs suspended in 0.3 ml of phosphate-buffered saline (PBS) was injected into the tail vein on the 3rd day after diabetes onset(only once).
(5) hUCMSCs + Res group (12 mice): 1×10^6 hUCMSCs suspended in 0.3 ml of PBS was injected into the tail vein on the 3rd day after diabetes onset(only once), and 200 mg/kg * d Res was administered by gavage once a day until the end of the experiment.

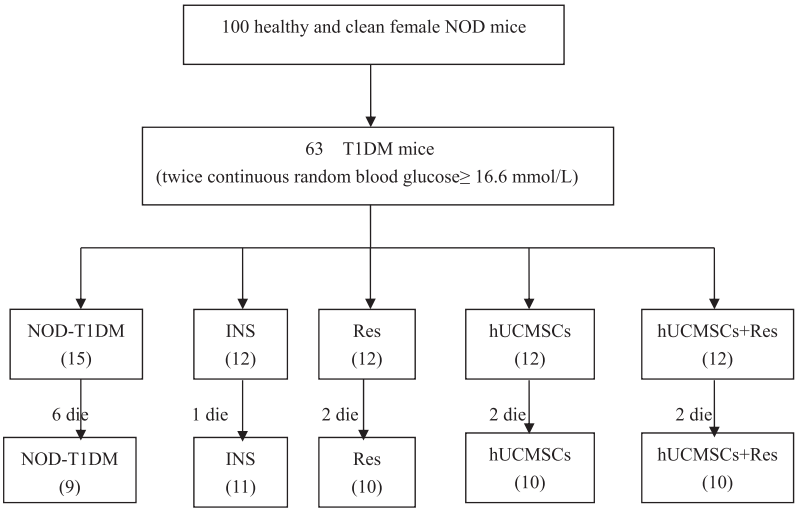
The mice not exhibiting diabetes (fed blood glucose ≤10 mmol/l) were classified as NOD control group (NOD group). The day of diabetes onset was recorded as the first day (0 w). Weight and blood glucose were measured every two weeks in mice. During the experiment, the Res group, hUCMSCs group and hUCMSCs + Res group were given low-dose insulin glargine (0–1 U/day) to prevent severe infection and diabetic ketoacidosis. To the end of the experiment, 6 mice died in the NOD-T1DM group, 1 mouse died in the INS group, and 2 mice each died in the Res group, hUCMSCs group and hUCMSCs + Res group. The number of mice in the NOD group was 9.

2.4. Collection of blood, 24 h urine samples and tissue specimens

Urine samples were taken 24 h before the end of the experiment. In order to prevent the effects of feces and food on urinary protein, mice were put into metabolic cages (Shanghai, China) and fasting immediately. Urinary albumin excretion rate (24 UAER) was measured by enzyme-linked immunosorbent assay (ELISA, Shanghai Enzyme-linked Biotechnology Co., Ltd. China). After fasting for 8–10 h, blood was collected from the inner canthus, and BUN and SCr were measured by Hitachi 7600 automatic biochemical analyzer (Hitachi Limited, Japan) after centrifugation. After blood samples were obtained, the mice were anesthetized with a peritoneal injection of 10% chloral hydrate, and the kidneys were dissected lengthwise. Some of the kidneys were fixed in 4% paraformaldehyde for PAS staining and immunohistochemical staining, some of them were fixed in 2.5% glutaraldehyde for electron microscopic observation. Another part of the kidney was placed in the EP tube and stored at −80 °C for Western blot analysis.

2.5. Podocyte counting method

5 μm thick sections were stained with periodic acid-Schiff (PAS) reagent. In each PAS-stained section, 10 glomeruli in a high-power field (×400) were randomly selected by light microscopy, and the glomerular area (μm^2) was measured using MCID Analysis Evaluation 7.0 image analysis software; the number of podocytes in each measured glomeruli were counted at the same time. The number of glomerular podocytes per 1000 μm^2 area in each glomerulus was calculated, and the mean value was used to represent the number of glomerular podocytes [15].



2.6. Transmission electron microscopy (TEM) examination

The tissue was prefixed with glutaraldehyde and postfixed with osmic acid, followed by dehydration, infiltration, embedding, ultrathin sectioning, double staining with acetate axis and lead citrate, and observation by TEM (×10,000). Glomerular basement membrane thickness (GBMT) and foot process fusion rate (FPFR) were measured with Medical Image System 6.0.

2.7. Western blot analysis for MCP-1, RAGE, nephrin, WT1, NF-κB(P65)

100 g of kidney tissue was shredded, placed in precooling lysate buffer, homogenized on ice, and centrifuged at 12,000 r/min for 5 min at 4 °C; the protein concentration was measured from the supernatant. Following preparation of 8% separation and 4% stacking gels, the samples were loaded, electrophoresed, and transferred to a polyvinylidene fluoride membrane. After incubating with diluted rabbit anti-mouse primary antibody (MCP-1 1:5000, RAGE 1:1000, nephrin 1:1000, WT1 1:2000, NF-κB(P65) 1:1000, Abcam, UK) at 4 °C for overnight, and then incubated at 37 °C for 1 h. The secondary antibody of goat anti-rabbit (Bioswamp, Shanghai, China) labeled with horseradish peroxidase was diluted at 1:2000. After rinsing the film, develop the color and expose the sample to the darkroom and fix it. The images were observed and analyzed by Image Pro-Plus 6.0 software.

2.8. Statistical analysis

All quantitative experimental data were analyzed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The data are expressed as the mean ± standard deviation ($\bar{x}$±S). Differences among groups were analyzed by one-way ANOVA, and LSD analysis was used to compare the two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characterization tests for hUCMSCs

Cell surface markers of P3 generation hUCMSCs were identified by flow cytometry: CD105 expression was positive and the positive rate was 99.3%; CD90 expression was positive and the positive rate was 96.3%; CD73 expression was positive and the positive rate was 96.7%; CD34 expression was negative and the negative rate was 0.1%; CD45 expression was negative and the negative rate was 0.1%; CD14 expression was negative and the negative rate was 0.3%; HLA-DR expression was negative and the negative rate was 0.1%. hUCMSCs highly expressed mesenchymal stem cell surface markers instead of hematopoietic stem cell surface markers, which in accordance with the current international definition of MSCs (Fig. 1).

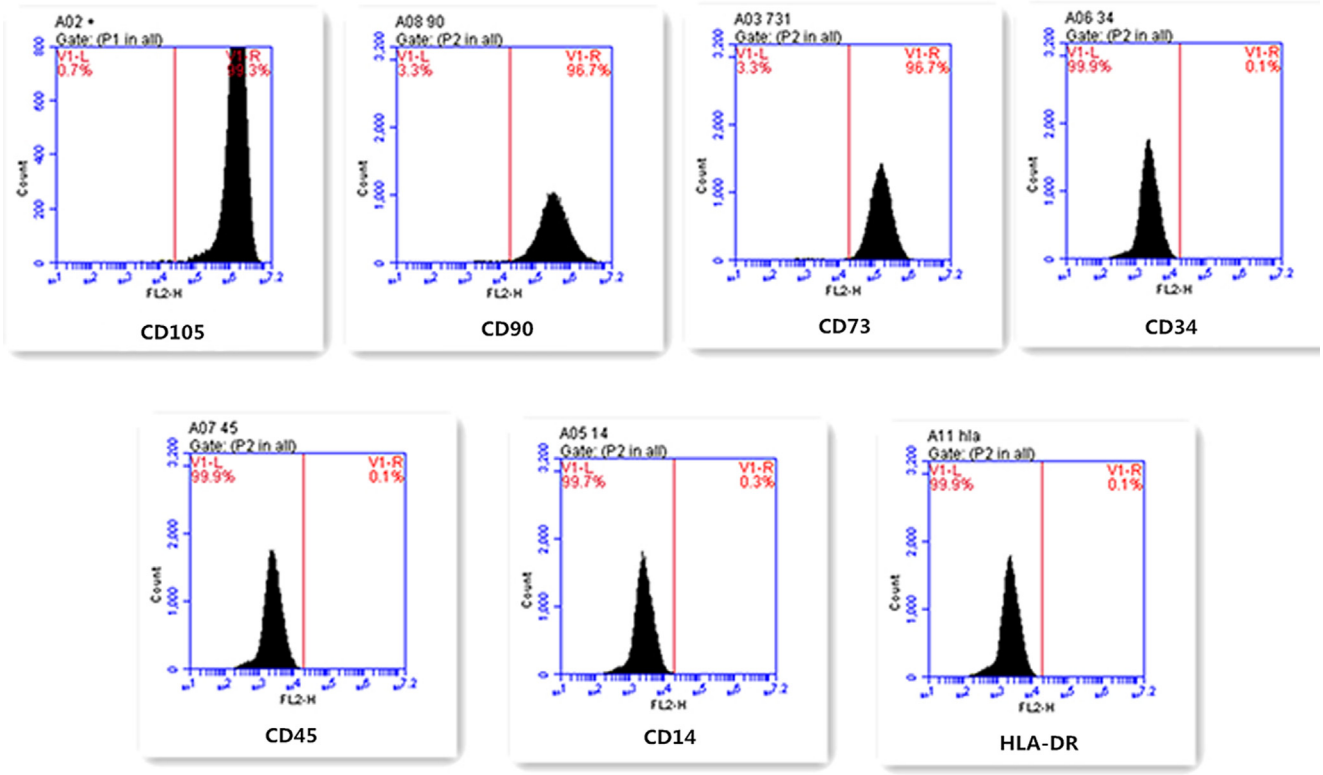


Fig. 1 – The surface markers and multi-directional differentiation of hucMSCs. The cell surface markers were identified by flow cytometry: CD105, CD90 and CD73 were expressed, the positive rates were 99.3%, 96.3% and 96.7% respectively; CD34, CD45 and CD14 were not expressed, the expression rates were 0.1%, 0.1% and 0.3% respectively; HLA-DR was not expressed, the expression rate was 0.1%.

Table 1 – Weight changes in each group of mice unit: g.

Group	n	0w	2w	4w	6w	8w
NOD	9	24.34 ± 0.85	25.14 ± 0.96	26.10 ± 1.07	27.32 ± 1.30	28.66 ± 1.34
NOD-T1DM	9	25.17 ± 1.19	23.92 ± 1.33[a]	22.79 ± 1.70[a]	21.11 ± 1.83[a]	19.23 ± 1.58[a]
INS	11	25.27 ± 1.39	24.69 ± 1.06[b]	25.10 ± 1.11[b]	24.84 ± 1.80[a,b]	24.70 ± 3.31[a,b]
Res	10	24.68 ± 1.03	23.60 ± 1.21[a]	22.88 ± 1.64[a]	24.26 ± 1.25[a,b]	23.88 ± 2.69[a,b]
hUCMSCs	10	25.54 ± 1.36	24.39 ± 1.12	25.01 ± 1.40[b]	25.61 ± 1.86[a,b]	23.75 ± 2.54[a,b]
hUCMSCs + Res	10	26.07 ± 1.45	23.93 ± 1.92	24.69 ± 2.26[b]	25.36 ± 1.93[a,b]	26.92 ± 2.32[b]

[a] Compared with NOD group, P < 0.05.
[b] Compared with NOD-T1DM group, P < 0.05.

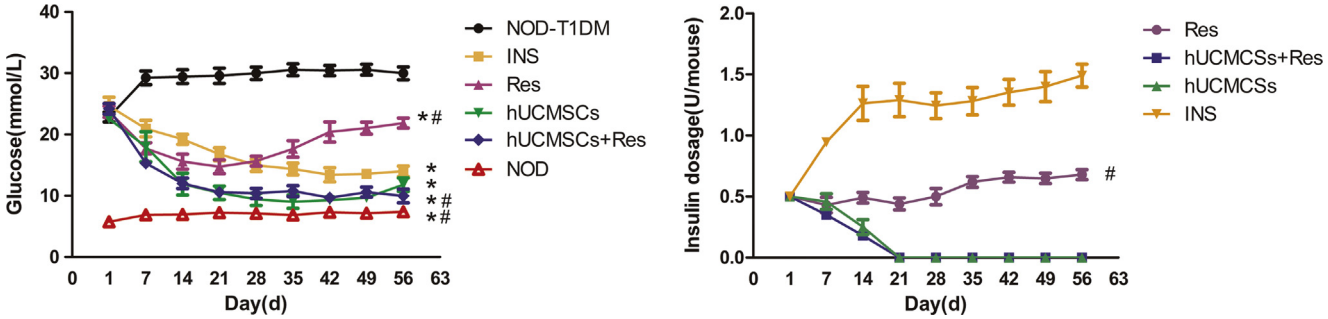


Fig. 2 – Blood glucose level and insulin dose in mice. Blood glucose in the INS group, hUCMSCs group and hUCMSCs + Res groups was decreased compared with the NOD-T1DM. The blood glucose in the Res group was lower compared with the NOD-T1DM group, yet higher compared with the INS, hUCMSCs group and hUCMSCs + Res group. The dosage of insulin required in the hUCMSCs group and hUCMSCs + Res group was decreased compared with the INS group and Res group. *P < 0.05 vs. the NOD-T1DM group; #P < 0.05 vs. the INS group.

3.2. *General condition and weight of the mice*

In the NOD group, the mice had soft fur and no significant changes in diet, drinking water and voiding. Mice in the NOD-T1DM group were significantly thinner, their fur was shiny and yellow, their food and water intake increased, their urine volume increased, and their urine showed a darker color. Mice in the four treatment groups showed an obviously improved fur color and mental status.

At 0 w, the mice in the six groups were similar in weight. At 8w, the weight of mice in NOD-T1DM group was significantly lower than that in the other five groups (P < 0.05). The weight of mice in the four treatment groups was significantly higher than that in NOD-T1DM group (P < 0.05). The weight of mice in hUCMSCs + Res group was close to that in NOD group, but there was no significant difference (P > 0.05) (Table 1).

3.3. *Blood glucose level of the mice*

At 0 w, the blood glucose levels of the five experimental groups were significantly higher than those of the NOD group (P < 0.05), and there was no significant difference among the five groups (P > 0.05). At 8w, the blood glucose levels of Res group, hUCMSCs group, hUCMSCs + Res group and INS group decreased compared with that of 0 w, but the blood glucose levels of Res group were still higher, and there was no significant difference between hUCMSCs + Res group and NOD group (P > 0.05) (Fig. 2).

Insulin glargine was given to the four treatment groups to control blood glucose. On the first day, the insulin dosage was all 0.5 U, and then adjusted the dosage according to the blood glucose. The results showed that the dosage of insulin decreased gradually in hUCMSCs group and hUCMSCs + Res group, and insulin therapy was stopped after 2w. Insulin dosage in INS group gradually increased to 1.46 ± 0.31 U and that in Res group was 0.68 ± 0.13 U at 8w (Fig. 2).

3.4. *Analysis of BUN, SCr and 24 h UAER of the mice*

Compared with the NOD group, the other five groups showed significantly increased BUN, SCr and 24 h UAERs (P < 0.05). Compared with the NOD-T1DM group, there was significant decrease after the Res and hUCMSCs treatments. The hUCMSCs + Res group showed the most significant decrease in the BUN, SCr and 24 h UAER (P < 0.05) (Table 2).

3.5. *Podocyte count*

Compared with the NOD group, the podocyte count was significantly decreased in the NOD-T1DM group, and was increased in the four treatment group. The hUCMSCs + Res group showed the most significant increase (P < 0.05) (Table 2).

Table 2 – Comparison of BUN, SCr, 24hUAER and podocyte number, GBMT and FPFR in each group.

Group	n	BUN (mmol/L)	SCr (umol/L)	24 h UAER (mg/24 h)	Podocyte number (/1000 μm^2)	GBMT (nm)	FPFR (%)
NOD	9	6.8 ± 1.42	22.0 ± 6.44	5.8 ± 3.17	22.3 ± 2.35	135.7 ± 14.92	0
NOD-T1DM	9	27.0 ± 3.59[a]	78.8 ± 11.71[a]	72.8 ± 11.2[a]	6.8 ± 1.86[a]	268.8 ± 28.76[a]	72.63 ± 8.09[a]
INS	11	19.5 ± 3.74[a,b]	47.2 ± 6.65[a,b]	27.5 ± 7.60[a,b]	12.9 ± 1.92[a,b]	202.3 ± 21.9[a,b]	49.89 ± 9.29[a,b]
Res	10	20.8 ± 3.19[a,b]	49.6 ± 8.04[a,b]	31.8 ± 6.66[a,b]	11.9 ± 1.91[a,b]	209.0 ± 18.41[a,b]	52.36 ± 7.79[a,b]
hUCMSCs	10	13.7 ± 2.71[a,b,c]	40.0 ± 7.10[a,b,c]	16.7 ± 4.8[a,b,c]	16.1 ± 2.02[a,b,c]	190.7 ± 19.64[a,b]	45.31 ± 6.62[a,b]
hUCMSCs + Res	10	12.9 ± 2.30[a,b,c]	31.8 ± 6.49[a,b,c]	11.8 ± 4.43[a,b,c]	19.2 ± 1.93[a,b,c]	177.4 ± 18.05[a,b,c]	39.08 ± 5.35[a,b,c]

[a] Compared with NOD group, $P < 0.05$.
[b] Compared with NOD-T1DM group, $P < 0.05$.
[c] Compared with INS group, $P < 0.05$.

3.6. *Electron microscopy*

Fig. 3 showed that, the basement membrane in NOD group had clear structure, evenly and equally distributed, complete foot process and almost had no foot process fusion. Compared with NOD group, the glomerular capillary basement membrane in NOD-T1DM group was blurred, showed irregular thickening, foot process destroy, fusion and even effacement. The foot processes fusion was also seen in each treatment group, but there was significantly improvement compared with the NOD-T1DM group ($P < 0.05$), and the hUCMSCs + Res group exhibited the least damage. Glomerular basement membrane thickness (GBMT) and foot process fusion rate (FPFR) were shown in Table 2.

3.7. *Western blot analysis*

Compared with NOD group, the expressions of MCP-1, RAGE and NF-κB(p65) in NOD-T1DM group were significantly increased ($P < 0.05$), the expressions were increased 2.3, 2.5 and 1.96 times respectively, and the expressions of Nephrin and WT1 were significantly decreased ($P < 0.05$), 68% and 66% respectively. The treatment group improved compared with the NOD-T1DM group, and the difference was statistically significant ($P < 0.05$). The expression levels of MCP-1, RAGE and NF-κB(p65) protein in hUCMSCs + Res group were the lowest, Nephrin and WT1 protein were the highest, followed by hUCMSCs group (Fig. 4).

4. Discussion

Podocyte damage is considered to be the main feature of proteinuria caused by DN [16,17]. After podocyte damage occurs, extracellular matrix deposition increases, and the remaining normal podocytes can only cover the exposed basement membrane by continuously extending their foot processes to reduce damage to the filtration barrier. The imbalance

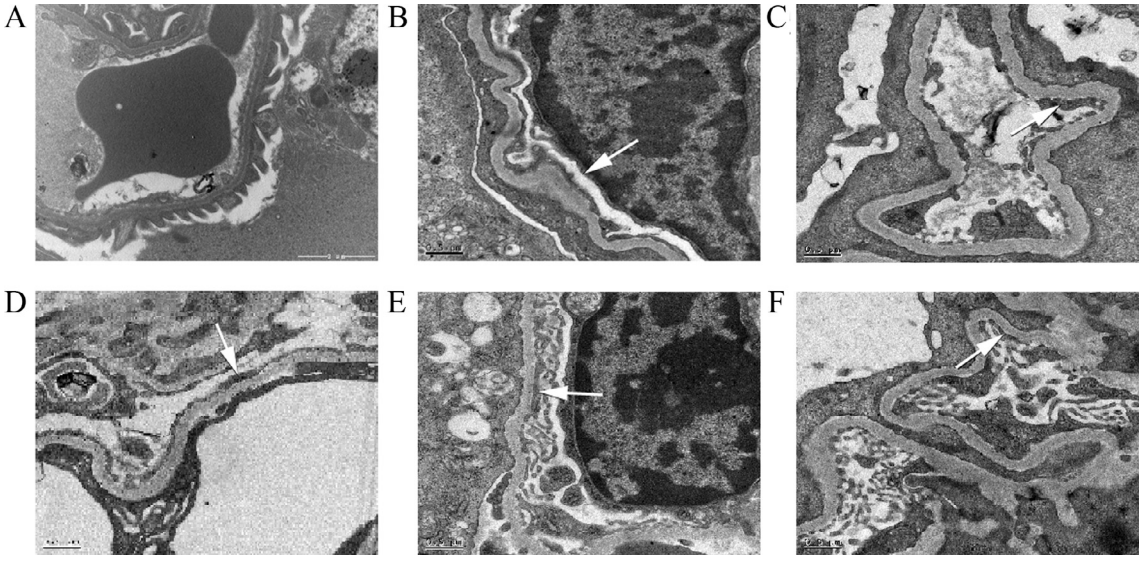


Fig. 3 – Electron microscopy of the mice kidney(×10,000). A: NOD group; B: NOD-T$_1$DM group; C: INS group; D: Res group; E: hUCMSCs group; F: Res + hUCMSCs group. The basement membrane in NOD group had clear structure, evenly and equally distributed, complete foot process. Compared with NOD group, the glomerular capillary basement membrane in NOD-T1DM group was blurred, showed irregular thickening, foot process destroy, fusion and even effacement. The foot processes fusion was also seen in each treatment group, but there was significantly improvement compared with the NOD-T1DM group ($P < 0.05$). Note: "→" indicate fusion of foot processes.

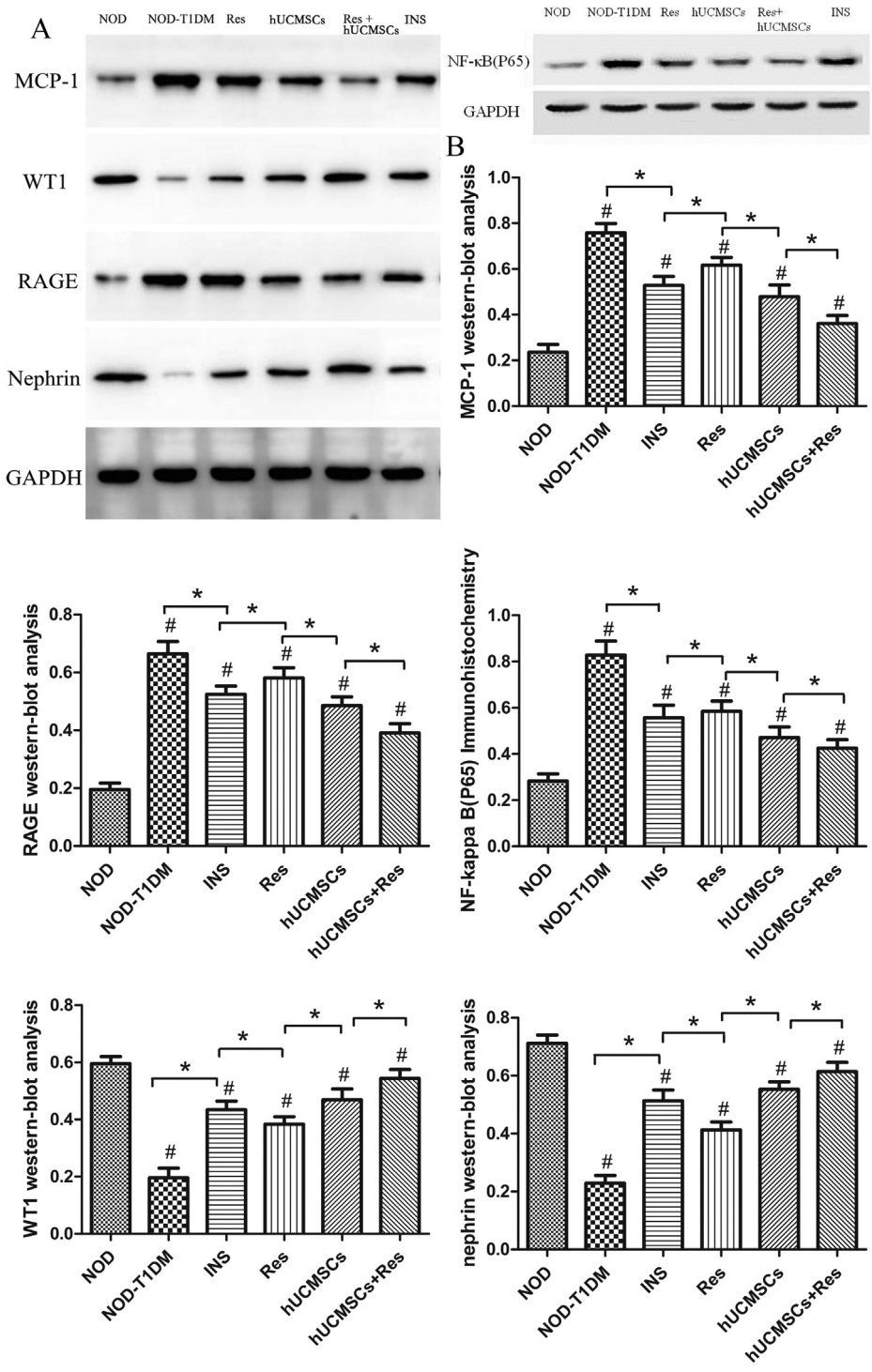


Fig. 4 – Western blot analysis of MCP-1 , RAGE, nephrin, WT1, NF–κB(p65). The expressions of MCP-1, RAGE and NF–κB(p65) in NOD-T1DM group were increased 2.3, 2.5 and 1.96 times respectively, and the expressions of Nephrin and WT1 were decreased 68% and 66% respectively. The treatment group improved compared with the NOD-T1DM group ($P < 0.05$). Note: comparison between the two groups: $^{*}P < 0.05$; compared with group NOD: # $P < 0.05$.

inherent in this compensatory response leads to glomerulosclerosis [18].

Studies have shown that decreases in podocyte number and in the podocyte-related molecule nephrin are two key factors in the progression of DN-associated proteinuria [19]. Nephrin is localized in the slit diaphragm of the foot process of podocytes and plays a key role in maintaining the integrity of the GFB. WT1 is specifically expressed in the podocyte nucleus and indirectly reflects the number of podocytes in the glomerulus [20,21]. Studies have shown that the expression of nephrin and the WT1 transcription factor in glomeruli is significantly decreased in mice with DN [22]. Therefore,

restoring the podocyte number and recovering the normal expression of podocyte-associated proteins can stabilize the permeability of glomerular filtration membranes and reduce proteinuria, thereby preventing or treating DN.

Many injury-related factors, such as immunity, inflammation, infection, metabolism and the environment, can directly or indirectly affect podocytes. Inflammation plays an increasingly important role in podocyte injury. Many studies have confirmed that the major target of hyperglycemia and inflammation in cells is the transcription factor NF-κB, which consists of two subunits, polypeptide chains P50 and p65, among which p65 is the main form. MCP-1 is a powerful chemokine secreted by activated macrophages that can induce monocyte aggregation and promote glomerular inflammatory damage. MCP-1 can release a large number of inflammatory mediators and promote oxidative stress, thereby damaging endothelial cells and the kidney [23]. Studies have found that the RAGE promoter has two NF-κB binding sites, and AGEs-RAGE binding can induce phosphorylation of NF-κB, a key regulator in the inflammatory response, and activate its downstream signal transduction pathways [24]. The expression and activation of NF-κB can promote MCP-1 expression [25].

Res can alleviate proteinuria and improve renal function in STZ-induced diabetes and db/db mice [26]. but the target and mechanism are not completely clear. Research confirmed that Res can inhibit the apoptosis of pancreatic β-cells and significantly decrease the expression of an inhibitor (nuclear factor-kappa-B-inhibitor alpha) of nuclear factor (NF)-κB and NF-κB p65 in NF-κB signaling [27]. Our study found that Res can reduce BUN, SCr, and 24-h UAER values and the expression of MCP-1, RAGE and NF-κB, increase the podocyte count, improve renal pathological structure, and up-regulate the expression of nephrin and WT1 in T1DM mice. However, no evidence was found to support that Res exerted a sustained hypoglycemic effect in mice spontaneous T1DM mice in our experiment. Whether Res can reduce blood glucose levels is still controversial. Some scholars believe that Res mainly relies on insulin to reduce blood glucose levels in diabetes mellitus; therefore, the effect of Res on reducing blood glucose in T2DM animal models is more significant, but the effect on T1DM animal models is relatively small [28,29]. Early hyperglycemia in diabetic patients can lead to organ damage. Even if blood sugar reaches an ideal level in later stages, the damage still exists. This phenomenon is called “metabolic memory”. Metabolic memory plays a crucial role in the progression of various complications of diabetes, including DN [30,31]. Studies have shown that resveratrol may inhibit the effects of hyperglycemic metabolic memory on cell proliferation and oxidative stress through the SIRT1 axis [32]. Therefore, we speculate that although resveratrol has no sustained effect on reducing blood glucose, it can improve kidney injury, reduce proteinuria and protect kidney function by blocking metabolic memory effect.

hUCMSCs exist in the surrounding tissues of vessels of the human umbilical cord [33,34], which possess stem cell-like characteristics, including self-duplication and differentiation [35]. Our previous research demonstrated that hUCMSCs could improve hyperglycemia and the number of β-cells in the pancreatic islets of mice with type 1 diabetes [36,37]. Currently, studies have examined the treatment of DN using hUCMSCs [38]. Studies have shown that Res could protect MSCs against inflammation and oxidative injury. In vitro study revealed that Res enhanced the viability and proliferation, reduced the senescence and apoptosis of hUCMSCs [39,40]. Zhang et al. showed that resveratrol-modified hUCMSCs activated ERK pathway in renal tubular cells and promoted angiogenesis in endothelial cells via paracrine platelet-derived growth factor-DD [41]. Recent findings have demonstrated that Res may promote the self-renewal and differentiation of MSCs by regulating SIRT1 signaling, which is associated with cell self-renewal, senescence, apoptosis and neural differentiation [42].

In this study, it was found that the treatment of hUCMSCs could increase body weight and decrease blood glucose level in mice with type 1 diabetes. It could also decrease BUN, SCr, 24-hour UAER, increase podocyte count, decrease the expression of MCP-1, RAGE and NF-κB, and increase the expression of nephrin and WT1. Moreover, the therapeutic effect of hUCMSCs is better than that of resveratrol and insulin. In addition, our study also found that hUCMSCs treatment can reduce the dosage of insulin.

In conclusion, our study considers that persistent high glucose stimulation leads to an increase in AGEs production. The combination of AGEs and RAGE activates the inflammatory response signaling pathway of NF-κB, increases the level of MCP-1, induces macrophage and bacteriophage migration, increases immune/inflammatory response, and induces podocyte immune damage, which is manifested by decreased levels of nephrin and WT1. hUCMSCs and resveratrol can reduce the inflammatory response of podocytes by blocking RAGE- NF-κB signaling pathway, inhibit the release of inflammatory factor MCP-1 from activated macrophages, or promote the secretion of anti-inflammatory factors through paracrine effect. hUCMSCs combined with resveratrol treatment can significantly improve the protective effect of podocyte.

However, as with the majority of studies, the design of the current study is subject to limitations. First, the present study did not trace and check the MSC cells in vivo. Secondly, the expression of inflammatory factors in the downstream of NF-κB signaling pathway was not measured. Due to the complex pathophysiology of DN further investigations should focus on the possible mechanisms of action and potential side effects.

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Declaration of Competing Interest

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Author contributions

Yuxin Xian wrote the manuscript and performed the experiments. Yi Lin performed the electron microscopy studies and western blot. Caixia Cao, Li Li and Jing Wang performed the animal experiments and the histological examination. Jiapeng Niu, Yunlei Guo, Yanan Sun analyzed and interpreted data, created figure. Yangang Wang and Wei Wang designed the experiments, revised and proved the manuscript.

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