Infiltrating macrophages in diabetic nephropathy promote podocytes apoptosis via TNF-α-ROS-p38 MAPK pathway

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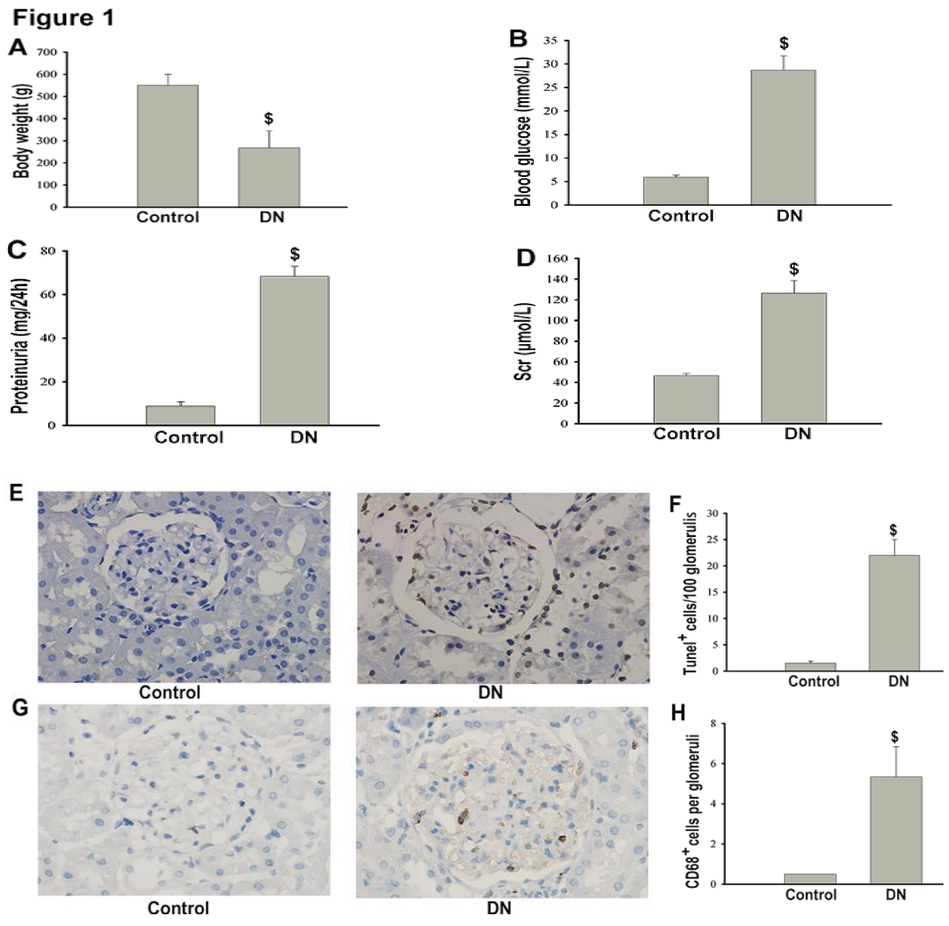
Background

Macrophages infiltration has been linked to the pathogenesis of diabetic nephropathy (DN). However, how infiltrating macrophages affect the progression of DN is unknown. Though infiltrating macrophages produce pro-inflammatory mediators and induce apoptosis in a variety of target cells, there are no studied in podocytes. Therefore, we tested the contribution of macrophages to podocytes apoptosis in DN.

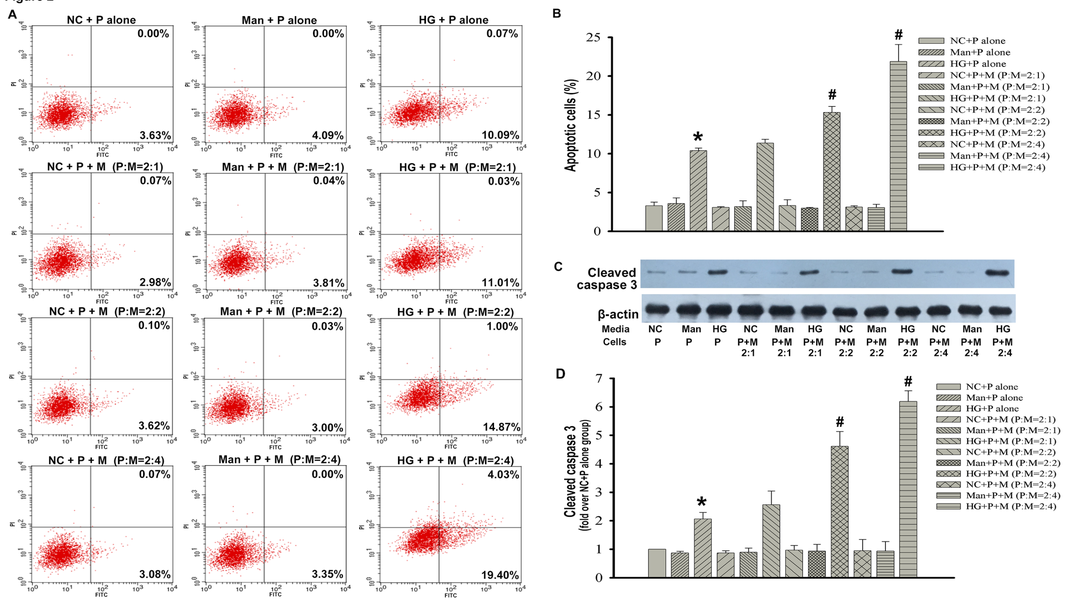
Results

1. Podocytes apoptosis and macrophage infiltration in STZ-induced DN rats



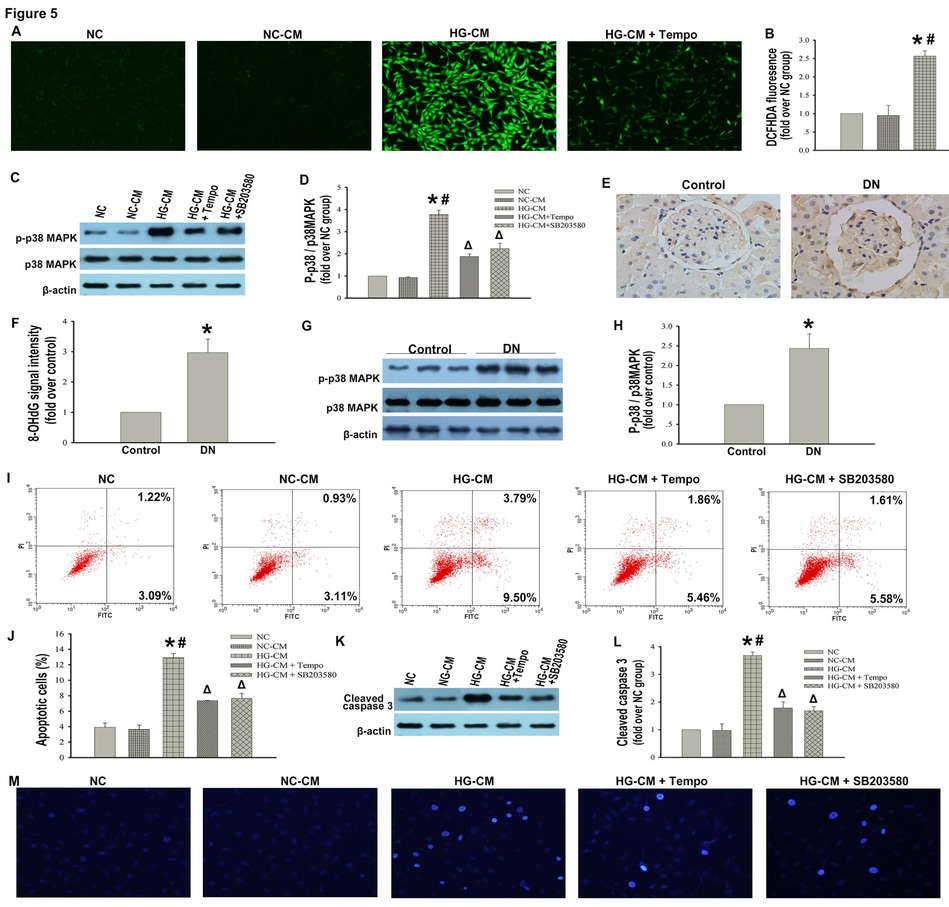
$P<0.05 vs. control group.

2. Macrophages induced podocytes apoptosis in the condition of high glucose



*P<0.05 vs. NC+P alone group; #P<0.05 vs. HG+P alone group.

5. Apoptosis in podocytes triggered by macrophages was ROS–p38 MAPK dependent

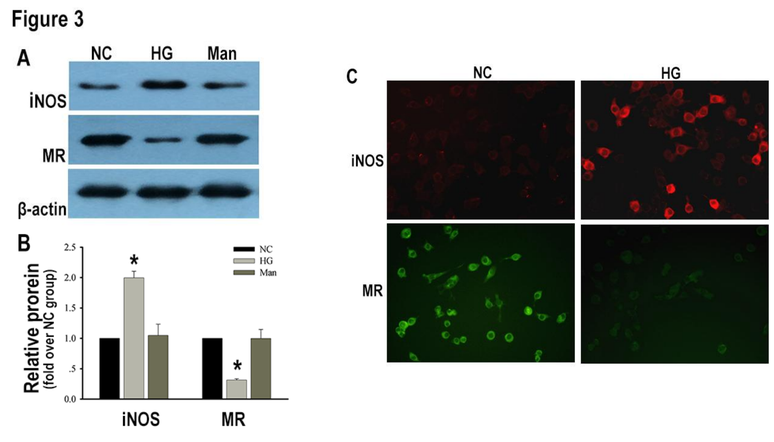


*P<0.05 vs. NC group; #P<0.05 vs. NC-CM group; ΔP<0.05 vs. HG-CM group.

Materials and Methods

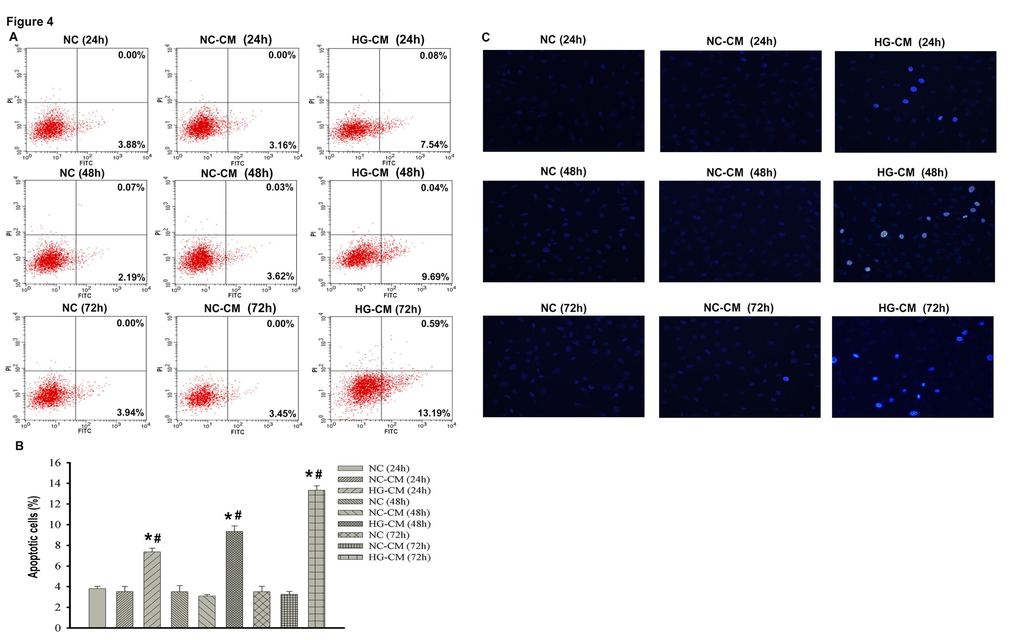
In vivo, DN model rats were established by intraperitoneal injection with STZ. Rats were sacrificed at 18w for histological and molecular analysis. Macrophages infiltration was detected by CD68 IHC stain. 8-OHdG stain was used to detect reactive oxygen species (ROS) in podocytes. In vitro, RAW 264.7 cells were co-cultured with podocytes with or without 25mM high glucose treatment in a transwell co-culture system. In addition, podocytes were cultured in conditioned media from normal glucose stimulated macrophage (NC-CM), and CM from 25mM high glucose stimulated macrophage medium (HG-CM) with or without ROS inhibitors (Tempo), p38 MAPK inhibitor(SB203580), anti-TNF-α neutralizing antibody or IgG1 Isotype control. Annexin V-FITC/P staining were used to analyze apoptotic rate of podocytes. ROS was measured using DCFHDA stain. Proteins expression of cleaved caspase-3, p38MAPK (p38 mitogen-activated protein kinases), p-p38MAPK, and phenotypic markers of M1/M2 macrophage such as iNOS and MR were detected by Western Blot. TNF-α level in culture supernatant was detected by ELISA.

3. Macrophages displayed pro-inflammatory M1 activation under high glucose condition



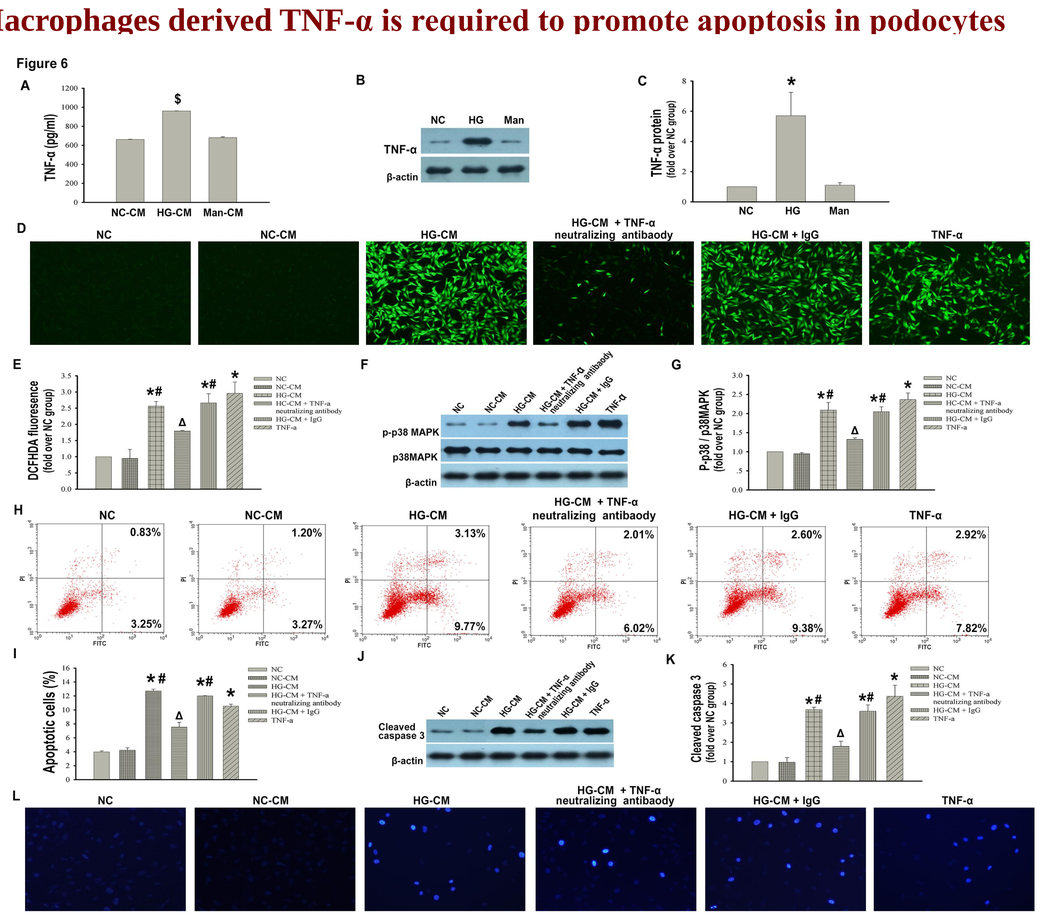
*P<0.05 vs. NC group.

4. Conditioned media from high glucose stimulated macrophages promoted podocytes apoptosis



*P<0.05 vs. NC group; # P<0.05 vs. NC-CM group.

6. Macrophages derived TNF-α is required to promote apoptosis in podocytes



*P< 0.05 vs. NC group; #P < 0.05 vs. NC-CM group; Δ P<0.05 vs. HG-CM group.

Conclusion

TNF-α released by high glucose activated M1 macrophages led to podocytes apoptosis via ROS-p38 MAPK pathway in diabetic nephropathy.

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