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STEM CELLS

REGENERATIVE MEDICINE

Mesenchymal stem cells elicit macrophages into M2 phenotype via improving transcription factor EB-mediated autophagy to alleviate diabetic nephropathy

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Abstract

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease. Chronic inflammation is recognized as a key causal factor in the development and progression of DN, and the imbalance of M1/M2 macrophages (Mφ) contributes to this process. Mesenchymal stem cells (MSCs) have been reported to prevent renal injuries via immune regulation in diabetic models, but whether these benefits are owing to the regulation of Mφ, and the underlying signaling pathways are unknown. Here, we showed that MSCs elicited Mφ into M2 phenotype and prevented renal injuries in DN mice, but these effects were abolished when the Mφ were depleted by clodronate liposomes (Lipo-Clod), suggesting that Mφ were necessary for renal protection of MSCs in DN mice. Moreover, the MSCs promoted M2 polarization was attributable to the activation of transcription factor EB (TFEB) and subsequent restore of lysosomal function and autophagy activity in Mφ. Furthermore, in vivo adoptive transfer of $M\varphi^{in\ vivo}$ (Mφ from DN + MSCs mice) or $M\varphi^{MSCs}$ (Mφ cocultured with MSCs in vitro) to DN mice improved renal function. While, TFEB knockdown in Mφ significantly abolished the protective role of $M\varphi^{MSCs}$. Altogether, these findings revealed that MSCs suppress inflammatory response and alleviate renal injuries in DN mice via TFEB-dependent Mφ switch.

KEYWORDS
diabetic nephropathy, lysosome-autophagy, macrophages polarization, mesenchymal stem cells, TFEB

1 | INTRODUCTION

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease, affecting ~30% of type 1 and type 2 diabetic patients.[1,2] Recently, chronic inflammation is recognized as a key contributing factor in the progression of DN.[3] Macrophages (Mφ) are important immune cells, and the imbalance of M1/M2 Mφ phenotypes contributes to the inflammation in DN.[4] Therapeutic strategies that can directly or indirectly inhibit M1 Mφ polarization have shown great potentials to ameliorate renal injuries in diabetes.[5,6]

Advances in regenerative medicine have uncovered a promising potential of mesenchymal stem cells (MSCs) for kidney repair, which primarily resides in their remarkable anti-inflammatory ability.[7,8] More importantly, increasing evidences suggest that the immunomodulatory effects on Mφ is also a critical mechanism in MSCs mediated amelioration of inflammation-related disease.[9] We have previously reported that MSCs prevent renal injuries via immune regulation in diabetic rats, in which MSCs elicit Mφ into M2 phenotype and restore the homeostasis of the immune microenvironment.[10] However, the specific mechanisms are still unclear.

An area that has garnered substantial focus is autophagy, a highly conversed intracellular degradation system associated with maintenance of cellular homeostasis.[11] Several studies have shown that impaired or deficient Mφ autophagy promotes Mφ polarization into M1 phenotype, leading to hepatic inflammation, fibrosis, and systemic insulin resistance in obese mice.[12,13] Moreover, a recent study has demonstrated that Mφ develop a progressive autophagy dysfunction in DN rats.[14] However, the triggers of this impairment are not well defined and whether stimulation of Mφ autophagy can be renal-protective remains unclear. It is demonstrated that a critical factor for autophagy activation is the functional status of Mφ lysosomes. Since lysosomes govern the overall degradative capacity of cells including autophagosome processing, we hypothesized that the development of high glucose (HG)-induced lysosomal dysfunction may be an important contributor to the observed autophagy deficiency in diabetic Mφ.[15]

It has been discovered that the lysosome-autophagy pathway is itself under the control of a "master regulator" called transcription factor EB (TFEB). Activated TFEB not only generates autophagosomes, but also accelerates their delivery and clearance by lysosomes via increase of lysosomal biogenesis.[16] Researchers have showed that overexpression of TFEB in Mφ reverses the autophagy dysfunction in atherosclerotic plaques, blunts Mφ apoptosis, and secretion of pro-inflammatory interleukin (IL)-1β, leading to reduced atherosclerosis.[17] Nevertheless, it remains unclear whether TFEB mediated augment of Mφ lysosome-autophagy can effectively combat DN.

Considering the critical role of TFEB mediated autophagy in Mφ polarizaiton, and the latter is related to MSCs mediated immune regulation, in this study, we tested the hypothesis that MSCs alleviated renal injuries in DN mice relying on the augment of TFEB-mediated lysosome-autophagy in Mφ.

Significance statement

Mesenchymal stem cells (MSCs) have been reported to prevent renal injuries via immune regulation in diabetic models; however, the mechanisms remain to be elucidated. In this study, depletion of Mφ abolished the renal protective role of MSCs, convincing the indispensable role of Mφ. Moreover, adoptive transfer of MSCs-educated Mφ conferred renal protection in diabetic nephropathy (DN) mice, while transcription factor EB knockdown in Mφ abolished the effects. This study provides novel insights into MSCs-based immune regulation, and provides scientific evidence for clinical application of MSCs in DN disease.

2 | MATERIALS AND METHODS

2.1 | Animal models

Male BALB/c mice (6- to 8-week-old) were housed in the animal center of West China Hospital, Sichuan University in accordance with the Guide for the Care and Use of Laboratory Animals. All experimental procedures were approved by the Animal Use Subcommittee at the Sichuan University, China. Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ; 150 mg/kg), and the diagnostic criteria for diabetes mellitus (DM) are based on blood glucose level ≥ 16.7 mmol/L. For MSCs treatment, MSCs were injected via the tail vein (5×10^5 cells in 0.1 mL saline, once every 2 weeks), from the fourth week for three times after DM is established (Figure 3A). After 12 weeks of diabetes, all the animals were sacrificed by cervical decapitation for the further study.

2.2 | Isolation and analysis of Mφ

Peritoneal Mφ were collected from mice according to a previous report.[10] Briefly, mice were anesthetized, and injected into the peritoneal cavity with 6 mL of DMEM. The fluid was centrifuged (350*g* for 5 minutes at 4°), and resuspended in DMEM medium (15 fetal bovine serum [FBS], penicillin 100 U/mL, streptomycin 100 mL). After incubation at 37°C in a 5% CO_2 humidified incubator for 2 hours to enable macrophages adhere before removing the suspending cells. To analyze the Mφ infiltrated in kidney, kidney tissues were minced and dissociated into single-cell suspension using collagen IV (1 mg/mL) added with DNase (0.1 mg/mL), and the phenotype was analyzed by flow cytometry using mAbs: F4/80-APC (Biolegend, San Diego, California), CD206-PE (Biolegend), and CD11c-fluorescein isothiocyanate (FITC) (Abcam, Cambridge, Massachusetts).

2.3 | Histopathology and immunofluorescence

Kidney tissues were fixed immediately in 10% buffered formalin solution after harvest and were embedded in paraffin to make tissue sections. Then the sections were subjected to hematoxylin-eosin (HE), periodic acid Schiff (PAS), and Masson's trichrome staining. For immunofluorescent analysis, frozen sections (5 mm thick) of kidney tissue were blocked using 5% bovine serum albumin for 30 minutes and then incubated overnight at 4°C with anti-F4/80-FITC, anti-Lamp1, and anti-TFEB antibodies. Slides were then correspondingly incubated with secondary antibody at 37°C for 1 hour, and washed in phosphate-buffered saline (PBS) for four times. The cell nuclei were stained with DAPI for 5 minutes.

2.4 | Depletion of Mφ in mice

To deplete the endogenous Mφ in mice, mice were intraperitoneally injected with Lipo-Clod (0.2 mL/25 g bodyweight; once every 4 days, FormuMax Scientific, Palo Alto, California) or Lipo-PBS (nondepleted control mice) from the fourth week after DM is established until the end of the experiment for about 39 times. At the end of the experiment, the efficiency of Mφ depletion in kidney was analyzed by flow cytometry