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Research Article

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Human umbilical cord mesenchymal stem cells attenuate diabetic nephropathy through the IGF1R-CHK2-p53 signalling axis in male rats with type 2 diabetes mellitus

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Abstract: Diabetes mellitus (DM) is a disease syndrome characterized by chronic hyperglycaemia. A long-term high-glucose environment leads to reactive oxygen species (ROS) production and nuclear DNA damage. Human umbilical cord mesenchymal stem cell (HUcMSC) infusion induces significant antidiabetic effects in type 2 diabetes mellitus (T2DM) rats. Insulin-like growth factor 1 (IGF1) receptor (IGF1R) is important in promoting glucose metabolism in diabetes; however, the mechanism by which HUcMSC can treat diabetes through IGF1R and DNA damage repair remains unclear. In this study, a DM rat model was induced with high-fat diet feeding and streptozotocin (STZ) administration and rats were infused four times with HUcMSC. Blood glucose, interleukin-6 (IL-6), IL-10, glomerular basement membrane, and renal function were examined. Proteins that interacted with IGF1R were determined through coimmunoprecipitation assays. The expression of IGF1R, phosphorylated checkpoint kinase 2 (p-CHK2), and phosphorylated protein 53 (p-p53) was examined using immunohistochemistry (IHC) and western blot analysis. Enzyme-linked immunosorbent assay (ELISA) was used to determine the serum levels of 8-hydroxydeoxyguanosine (8-OHdG). Flow cytometry experiments were used to detect the surface markers of HUcMSC. The identification of the morphology and phenotype of HUcMSC was performed by way of oil red "O" staining and Alizarin red staining. DM rats exhibited abnormal blood glucose and IL-6/10 levels and renal function changes in the glomerular basement membrane, increased the expression of IGF1 and IGF1R. IGF1R interacted with CHK2, and the expression of p-CHK2 was significantly decreased in *IGF1R*-knockdown cells. When cisplatin was used to induce DNA damage, the expression of p-CHK2 was higher than that in the *IGF1R*-knockdown group without cisplatin treatment. HUcMSC infusion ameliorated abnormalities and preserved kidney structure and function in DM rats. The expression of IGF1, IGF1R, p-CHK2, and p-p53, and the level of 8-OHdG in the DM group increased significantly compared with those in the control group, and decreased after HUcMSC treatment. Our results suggested that IGF1R could interact with CHK2 and mediate DNA damage. HUcMSC infusion protected against kidney injury in DM rats. The underlying mechanisms may include HUcMSC-mediated enhancement of diabetes treatment via the IGF1R-CHK2-p53 signalling pathway.

Key words: Insulin-like growth factor 1 receptor (IGF1R); Checkpoint kinase 2 (CHK2); Protein 53 (p53); Diabetes mellitus; Human umbilical cord mesenchymal stem cell (HUcMSC); DNA damage repair

1 Introduction

The incidence of diabetes is particularly high in China. The number of diabetes patients in China has reached 92.4 million, with an average of 1 in 10 adults suffering from diabetes, and more than 90% of patients have type 2 diabetes mellitus (T2DM) (Guariguata et al.,

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2014). T2DM is a metabolic disease characterized by chronically elevated blood glucose caused by insulin resistance (Ríos-Silva et al., 2014; Sohn et al., 2015). In diabetes patients, DNA breakage, pyrimidine ring oxidation, and purine site changes are considerably higher than those in control groups. These changes suggest that diabetes has an important relationship with DNA damage and oxidative stress (Collins et al., 1998; Adaikalakoteswari et al., 2007). In a hyperglycaemic environment, reactive oxygen species (ROS) and superoxide free radicals generated by the electrical gradient via the mitochondrial electron transport chain are increased, and excessive ROS can directly cause DNA damage (Nishikawa et al., 2000; Du et al., 2003). In addition, ROS can also increase the synthesis of peroxynitrite and induce further oxidation, thus increasing the level of nitrated tyrosine, causing DNA damage and apoptosis in tissues and cells, and further promoting the occurrence and development of diabetic complications (Ceriello, 2003).

Insulin-like growth factor 1 (IGF1) is a 70-amino-acid (aa) polypeptide hormone that is involved in the pathogenesis of diabetic kidney disease and can promote growth differentiation and insulin metabolism by binding with IGF1 receptor (IGF1R) (Vasylyeva and Ferry, 2007; Landau et al., 2009; Troib et al., 2011; Kong et al., 2016; Ansarullah et al., 2021). In diabetic kidney disease, IGF1 expression in the kidney is greatly increased (Flyvbjerg et al., 1990; Cingel-Ristic et al., 2005). Recently, it was reported that IGF1 and IGF1R are important for the DNA damage response. Inhibiting IGF1R by decreasing ataxia telangiectasia-mutated (ATM) phosphorylation leads to a decrease in DNA damage repair, which plays a crucial role in homologous recombination (HR) and non-homologous end-joining (NHEJ) (Turney et al., 2012). IGF1R overexpression accelerates DNA repair in primary human lung fibroblasts, some human cancer cells, and irradiated salivary glands (Chitnis et al., 2008). IGF1R-dependent signals prevent genotoxic stress by suppressing hyperglycaemic danger signals and enhancing the repair of DNA double-strand breaks (DSBs) by homologous recombination repair (HRR) (Yang et al., 2005; Meyer et al., 2017). However, the roles of IGF1 and IGF1R in the DNA damage response in diabetes remain unclear.

Mesenchymal stem cells (MSCs) are pluripotent cells. They can protect the function of islet cells and improve insulin sensitivity through immune regulation, antioxidation, inhibiting apoptosis, and other effects, and can act on multiple targets to improve insulin resistance and the inflammatory immune status of T2DM through multiple pathways (Nishikawa et al., 2000; Hodgkinson et al., 2003; Cho et al., 2018; Qi et al., 2019). Although the results of treatments based on MSCs in many studies look promising, the potential tumorigenicity of MSCs remains a safety concern (Lee, 2018). Compared with other stem cells, human umbilical cord MSCs (HUcMSCs) have the characteristics of convenience, no ethical disputes, large cell numbers, strong proliferation, low immunogenicity, and greater application potential. These cells are ideal seed stem cells for the treatment of T2DM (Macias et al., 2010; Ding et al., 2015). Studies suggest that HUcMSCs-small extracellular vesicles (sEVs) ameliorate insulin resistance and exert protective effects on T2DM rats. Histological analysis revealed that HUcMSC-sEVs were able to alleviate structural damage to the pancreas, kidney, and liver (Yap et al., 2022). Some literature reports that exosomes derived from HUcMSCs reduce acetaminophen-induced acute liver failure through activating the extracellular signal-regulated protein kinase (ERK) and IGF1R/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signalling pathways (Wu et al., 2021). However, the mechanism of the IGF1R pathway as an action of HUcMSCs in T2DM has not been clarified.

In previous studies, HUcMSC-secreted growth factors improve renal function, inhibit inflammation and fibrosis, and prevent progression in a diabetes-induced chronic renal injury model (Xiang et al., 2020). HUcMSCs inhibited pyroptosis of renal tubular epithelial cells through targeting the nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3)/Caspase1 pathway (Zheng et al., 2023) and miR-146a-5p-modifed umbilical cord MSCs enhance protection against renal injury in diabetic nephropathy through facilitating M2 macrophage polarization by targeting tumor necrosis factor receptor-associated factor-6 (TRAF6)-signal transducer and activator of transcription 1 (STAT1) signalling (Zhang et al., 2022). Furthermore, HUcMSCs attenuated renal oxidative damage and apoptosis in T2DM diabetic nephropathy by activating nuclear factor erythroid 2-related factor 2 (Nrf2), which indicated that HUcMSCs may be a potential drug target for diabetic nephropathy (Nie et al., 2021). HUcMSC infusion

induced significant antidiabetic effects and promoted insulin sensitivity in T2DM rats that were induced with a high-fat diet (HFD) combined with streptozotocin (STZ) and directed ATMs into an alternatively activated phenotype (M2, anti-inflammatory) (Xie et al., 2016). However, the underlying mechanism remains unclear.

Therefore, we established an STZ-induced T2DM rat model and explored the therapeutic effect and underlying mechanisms of HUcMSCs in diabetes. Our study aimed to examine the mechanism by which HUcMSCs can treat diabetes through DNA damage repair and provide a new therapeutic target for diabetes.

2 Results

2.1 Induced differentiation ability and biological properties of HUcMSCs

We constructed the HFD/STZ-induced T2DM rat model, which was treated with HUcMSCs (Fig. 1). HUcMSCs were derived from umbilical cord tissues, which possessed a variety of differentiation-inducing capabilities as well as basic cell biological properties. Biological effectiveness experiments confirmed that HUcMSCs could be differentiated into adipogenic, osteogenic, and chondrogenic phenotypes (Figs. 2a–2c). Flowcytometry experiments showed that HUcMSCs were positive for cluster of differentiation 105 (CD105) (100%), CD90 (100%), and CD73 (100%), and negative for CD11 (1.300%), CD19 (0.454%), CD31 (0.434%), CD34 (0.463%), CD45 (0.826%), and human leukocyte antigen-DR (HLA-DR) (0.382%), which confirmed the identity of HUcMSCs (Fig. 2d).

2.2 Effect of HUcMSCs on biological change in the diabetes model

To validate the effect of HUcMSCs in the diabetic rats, we checked body weight, blood glucose, insulin, interleukin-6 (IL-6), IL-10, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and glomerular basement membrane, and found that the diabetes mellitus (DM) group showed persistent hyperglycaemia combined with a gradual decrease in body weight, and the MSC group showed a significant decrease of blood glucose level compared with the DM group, but the reduction was only maintained for three weeks (Figs. 3a and 3b). We also weighed the kidneys of rats with DM and found that, compared with the control group, the kidney/body weight ratio was significantly increased in the diabetes group, while the ratio in the MSC group decreased, indicating that kidney weight can be improved by HUcMSC administration (Fig. 3c). Moreover, the concentration of serum insulin

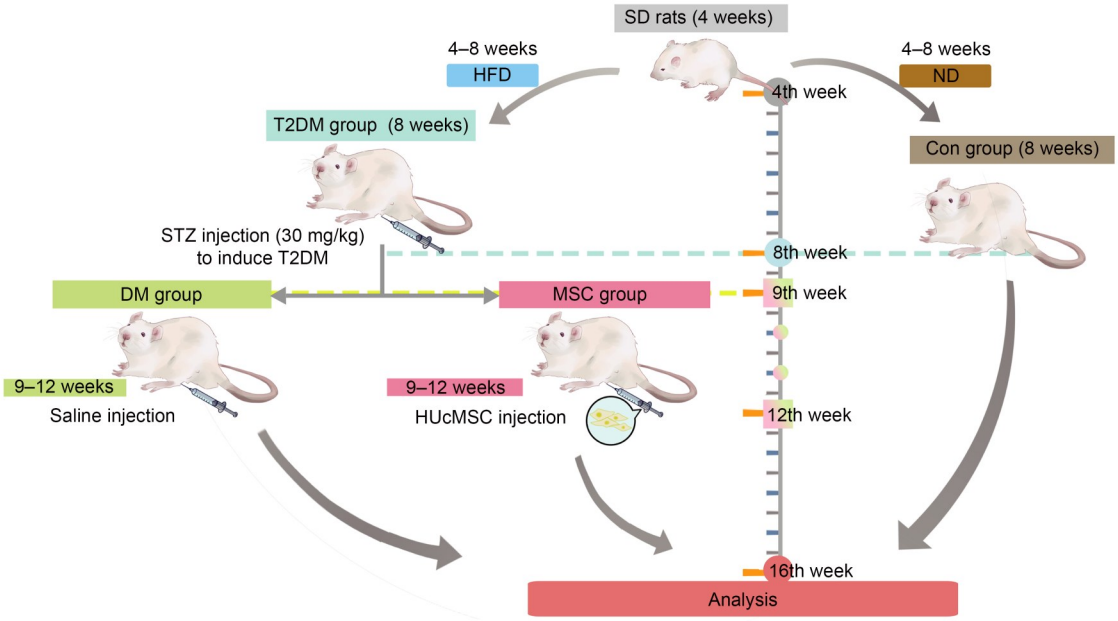

Fig. 1 Streptozotocin (STZ)-induced diabetic rat model and intervention program. Con: control; DM: diabetes mellitus; HFD: high-fat diet; MSC: mesenchymal stem cell; HUcMSC: human umbilical cord MSC; ND: normal diet; SD: Sprague Dawley; T2DM: type 2 diabetes mellitus.

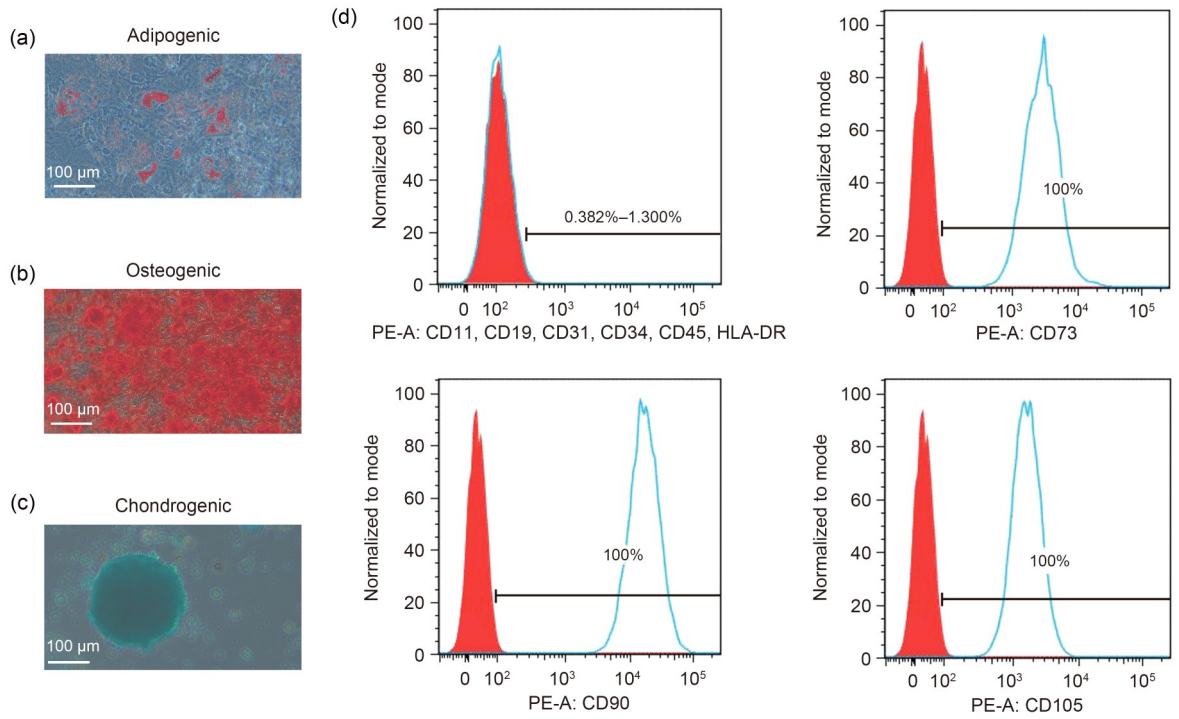

Fig. 2 Morphology and phenotype identification of human umbilical cord mesenchymal stem cells (HUcMSCs). (a) The morphology of HUcMSCs, which could be differentiated into adipocytes through Oil Red O staining. (b) The differentiated HUcMSCs have osteogenic properties, which were verified by Alizarin Red staining. (c) HUcMSCs were successfully differentiated into the chondrocytes, which were identified by a simple blue dye. (d) Flowcytometry experiments detect the surface markers of HUcMSCs, including cluster of differentiation 11 (CD11), CD19, CD31, CD34, CD45, human leukocyte antigen-DR (HLA-DR), CD73, CD90, and CD105. Among them, the positive rates of CD73, CD90, and CD105 were almost 100%, and others were negatively expressed. PE: phycoerythrin.

in the DM group was greatly decreased compared with that of the control group, while HUcMSC treatment significantly suppressed the decrease (Fig. 3d).

IL-6, MCP-1, and TNF-α are widely recognized proinflammatory cytokines. Our results found that the concentrations of serum IL-6, TNF-α, and MCP-1 were all significantly elevated in the DM group, whereas these inflammatory cytokine levels were reduced by HUcMSC treatment compared with the DM group (Figs. 3e–3g). Moreover, adipose tissue M2 macrophages secrete the anti-inflammatory factor IL-10, which can inhibit, verify, and improve insulin resistance. Our results found that IL-10 was significantly decreased in the DM group, but recovered by the HUcMSC treatment (Fig. 3h).

Next, we examined the glomerular basement membrane in the three groups. Compared with the control group, in the DM group, the mesangial cells and stroma were significantly proliferated, the basement membrane was thickened, the epithelial cells of the visceral layer were swollen, and the foot processes were fused. After treatment with HUcMSCs, mesangial cells and stroma proliferation were improved, the basement membrane was mildly thickened, the visceral epithelial cells were mildly swollen, and the foot processes were segmentally fused (Figs. 3i and 3j). Taken together, these results showed that HUcMSC infusions reduce inflammation levels and improve renal biological changes in T2DM rats.

2.3 Effect of HUcMSCs on pathological changes in renal tissue

To determine pathological changes of renal tissue, haematoxylin and eosin (H&E) staining and periodic acid-Schiff (PAS) staining were performed. The H&E results indicated that, compared with the control group, the DM group showed glomerular mesangial cell proliferation and renal tubular epithelial cell swelling, disintegration, and vacuolar degeneration, and these pathological changes were significantly improved in the MSC group, in which glomerular mesangial cells were decreased and the extracellular matrix was only slightly

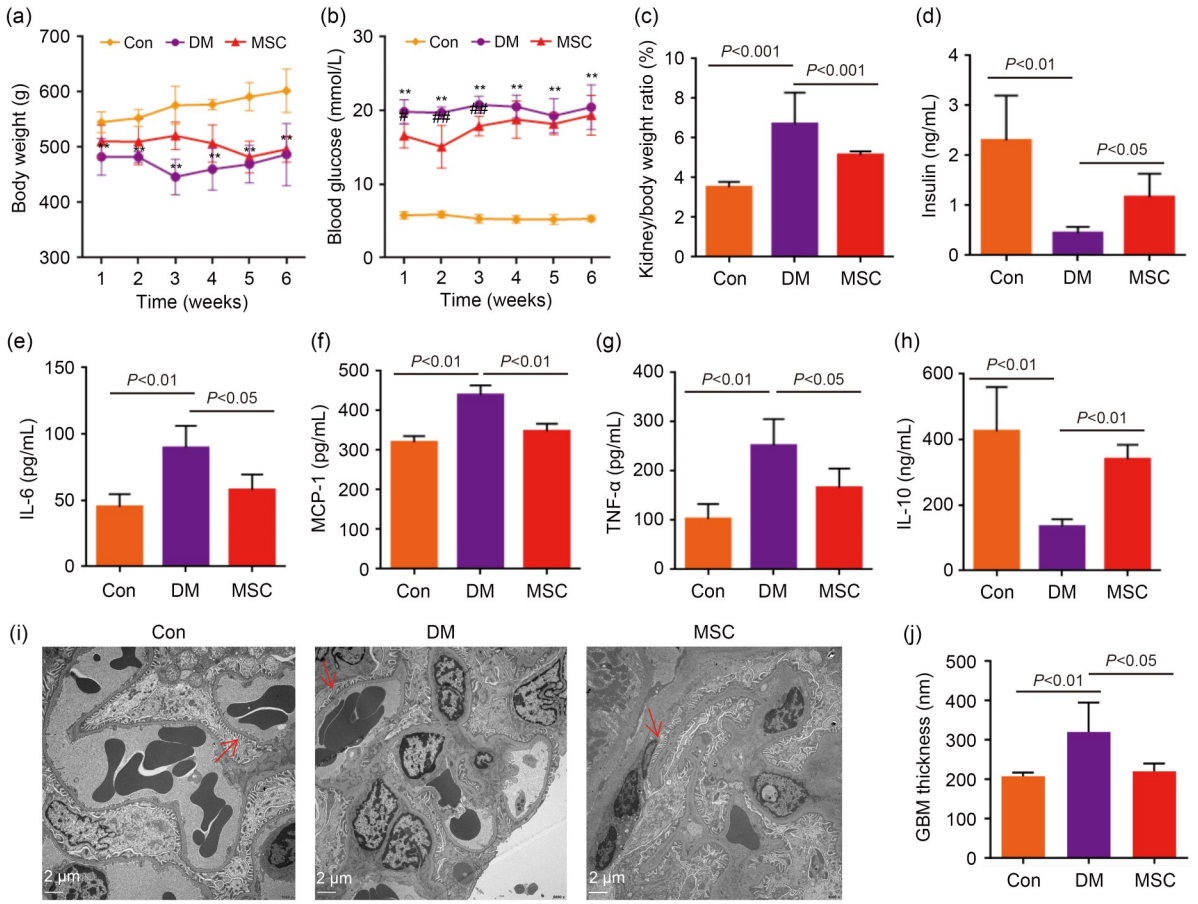

Fig. 3 Effects of human umbilical cord mesenchymal stem cell (HUcMSC) infusions on inflammation levels and renal biological changes in type 2 diabetes mellitus (T2DM) rats. (a, b) Weekly body weight and blood glucose changes in each group (control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC)). $^{**}P$<0.01, versus the control level; $^{\#}P$<0.05 and $^{\#\#}P$<0.01, versus the DM model level. (c) The renal index calculated as kidney weight (g)/body weight (g)×100%. (d–h) The concentrations of insulin, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and IL-10 were checked in serum. (i) Ultrastructure of the glomerular basement membrane (GBM) thickness in diabetic kidneys. Red arrows indicate podocytes. (j) Representative quantitative analysis of GBM thickness in each group. All values are presented as mean±standard deviation (SD), n=8.

proliferated (Fig. 4a). PAS staining showed matrix hyperplasia and glomerular basement membrane thickening in renal tissue sections from STZ-induced diabetic rats. It is worth noting that HUcMSC treatment reduced glomerular mesangial matrix expansion and glomerular basement membrane thickening (Fig. 4b). Then, we used NDP.view software to measure glomerular diameter and analysed the Paller score of renal tissue (Paller and Neumann, 1991) (Figs. 4c and 4d).

To further determine whether treatment with HUcMSCs could alleviate apoptosis in renal cells, we examined apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The TUNEL assay is based on fluorescein and can detect low levels of apoptosis in renal tissue with high sensitivity, and can quantify apoptosis through subsequent fluorescence microscopy. The TUNEL results showed that, compared with the control group, apoptosis in renal cells in the DM group was appreciably increased, while apoptosis in the MSC group was decreased (Fig. 4e). These findings suggest that HUcMSCs may alleviate renal pathological changes.

2.4 Effect of HUcMSCs on renal function change

Next, we examined renal function change to determine the role of HUcMSCs. Compared with the control group, the levels of serum creatinine (Scr), blood urea nitrogen (BUN), uric acid (UA), and cystatin C in the DM group increased significantly, but decreased

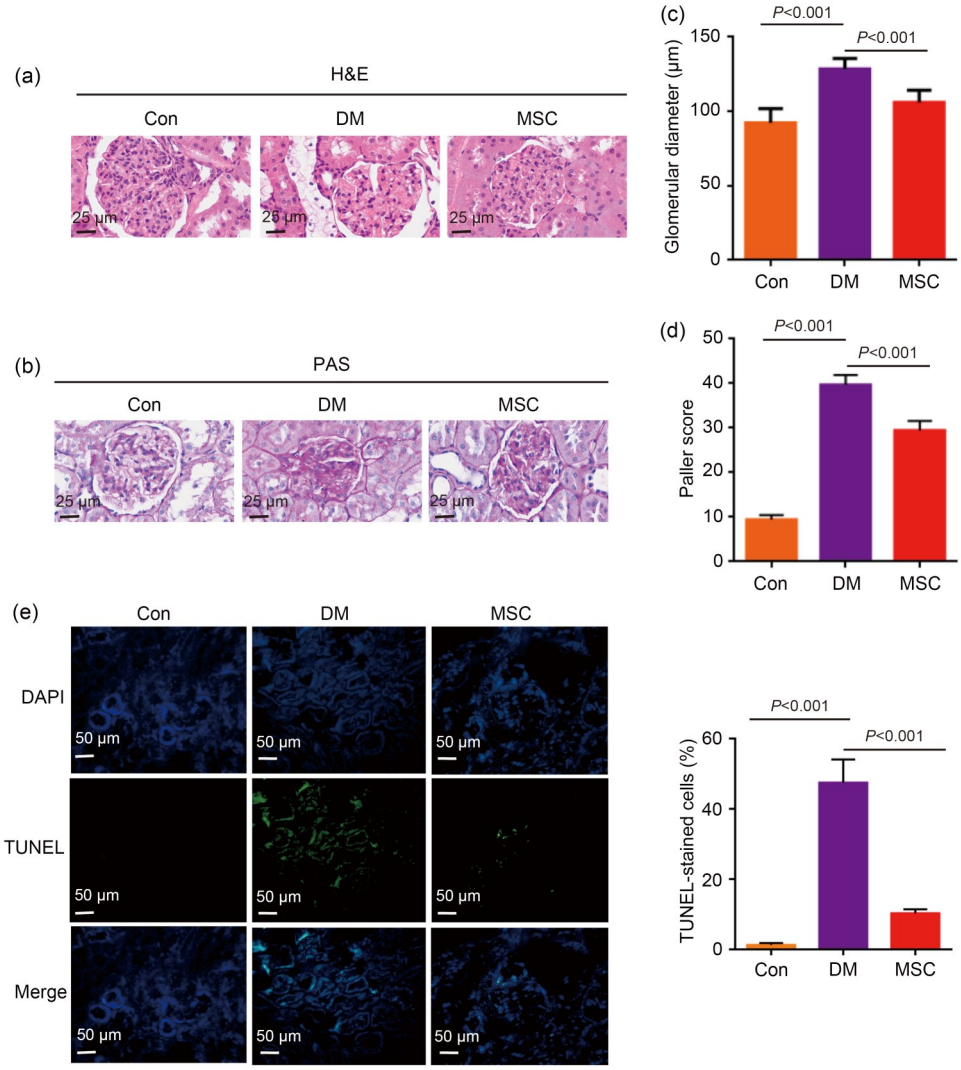

Fig. 4 Effect of human umbilical cord mesenchymal stem cells (HUcMSCs) on renal pathological changes. (a, b) Renal tissues were stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) in each group (control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC)). (c) Glomerular diameter measured by NDP.view software. (d) Paller score of renal tissue. (e) Apoptosis in renal cells detected by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and its quantitative analysis. The green fluorescence indicates positive cells. All values are presented as mean±standard deviation (SD), *n*=8. DAPI: 4',6-diamidino-2-phenylindole.

significantly after HUcMSC injection (Fig. 5), indicating that HUcMSC treatment was beneficial in protecting against diabetes-induced damage.

2.5 Expression of IGF1 and IGF1R in renal tissues

To further examine the changes in the expression of IGF1 and IGF1R in renal tissues after HUcMSC intervention, we performed immunohistochemical staining in the control, DM, and MSC groups. The results showed that, compared with the control group, the expression of IGF1 and IGF1R was increased in the renal tissue of diabetic rats but was decreased significantly after HUcMSC treatment (Fig. 6a). In contrast, the serum IGF1 level decreased in the DM group, while increased in the MSC group (Fig. 6b).

Previous studies have shown that renal-extractable IGF1 protein was increased in diabetic rats, while serum IGF1 levels did not change during the first two weeks of diabetes, but a significant decrease was observed after four weeks (Segev et al., 1997). IGF1 is transported from the blood to the tissue when hyperglycaemia occurs, and cell hypertrophy in the kidney leads to glomerular and renal function damages. Therefore, the level of IGF1 in the blood is decreased, and in renal tissue

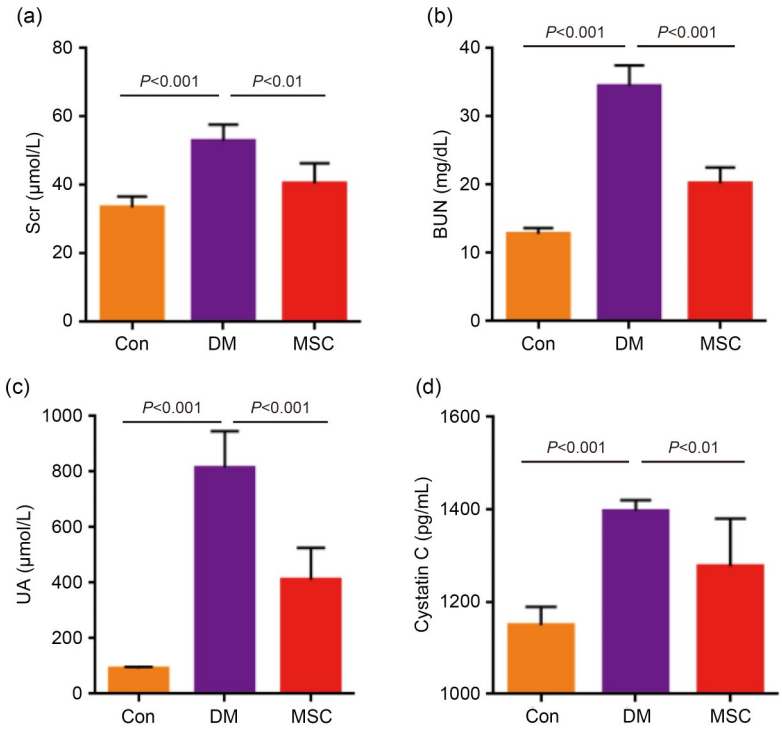

Fig. 5 Roles of human umbilical cord mesenchymal stem cell (HUcMSC) treatment in protecting against diabetes-induced damage. Kidney function indexes of mouse models in each group (control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC)): (a) serum creatinine (Scr); (b) blood urea nitrogen (BUN); (c) uric acid (UA); (d) cystatin C. Data are expressed as mean±standard deviation (SD), *n*=8.

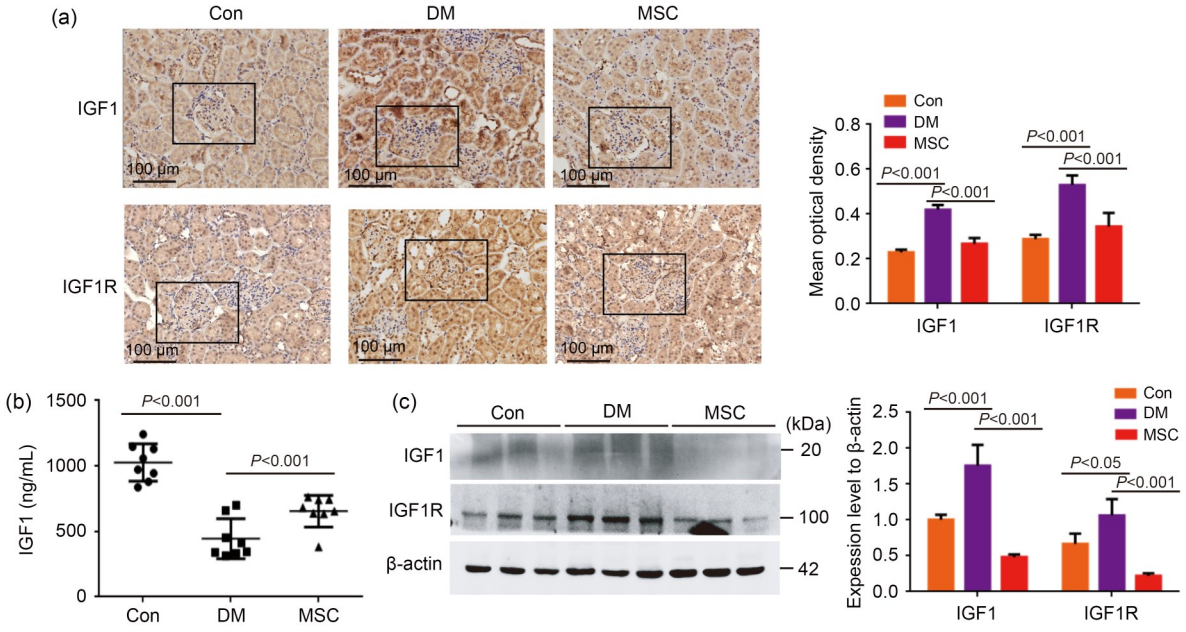

Fig. 6 Upregulated insulin-like growth factor 1 (IGF1) and IGF1 receptor (IGF1R) protein expression levels in streptozotocin (STZ)-induced diabetic kidneys. (a) Representative immunohistochemical images and quantification of IGF1 and IGF1R in the control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC) groups. Black borders indicate glomerulus. Mean optical density was calculated by the ratio of integrated optical density (IOD) to positive area. (b) Concentration of IGF1 in rat serum. (c) Western blot and quantitative analyses of the IGF1 and IGF1R expression levels. All values are presented as mean±standard deviation (SD), *n*=8.

is increased. We further measured IGF1 and IGF1R expression in kidney tissues in the three groups using western blotting, which was consistent with the immunohistochemical staining results. Compared with the control group, the expression of IGF1 and IGF1R in the DM group was increased, while in the MSC group was recovered (Fig. 6c). These data suggested that IGF1 and IGF1R participate in renal histopathological damage and that MSCs may alleviate pathological changes in renal tissue.

2.6 IGF1R involved in the DNA damage repair pathway

IGF-1 and IGF-1R are necessary for nucleotide excision and repair in keratinocytes (Loesch et al., 2016). However, it is not clear whether IGF1 and its receptor IGF1R cause DNA damage in diabetes. The ATM-checkpoint kinase 1 (CHK1)/CHK2-protein 53 (p53) signaling pathway plays a key role in DNA damage repair. We found that IGF1R interacted with CHK2 but not with CHK1, using a coimmunoprecipitation assay (Figs. 7a–7c). To further determine whether IGF1R participates in the DNA damage response, we knocked down *IGF1R* with short hairpin RNA (shRNA) in 293T cells and measured the expression of phosphorylated CHK2 (p-CHK2) and phosphorylated p53 (p-p53). Compared with the control group, *IGF1R* knockdown significantly decreased p-CHK2 and p-p53 (Fig. 7d). Following treatment with 20 μmol/L cisplatin that induces DNA damage, the expression of p-CHK2 was higher than that in the *IGF1R*-knockdown group (Fig. 7e). These results showed that IGF1R was involved in DNA damage through the CHK2-p53 signaling axis.

8-Hydroxydeoxyguanosine (8-OHdG) is a sensitive marker of the occurrence of oxidative DNA damage and changes in antioxidant capacity (Papaharalambus and Griendling, 2007). We used an enzyme-linked immunosorbent assay (ELISA) to determine the levels of 8-OHdG in the serum of rats in these three groups. The results indicated that, compared with the control group, the level of 8-OHdG in the DM group increased, suggesting an increase in the levels of DNA damage and oxidative stress, but it decreased in the HUcMSC treatment group, suggesting a decrease in DNA damage and oxidative stress (Fig. 7f). These results showed that HUcMSC treatment could effectively alleviate DNA oxidative damage caused by diabetes.

To determine the expression of DNA damage effectors in each group, we performed immunohistochemical staining of renal tissue from diabetic rats. The results showed that, compared with the control group, the expression of p53, p-p53, CHK2, and p-CHK2 in the DNA damage pathway was increased in the DM group, while the expression of these effectors was significantly decreased after HUcMSC treatment (Figs. 8a and 8b). The western blot results showed that the expression of p53 and p-p53 was increased in the renal tissue of diabetic rats but was decreased in the MSC group (Fig. 8c). These results illustrated that HUcMSCs could ameliorate DNA damage in diabetic rats.

3 Discussion

During the development of T2DM, changes in ROS and metabolism in a high-glucose environment lead to DNA damage and genomic instability. DNA oxidative stress damage caused by metabolic disorders in diabetes has obvious characteristics, but the specific molecular mechanism is not clear. 8-OHdG is an oxidative nucleoside of DNA. 8-OHdG is the most direct and sensitive biological indicator of oxidative DNA damage. It can directly reflect the degree of oxidative DNA damage and the level of oxidative stress in the body (Bruner et al., 2000). Previous studies have shown that the levels of 8-OHdG in the blood and urine in the T2DM group are much higher than those in a normal control group (Leinonen et al., 1997; Pestieau et al., 2011). In our study, we showed that, in the diabetes rat model, the level of 8-OHdG in the blood was significantly higher than that in the control group. After treatment with HUcMSCs, the level of 8-OHdG decreased significantly. These results indicate that oxidative DNA damage is closely related to the treatment of diabetic complications, and it is critical to further clarify its exact molecular mechanism.

Many studies have proven that the changes in blood glucose and insulin associated with IGF1 and its receptor have a great impact on diabetes. Hypoglycaemic drugs may also affect the levels of serum IGF1 and its binding proteins by affecting blood glucose and insulin secretion in patients. In many animal studies, renal IGF1 protein levels were elevated in DM, but serum and renal *IGF1* messenger RNA (mRNA) levels were decreased (Segev et al., 1997; Landau et al., 2001;

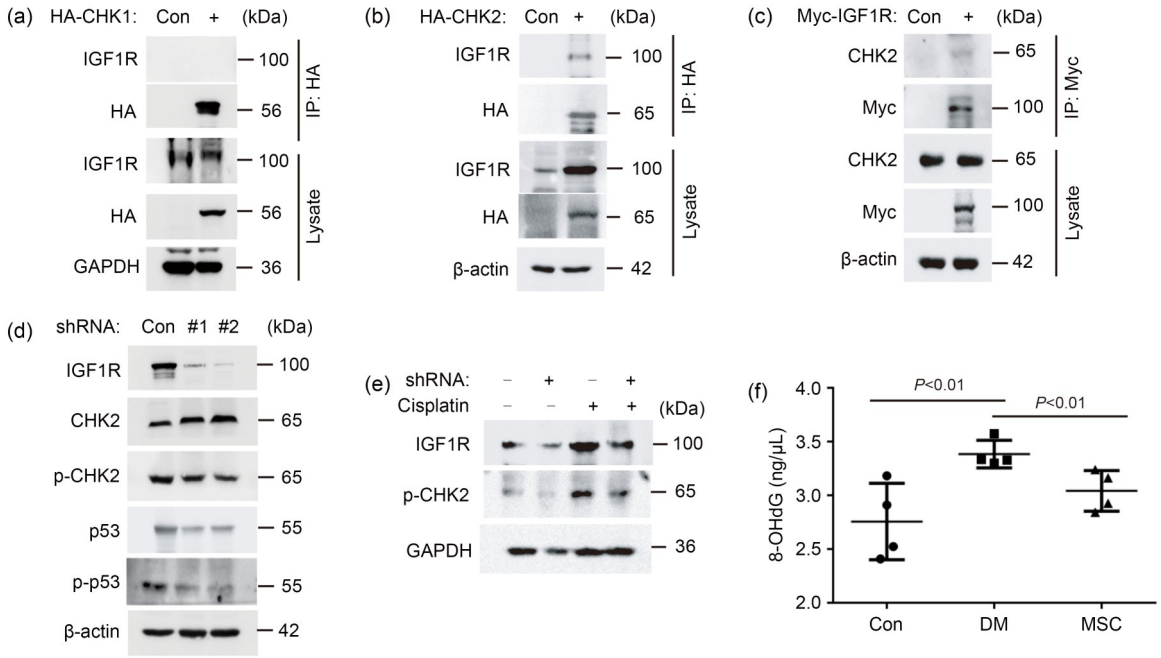

Fig. 7 Insulin-like growth factor 1 receptor (IGF1R) involved in the DNA damage repair pathway. (a) Cells were transfected with hemagglutinin (HA)-checkpoint kinase 1 (CHK1). HA-CHK1 was purified on anti-HA-agarose, and then coimmunoprecipitation with endogenous IGF1R was measured by anti-IGF1R antibodies. (b) Cells were transfected with HA-CHK2. HA-CHK2 was purified on anti-HA agarose, and then coimmunoprecipitation with endogenous IGF1R was measured by anti-IGF1R antibodies. (c) Lysates from HEK293T cells expressing Myc-IGF1R were subjected to immunoprecipitation and western blot analysis using the indicated antibodies. (d) In cells stably expressing control or IGF1R short hairpin RNA (shRNA), western blot was used to examine the DNA damage pathway factors CHK2, protein 53 (p53), phosphorylated CHK2 (p-CHK2), and phosphorylated p53 (p-p53). (e) Cells stably expressing the control or indicated shRNA were treated with vehicle or 20 μmol/L cisplatin for 24 h before being harvested. Cell lines were analysed with p-CHK2 antibodies. (f) Measurement of 8-hydroxydeoxyguanosine (8-OHdG) in the serum of rats in each group (control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC)). All values are presented as mean±standard deviation (SD), *n*=4. Experiments were repeated three times independently with similar results; data of one representative experiment are shown. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IP: immunoprecipitation.

Li et al., 2018; Gurevich et al., 2021). This local increase in renal IGF1 has been suggested to play a role in mediating the development of diabetic kidney disease. Raz et al. (2003) showed that, following IGF1 administration, renal phosphorylated AKT (p-AKT), phosphorylated ribosomal protein S6 (p-rpS6), phosphorylated mitogen-associated protein kinase (p-MAPK), and IGF1R levels increased more markedly in diabetic animals, suggesting increased sensitivity to IGF1 in DM. Moreover, activated IGF1R maintained genomic integrity and enhanced DNA repair through HR in mesangial cells (Yang et al., 2005). In our study, we found that the levels of IGF1R, p-CHK2, and p-p53 in diabetic rats were notably higher than those in control rats. After treatment with HUcMSCs, the levels of IGF1R, p-CHK2, and p-p53 decreased significantly. Therefore, studying the regulatory mechanism between DNA damage repair and IGF1R is likely to provide an unprecedented method for the prevention and treatment of diabetes and its complications.

Metformin is a first-line drug for the T2DM treatment. Metformin can reduce DNA damage and maintain genome stability by stimulating DNA damage repair through homologous end connections, HR, and nucleotide excision repair pathways. Variation in ATM can change the effect of metformin on blood glucose. The ATM pathway regulates insulin resistance and is mediated by p53 phosphorylation. The inactivation of ATM can also inhibit the function of glucose transporter 4 (GLUT4) and affect insulin signal transduction (Armata et al., 2010). The poly(adenosine diphosphate-ribose) polymerase (PARP) protein is a DNA damage sensor. PARP inhibitors can promote wound healing in diabetes patients and improve the progression of diabetic nephropathy by reducing the levels of inflammation, oxidative stress, and fibrosis (Zakaria et al.,

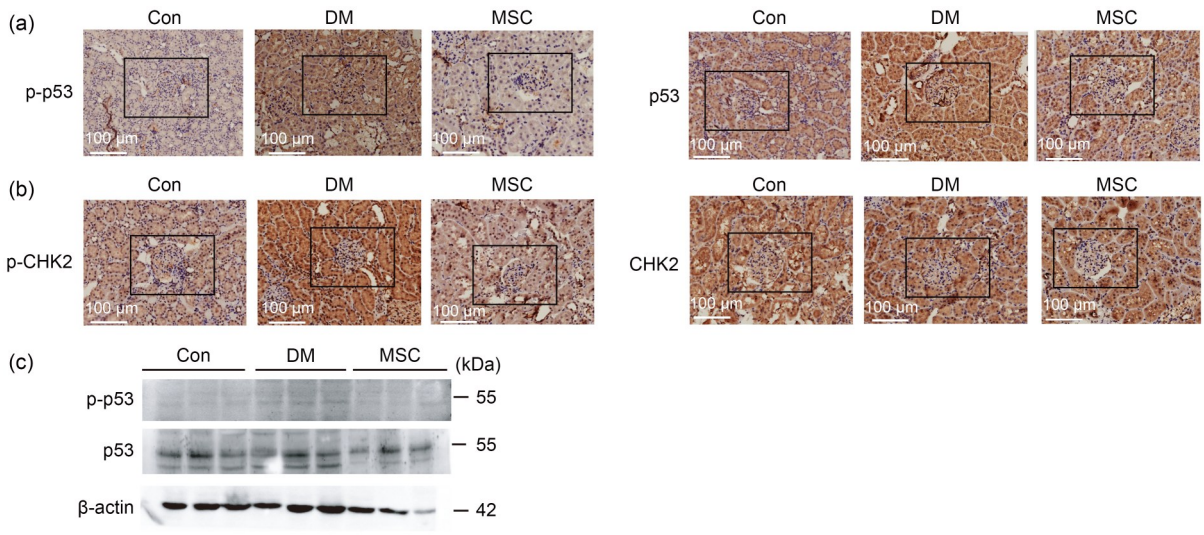

Fig. 8 Increased phosphorylated checkpoint kinase 2 (p-CHK2) and phosphorylated protein 53 (p-p53) protein expression levels in streptozotocin (STZ)-induced diabetic kidney tissue. (a, b) Expression of p53, p-p53, CHK2, and p-CHK2 in the kidney tissue of diabetic rats in each group (control (Con), diabetes mellitus (DM), and mesenchymal stem cell (MSC)) was examined by immunohistochemistry (IHC). Black borders indicate glomerulus. (c) Western blot analyses of p-p53 and p53 in the kidney tissue of rats in each group.

2017; Zhou et al., 2017; Lahiguera et al., 2020). Umbilical cord MSCs are adult stem cells with multiple differentiation potential. A previous study has shown that MSC infusion could improve insulin resistance in T2DM (Si et al., 2012). MSCs could also alleviate insulin resistance in the liver by improving glucose metabolism (Xie et al., 2017). The mechanism of MSCs is related to the regulation of inflammation and macrophage polarization (Bernardo and Fibbe, 2013). MSCs can enhance autophagy (Jia et al., 2018), but this mechanism cannot fully explain their effect. The latest research shows that oxidative stress caused by overload of iron ion content and imbalance of antioxidant system is the classic pathway of ferroptosis, which is involved in the survival of MSCs after transplantation and reduces the repair efficacy of MSCs (Xu et al., 2023). In our study, p-CHK2 and p-p53, renal glomeruli, and mesangial cells were decreased after umbilical cord stem cell treatment. This finding indicates that the treatment of diabetes and its complications with umbilical cord stem cells is closely related to the repair of DNA damage. CHK2 can be used as a new target for treating diabetes and its complications.

Our research had some limitations. Although we examined renal function changes such as serum Scr, BUN, UA, and cystatin C to determine the role of HUcMSCs, we did not measure 24-h urinary protein, which is also a diagnostic biomarker of diabetic nephropathy. In addition, we did not evaluate donor cell distribution, differentiation, or migration after stem cell therapy because of a lack of efficient markers of transplanted MSCs.

4 Conclusions

HUcMSCs downregulated the expression of IGF1/IGF1R in the renal tissue of diabetic rats, inhibited the activity of the target genes *CHK2* and *p53*, reduced apoptosis, and improved diabetic nephropathy. The regulatory effect of HUcMSCs was mediated by the IGF1R-CHK2-p53 signaling pathway. Our findings provide novel evidence supporting the use of HUcMSCs in the treatment of diabetes and suggest a prospective strategy for the clinical treatment of diabetic vascular complications.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

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Author contributions

Hao ZHANG, Bo HU, and Peicheng LI conducted the animal experiments. Xinshu WANG, Yierfan ABUDUAINI, and Lei LI contributed to the cellular and molecular experiments. Hongmei ZHAO and Zhongmin LIU contributed to HUcMSC preparation and quality control. Hongmei ZHAO, Ayinaer JIEENSIHAN, and Xishuang CHEN contributed to the immunohistochemical analyses. Shiyu WANG and Nuojin GUO contributed to the pathological analyses. Zhaosheng TANG and Hua WANG provided ideas and designed this study. Hao ZHANG and Yuntong YANG contributed the prepared figures. Jian YUAN and Yunhui LI drafted this manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Hao ZHANG, Xinshu WANG, Bo HU, Peicheng LI, Yierfan ABUDUAINI, Hongmei ZHAO, Ayinaer JIEENSIHAN, Xishuang CHEN, Shiyu WANG, Nuojin GUO, Jian YUAN, Yunhui LI, Lei LI, Yuntong YANG, Zhongmin LIU, Zhaosheng TANG, and Hua WANG declare that there are no potential conflicts of interest in the study.

All experimental procedures were approved by the Ethics Committee of Animal Experiments of Shanghai Zhanyuan Biotechnology Co., Ltd. (cooperative unit of Tongji University, Shanghai, China) (No. SHCQ-20200102). This study was carried out in strict accordance with internationally accepted guidelines regarding animal welfare and was conducted according to the guidelines of the Chinese Council on Animal Care.

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Supplementary information

Materials and methods