Mesenchymal Stem Cells Ameliorate Podocyte Injury and Proteinuria in a Type 1 Diabetic Nephropathy Rat Model

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ABSTRACT
Mesenchymal stem cells (MSC) attenuate albuminuria and preserve normal renal histology in diabetic mice. However, the effects of MSC on glomerular podocyte injury remain uncertain. The aim of this study was to evaluate the effects of MSC on podocyte injury in streptozotocin (STZ)-induced diabetic rats. Thirty days after diabetes induction by STZ injection (65 mg/kg, intraperitoneally) in Sprague-Dawley rats, the diabetic rats received medium or $2 \times 10^6$ enhanced green fluorescent protein-labeled MSC via the renal artery. In vivo tracking of MSC were followed by immunofluorescence analysis. Diabetes-related physical and biochemical parameters were measured on day 60 after the MSC infusion. The expression of podocyte markers (nephrin and podocin), podocyte survival factors (VEGF and BMP-7), and the ultrastructural pathology of podocytes were also assessed. MSC were only detected in the glomeruli from the left kidney receiving MSC infusion. Compared with medium-treated diabetic rats, rats treated with MSC showed a suppressed increase in kidney weight, kidney to body weight index, creatinine clearance rate and urinary albumin to creatinine ratio; however, the treatment had no effect on blood glucose or body weight levels. Furthermore, the MSC treatment reduced the loss of podocytes, effacement of foot processes, widening of foot processes, thickening of glomerular basal membrane and loss of glomerular nephrin and podocin. Most importantly, MSC-injected kidneys expressed higher levels of BMP-7 but not of VEGF. Our results clearly demonstrated that intrarterial administration of MSC prevented the development of albuminuria as well as any damage to or loss of podocytes, though there was no improvement in blood sugar levels. The protective effects of MSC may be mediated in part by increasing BMP-7 secretion.

INTRODUCTION
Diabetic nephropathy (DN) is a major complication of diabetes and represents the leading cause of end-stage renal disease worldwide [1]. To date, there is no cure for DN. Drugs that decrease blood glucose, lower blood pressure, or inhibit the actions of the hormone angiotensin can delay, but not eliminate, the onset of DN. Thus, the development of novel therapeutic strategies that could specifically target DN is necessary.

Podocytes are highly specialized cells with important roles in maintaining the glomerular filtration barrier and producing growth factors for both the mesangial and endothelial cells [2]. Recent studies have shown that progressive podocyte injury, called podocytopathy, is one of the key events in the pathogenesis of DN [3]. Podocyte loss and injury are associated with the development of albuminuria and the acceleration of glomerular structural abnormalities [2,4,5]. Thus, there is a need to clarify the underlying pathogenesis of podocyte injury and the associated alterations in the function of the glomerular filtration barrier to develop a novel therapeutic strategy for the prevention and amelioration of podocyte injury.

Stem cell therapy is a novel strategy for various diseases and has the potential to be more effective than single-agent drug therapies [6]. Several studies have shown that mesenchymal stem cell (MSC) therapy improves microalbuminuria and preserves the normal renal histology of diabetic mice [7,8]. However, the exact mechanisms underlying their therapeutic effects on microalbuminuria have not been clearly defined. It remains unknown whether the contribution of MSC to reducing microalbuminuria is associated with the improvement of podocyte injury. Based on these findings, we hypothesized that the transplantation of MSC may be a potential therapeutic approach for diabetic podocyte injury.

In the present study, we aim to evaluate whether MSC could exert their protective effects against diabetic podocyte injury. Here, our study provides the first evidence that MSC therapy may constitute a new therapeutic intervention to target podocyte damage.

MATERIALS AND METHODS
Experimental Animal
Adult male Sprague-Dawley (SD) rats (weight, 180 g to 200 g) from the laboratory animal center of Xinqiao hospital were maintained under specific pathogen-free conditions at the animal care facilities. After one week of adaptation, streptozotocin (STZ) (65 mg/kg; Sigma, St Louis, MO) was injected into the rats following overnight fasting. Rats with a blood glucose level over 16.7 mmol/L were considered diabetes-induced rats and were used in the study. Diabetic rats received daily injections of long-acting insulin in doses adjusted individually (ranging from 1 U to 4 U) to maintain blood glucose levels between 16 mmol/L and 28 mmol/L and to avoid ketonuria. All animal procedures were carried out according to the guidelines of the Animal Ethics Committee of the Third Military Medical University, which are consistent with the NIH Guide for the Care and Use of Laboratory Animals.

Isolation, Culture and Characterization of MSC
MSC were obtained from the femurs and tibias of adult male SD rats and cultured in DMEM/F12 medium (Gibco, Gaithersburg, MD) containing 10%
fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 mg/ml streptomycin. The phenotypic properties of MSC were performed by flow cytometry analysis using the following markers: CD29, CD44, CD34, and CD45 [9,10]. MSC were expanded in osteogenic or adipogenic differentiation media (Cyagen Biosciences, Guangzhou, China) in six-well plates after the third passage. Osteogenic and adipogenic differentiations were tested using standard protocol from Cyagen Biosciences Inc.

Cell Labeling for In Vivo Tracking

To assess the intrarenal localization of MSC, we used enhanced green fluorescent protein (EGFP) (Cyagen Biosciences) as a cell tracker in the tracking experiments. Cells were labeled using transduction with lentiviral-transduced EGFP, according to the manufacturer’s protocol. Briefly, MSC after the third passage were seeded at 2 x 10^6 cells in six-well plates 24 hours before transduction. The next day, 1 mL MSC medium per well containing 8 μg/ml polybrene (Sigma) and viral were added to the cells at 20 multiplicities of infection. After viral addition, cells were incubated at 37°C in 5% CO2 for 6 hours. The medium was replaced by a fresh complete culture solution, and cells were cultured until further experimentation. Selection was completed 72 hours after incubation with 10 μg/ml puromycin.

MSC Administration and Experimental Design

Thirty days after the injection of STZ, DN rats were randomly divided into 2 groups: DN rats treated with medium (DN+medium, n = 10) and DN rats treated with MSC (DN+MSC, n = 14). Nondiabetic rats were used as the normal control group (NC, n = 6). MSC-treated DN rats were injected with 2 x 10^6 MSC (suspended in 1 mL serum-free medium) via the left renal artery, as described previously [11], and DN+medium rats received an equal volume of medium. In this procedure, 2 rats that died of anesthesia or during surgery were excluded; one died in each group. Then, the rats were housed under specific pathogen-free conditions with a standard diet and water for 60 days before they were sacrificed (Figure 1). At the end of the experiment, the surviving rats were sacrificed, and the left and right kidneys of MSC-treated rats (n = 9) and medium-treated rats (n = 8) were harvested, weighed, and processed for histological and biochemical evaluation.

EGFP-MSC Detection

At 24 hours and on day 60 after MSC injection, 2 MSC-treated rats were sacrificed, and their kidney tissues were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA) and frozen. The tissues were sectioned into 8-μm samples using a cryostat microtome at -22°C. After fixation in acetone for 5 minutes, the sections were then covered with fluorescent antibody mounting medium. The 488-nm line of the laser was used for EGFP fluorescence excitation, and renal tissue background autofluorescence was detected at 555 nm. We used laser scanning confocal microscopy (Leica Microsystems, Wetzlar, Germany) for the 2 fluorescent analyses.

Physical and Biochemical Analysis

The body weight, kidney weight, and blood glucose were measured at the conclusion of the experiment. The serum creatinine levels were measured in the blood obtained from the retro-orbital sinus at the time of the sacrifice using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). The creatinine clearance rate was calculated as urinary creatinine x urine volume / serum creatinine and expressed as milliliters per minute. Urinary albumin excretion was expressed as the urinary albumin to creatinine ratio. Urinary albumin levels were measured in morning spot urine samples by a protein assay kit (Bio-Rad, Hercules, CA). After diabetes induction, albumin to creatinine ratios were determined every 30 days.

Renal Morphology

The paraffin sections were stained with Periodic Acid Schiff (PAS). The extent of glomerular damage was expressed as the percentage of glomeruli presenting mesangial expansion [12] or glomerulosclerosis, as described previously [13]. For ultrastructural examination, 1 mm3 pieces of renal cortex were fixed in 2.5% glutaraldehyde in .1 M phosphate buffer (pH, 7.4) for transmission electron microscopy. Electron micrographs of 5 blocks per kidney (10 photographs per block) were obtained at a final magnification of 8000- or 12,500-fold for each rat using a systematic uniform random sampling protocol. To ensure randomized sampling and avoid repetitious counting for podocytes in cross-sectional profiles, 3-μm sections taken at least 200 mm (average glomerular diameter) apart were cut and collected. The average number of podocytes was measured by manually tracing along the glomerular basement membrane (GBM) on a video screen. Podocytes were defined as those cells residing within the glomerular tuft but outside the GBM. The podocyte quantity, expressed as the number of podocytes per 2.5 μm GBM, was calculated by computerized measurement using the Image Measurement System (Leica Qwin Lite, Wetzlar, Germany) [14]. At the higher magnification, GBM thickness was defined as the distance perpendicular to the GBM between the endothelial and podocyte plasma membranes, measured with the aid of Image-Pro Plus (Media Cybernetics, Silver Spring, MD). The analysis of podocyte foot process effacement was expressed as the arithmetic mean of the podocyte foot processes width (FPW), which was calculated by dividing the total length of the GBM length by the total number of foot processes according to published methods [15].

Immunofluorescence

Immunofluorescent staining was performed with the specific podocyte marker nephrin and podocin for localization. Frozen sections (5 μm) were fixed in cold acetone for 10 minutes at -20°C and incubated with wash buffer containing 1% Triton X-100 at room temperature for 20 minutes to prevent nonspecific binding. Subsequently, the sections were incubated overnight at 4°C with polyclonal rabbit anti-nephrin (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and polyclonal rabbit anti-podocin (1:100 dilution; Santa Cruz Biotechnology) antibodies diluted in bovine serum albumin at 1%. After being rinsed in phosphate buffered saline, the TRITC-labeled goat antirabbit secondary antibody was incubated away from light at 37°C for 30 minutes. Negative controls were run by replacing the primary antibody with phosphate buffered saline. Staining was evaluated under laser scanning confocal microscopy (Leica).
ELISA Measurement of Cytokines

For the analysis of the growth factor production in MSC cultures, the supernatant was collected upon confluence in the third passage. The amounts of VEGF and BMP-7 in cell culture supernatants were quantified by ELISA (R&D Systems, Minneapolis, MN). The renal cortical homogenate VEGF and BMP-7 protein levels were also determined using ELISA. All experiments were performed in triplicate.

Statistical Analysis

All values are presented as the means ± SD. Statistical significance was evaluated using one-way ANOVA with modified t test performed with the Bonferroni correction. The paired t test was used to compare directly the left and right kidneys of an animal. A $P$ value < .05 was considered statistically significant.

RESULTS

Characterization of Rat MSC

Bone marrow-derived MSC from SD rats exhibited typical fibroblast-like morphology. MSC identity was proved by differentiation into osteogenic and adipogenic cells (Figure 2A). Flow cytometric analysis showed that the MSC were positive for CD29 (99.97%) and CD44 (99.97%) and negative for CD34 (1.53%) and CD45 (43%).

MSC Localization

Transduction EGFP into MSC by lentiviral vector resulted in a high-transduction efficiency, confirmed by nearly 90% of MSC exhibiting green fluorescence 3 days after transduction (Figure 2A). At 24 hours after MSC injection, EGFP-positive...
cells were only observed in the left kidney receiving EGFP-labeled MSC but not in the contralateral right kidneys (data not shown). EGFP-positive cells were observed in glomeruli but not in tubulointerstitial areas (data not shown). About 20% and 3% of glomeruli showed positive fluorescence at 24 hours and 60 days after MSC infusion, respectively (Figure 2B).

**Effect of MSC on Physical and Biochemical Parameters**

The blood glucose, body weight, kidney weight, kidney/body weight index, and creatinine clearance rate levels were reduced significantly by mesenchymal stem cells (MSC) treatment. Glucose and body weight did not change significantly between the 2 DN rats.

Data are presented as the means ± SD. Kidney weight, kidney/body weight and creatinine clearance rate levels but had no effect on blood glucose and body weight levels on day 60 after MSC infusion.

<table>
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<tr>
<th>Variable</th>
<th>NC (n = 6)</th>
<th>DN + medium (n = 8)</th>
<th>DN + MSC (n = 9)</th>
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<tr>
<td>Blood glucose (mmol/L)</td>
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<td>26.91 ± 4.71*</td>
<td>24.43 ± 4.03*</td>
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<td>Kidney weight (g)</td>
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<td>Body weight (g)</td>
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<td>Kidney/body weight (g/kg)</td>
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<td>7.67 ± .42</td>
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<tr>
<td>weight (g/kg)</td>
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<td>1.92 ± .07</td>
<td>1.63 ± .05*</td>
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<tr>
<td>Creatinine clearance rate</td>
<td>3.06 ± .06</td>
<td>3.12 ± .07</td>
<td>3.18 ± .09*</td>
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</tbody>
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NC indicates normal control; DN + medium, medium-treated rats; DN + MSC, MSC-treated rats.

Table 1

Renal Histopathological and Ultrastructural Changes

Light microscopy studies (PAS-staining) were performed to determine the extent of the glomerular damage (Figure 4A). As shown in Figure 4A, 90 days after STZ injection, the left and right kidneys of medium-treated rats and the right kidneys of MSC-treated rats exhibited profound extracellular matrix deposition and frequent fibrin cap formation inside the glomeruli. In contrast, 60 days after MSC injection, there was a significant decrease in mesangial matrix deposition (Figure 4B) and in the glomerulosclerotic index (Figure 4C) of the left kidneys of MSC-treated rats, compared to the left and right kidneys of medium-treated rats and the right kidneys of MSC-treated rats.

Electron microscopy studies were performed to determine the early structural changes in podocyte morphology (Figure 5A). Sixty days after MSC injection, compared with the left and right kidneys of medium-treated rats and the right kidneys of MSC-treated rats, the MSC-injected kidneys exhibited a significant improvement in ultrastructural abnormalities, such as loss of podocytes (Figure 5B), GBM thickening (Figure 5C), and podocyte foot process effacement (Figure 5D).

Oil Red O staining was performed to determine the renal accumulation of neutral lipids. As shown in Figure 2C, 60 days after MSC injection, approximately 10% of the MSC-treated glomeruli contained fat cells (Figure 2Ca). There was a focal accumulation of oil red O staining in the glomeruli of MSC-treated rats (Figure 2Cb) and almost no staining in the glomeruli of medium-treated rats or in the right glomeruli of MSC-treated rats (data not shown). Electron microscopy showed the individual large vacuoles as well as several small droplets within the glomerular podocyte cells (Figure 2Cc).

**Effect of MSC on the Renal Expression of Nephrin and Podocin**

To further reveal the mechanism responsible for the prevention of albuminuria in MSC-treated diabetic rats, we studied the expression of nephrin and podocin proteins, which are the 2 major proteins involved in the formation of a slit diaphragm. Comparing the left kidneys of medium-treated rats with the right kidneys of MSC-treated rats, MSC treatment led to a significant improvement in the loss of glomerular nephrin and podocin staining (Figure 6A).

In line with the results from immunofluorescence staining, western blotting analysis showed that MSC injection significantly attenuated the diabetic-induced decrease of glomerular nephrin and podocin expression, compared with the left kidneys of medium-treated rats and the right kidneys of MSC-treated rats (Figure 6B).

**MSC Express High Levels of VEGF and BMP-7**

Next, we aimed to evaluate whether MSC could exert their protective effects against podocyte damage in part by increasing VEGF and BMP-7 secretion, which have been shown to be podocyte survival factors to rescue podocytes from injury [17,18]. Both factors could be secreted by MSC, as previously reported [19]. After 24 hours of incubation, MSC released higher amounts of VEGF (1231 ± 123 pg/mg protein) versus 10 ± 2 pg/mg protein and BMP-7 (1868 ± 67 pg/mg protein versus 49 ± 12 pg/mg protein) in vitro cell culture supernatants than in fresh medium (Figure 7A).

As shown in Figure 7B, the renal cortical VEGF levels in the left kidneys from MSC-treated rats (1928 ± 63 pg/mg protein) did not significantly increase on day 60 post-MSC injection compared with the left kidneys of medium-treated rats (1804 ± 97 pg/mg protein) and the right kidneys of MSC-treated rats (1838 ± 87 pg/mg protein). In contrast, the BMP-7 levels were higher in the left kidneys of MSC-treated rats (1431 ± 39 pg/mg protein) than those in the left kidneys of medium-treated rats (837 ± 61 pg/mg protein)
and the right kidneys of MSC-treated rats (889 ± 100 pg/mg protein), suggesting that the protective effects of MSC on podocyte injury in DN may be mediated in part by an increased BMP-7 secretion.

**DISCUSSION**

MSC are multipotent cells present in bone marrow and in mesenchymal tissues that can differentiate in vitro into adipocytic, chondrocytic, and osteocytic lineages. These cells do not exhibit hemopoietic markers but rather express CD29, CD44, CD105, and alpha smooth muscle actin [10]. In vitro, MSC may give rise to insulin-producing cells [20,21]. In vivo, MSC may differentiate into renal cells [22,23] and reconstitute necrotic segments of damaged kidneys. Thus, bone marrow-derived MSC have been used successfully in cell therapy to treat animal models with acute and chronic renal failure, such as ischemia-reperfusion injury, 5/6 nephrectomy, and unilateral ureteral obstruction, as well as in kidney transplantation models [24]. The present study provides clear evidence that although there was no improvement in blood sugar levels, the injected MSC did prevent the development of albuminuria and the loss of podocytes.

The primary advantage of MSC for utilization in cell therapy is the ease with which they can be harvested from the bone marrow, isolated by plastic adherence, and expanded in culture [25]. However, in vitro manipulations may also alter or influence their natural phenotypes, leading to different, as-yet-undefined activities and responses. In this study, we established that adherent, bone marrow-derived, spindle-shaped cells expressed mesenchymal cell phenotypes (CD29, CD44+) rather than CD34 (hematopoietic cell marker) and CD45 (leukocyte marker). In addition, the MSC possessed osteogenic and adipogenic potential; we confirmed with this approach that the subsequently administered cells retained the characteristics of MSC.

We found that adequate homing of MSC to the injured tissue is important for effective therapy. In most studies, MSC is administered through a standard intravenous route. A disadvantage of the systemic intravenous delivery of MSC can be low uptake at the site of injury. Indeed, significant engraftment of injured tissue was observed in some studies [26-28], but not all [29,30]. Schrepfer et al [31] demonstrated that the systemic intravenous route of administration was not appropriate for MSC to reach their site of activation. Zonta [32] showed that the intra-arterial administration of MSC were the most effective route to achieve immunomodulating effects in experimental kidney transplantation, which primarily occurs because large MSC (15 μm to 19 μm) remain trapped in the capillaries of the small lung filter, which in turn causes the inadequate homing of MSC to the
injured tissue. However, using the renal artery as the injection route to administer MSC to treat DN may be associated with the following 2 major complications: (1) renal infarcts and loss of function, and (2) ectopic differentiation into adipocytes within glomeruli [33]. Recently, Ho et al [34] demonstrated that multiple intravenous transplantsations of MSC effectively restored the long-term blood glucose homeostasis during 15 weeks in STZ-induced diabetic mice; thus, multiple intravenous transplantsations of MSC may serve as a new therapeutic strategy for DM patients. The following 2 factors may contribute to MSC for the treatment of DN: (1) blood sugar reduction and (2) renal protective actions of the MSC themselves within the glomeruli. To examine the second factor, we could only choose the renal artery as the injection route instead of systemic administration. In our study, intra-arterial injection led to 20% of glomeruli containing EGFP cells at 24 hours, as observed in the targeted kidney but not in the lung, liver, or spleen (data not shown). However, 60 days after MSC injection, only 3% of glomeruli containing EGFP+ cells were found in MSC-treated kidneys. This low level of engraftment suggests a paracrine, rather than a cell differentiation, contribution to the beneficial effects of the MSC. However, the positive status of the 3% of the glomeruli in the kidney on 60 days after injection is still higher than the 0% in the lung, liver, and spleen and suggests that there was some targeting of the cell therapy to the kidney, likely due to the renal artery injection. In addition, on day 60 after the injection into a renal artery, approximately 10% of the MSC-treated glomeruli contained cells that exhibited features typical of adipocytes. Electron microscopy showed that large or multiple small fatty vacuoles were found within the glomerular podocyte cells. This phenomenon had been previously reported by Kunter and Rong et al [33]. Although it seems highly likely that these cells originated from the transplanted MSC, the exact mechanisms of MSC transformation are not completely understood. There is evidence that BMP-7 promotes the differentiation of MSC into adipocyte cells [35]. In our study, we found that the expression of BMP-7 was significantly increased in MSC-treated kidneys compared to medium-treated kidneys, which might help explain these results. These phenomena seems to suggest that the long-term effects of intrarenal, syngeneic MSC transplantation may result in the maldifferentiation of glomerular MSC into adipocytes, thus calling into question the potential benefit of MSC treatment for DN. Taken together, our observation is consistent with the homing capacity of MSC to injured areas via intra-arterial injection in several animal models [11,36].

Type 1 Diabetes (T1DM), a multifactorial autoimmune disease involving genetic and environmental factors, is hallmarkked by the T cell- and macrophages-mediated destruction of pancreatic β-cells, resulting in an irreversible insulin deficiency. STZ or other diabetogenic agents (eg, alloxan) with β-cell-toxic abilities have also been used for...
producing chemically induced T1DM models through their administration in a large dose or repeated low doses for several days. However, although the STZ model results in hyperglycemia and insulinopenia, it does not bear strong autoimmune features [37-39], suggesting that the STZ-induced diabetic model is not the equivalent of T1DM. Although an ideal experimental model of T1DM does not exist, we believe that the use of this STZ model may allow us to obtain valuable information about understanding the molecular bases that contribute to the induction of T1DM in humans.

Recent studies have shown that the systemic administration of MSC into STZ-induced type 1 diabetic C57BL/6 mice [7] and type 2 diabetic NOD/scid mice [8] reduced
microalbuminuria and preserved normal renal histology. In contrast, untreated diabetic mice presented glomerular hyalinosis and mesangial expansion. The observed renoprotection was associated with a decrease in glycemic levels that could be explained by beta-pancreatic islet regeneration resulting from MSC administration. However, in these studies, neither group demonstrated a significant histologic amelioration of podocyte damage in response to MSC injection; furthermore, their data could not completely distinguish these beneficial effects from a reduction in the serum glucose levels or direct protection of the podocytes. In this study, we successfully established an experimental DN, similar to human type 1 DN and characterized by hyperglycemia and albuminuria, that was also associated with the loss of podocytes, effacement of foot process, widening of foot process, and thickening of the GBM [1,40-42]. These changes contribute to progressive glomerulosclerosis, which is a feature of experimental DN. More importantly, our results revealed that MSC effectively ameliorated the phenotypic changes of podocytes in this animal model. Another interesting finding of the present study was that the renoprotective effect of MSC in podocyte injury was only found in the left kidneys, not in the right kidneys, of MSC-treated DN rats. In addition, the renoprotection of MSC were not attributable to glycemic control because MSC via intra-arterial injection had no impact on the elevated serum glucose levels in the diabetic rats. As shown in Table 1, MSC administration by intra-arterial injection reversed neither hyperglycemia nor body weight loss, the latter of which was associated with poor blood glucose control. Thus, our study clearly suggested that MSC themselves may have had a direct beneficial effect on the ultrastructural alterations in podocytes.

Podocytes play a major role in maintaining the integrity and permeability of the glomerular filtration barrier [43,44]. In DN, the effacement of podocyte foot processes and the loss of slit diaphragm proteins result in a leakage of albumin and proteinuria [45,46]. The results of the present study showed that the levels of urinary protein were significantly increased in diabetic rats and could be significantly lowered by MSC. To identify the molecular basis of the observed phenomena, we performed immunostaining for nephrin and podocin, which are constituents of the slit diaphragm and act as a barrier for glomerular capillary walls [47]. Our study revealed that MSC administration could restore the expression of nephrin and podocin in diabetic rats. Hence, our data suggested that the antiproteinuric effects of MSC in diabetes-induced proteinuria may be associated with the restoration of the expression of BMP-7 was significantly increased in MSC-treated kidneys compared to medium-treated kidneys. There were small increases in VEGF in MSC-treated kidneys. However, these did not reach statistical significance. These data suggested that MSC exert their protective effects against podocyte damage possibly by increasing BMP-7 secretion. Both in vitro and in vivo studies have indicated that BMP-7 is important for the maintenance of podocyte viability and differentiation [18,51,52]. In adult rodent glomeruli in vivo, BMP-7 and its receptors are expressed in podocytes [18]. In cultured mouse podocytes, high glucose decreases BMP-7 expression, and the treatment of podocytes with rhBMP-7 restores podocin and synaptopodin expression [51]. In STZ-induced diabetic mice, the maintenance of renal and glomerular podocyte BMP-7 levels by means of a transgene reduces the onset and progression of nephropathy, especially podocyte dropout [52]. Moreover, the ability of MSC to contribute to podocyte regeneration has been recently shown in a mouse model of Alport syndrome [53]. In addition to BMP-7 and VEGF, MSC can secrete a number of factors, such as HGF, bFGF, and IGF-1, all known to improve renal function in acute kidney injury, mediated by their antiapoptotic, mitogenic, and other cyto-kine actions [49,50]. Based on these observations, we deduce that the mechanisms that mediate the protective effects of MSC may be primarily paracrine actions. In summary, our current study provided further evidence that MSC could not only exert antialbuminuric effects but also, more importantly, prevent early phenotypic changes in podocytes and, subsequently, glomerulosclerosis. We believe that the successful treatment of DN with MSC demonstrated herein holds substantial promise for the development of novel, MSC-based interventions that can prevent the development of DN. However, because our study is an animal study, these findings must be confirmed after further study, such as clinical trial, on human subjects.

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Conflict of Interest Statement: There are no conflicts of interest to report.

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