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Original Article

Proinflammatory-factors-carrying-microvesicles from monocytes induced by high glucose enhance the activation of HIF/VEGF pathway in human renal mesangial cells

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Abstract: Objectives: Renal mesangial expansion has been identified as a major factor contributing to glomerulosclerosis, a typical symptom of diabetic nephropathy. It is unclear whether microvesicles, known as a mediator for cross-talk between cells and organs, are involved in a profibrotic process. In this study, we are the first to investigate the effect of monocyte-derived microvesicles induced by high glucose on renal mesangial cells. Methods: THP1 cells were evoked by high glucose to generate microvesicles, quantified by ELISA. Glucose uptake by THP1 cells was measured using fluorescently-labeled deoxyglucose analog 2-NBDG as a probe. The contents of inflammatory cytokines in microvesicles were detected by western blot. The expressions of HIF-1α and VEGF in human renal mesangial cells after treatment with THP1-derived microvesicles were examined by western blot. Results: The glucose uptake by THP1 cells was significantly increased after high glucose treatment. High glucose significantly evoked MV generation, which contained increased protein level of IL-6, 8 and MCP-1. The expressions of HIF-1α and VEGF in HRMC were augmented by microvesicles. Conclusions: Our study indicates that monocyte-derived MVs induced by high glucose can carry proinflammatory factors, and enhance the expression of the HIF/VEGF pathway in human renal mesangial cells. Our findings may provide a novel potential mechanism in the progression of diabetic nephropathy.

Keywords: Microvesicles, high glucose, proinflammatory factors, renal mesangial cells

Introduction

Microvesicles (MVs) are small membrane vesicles, derived from many different cell types by a process of exocytic budding of the plasma membrane [5], and have been implicated in the pathogenesis of diabetic nephropathy (DN), which is one of the leading causes of mortality in diabetic patients. MVs may mediate the cross-talk between kidney and other organs under physiologic and pathological conditions. The role of MVs in kidney diseases is largely unclear [1], and the mechanism underlying MV production and secretion remains elusive. In this study, we used high glucose induced-MVs from THP1 cells to treat mesangial cells, in order to investigate the cross-talk between monocytes and renal glomerular mesangial cells for the first time. Our findings will provide a novel potential mechanism in the progression of diabetic nephropathy.

Materials and methods

Cell culture

Human acute monocytic leukemia cell line-THP1 cells were obtained from the Cell Bank of Chinese Academy of Science (Shanghai, China), and Human renal mesangial cells (HRMCs) were purchased from American Type Culture Collection (ATCC® CRL-1927™). Both cell lines were grown and maintained in RPMI 1640 supplemented with 10% FBS and 2 mM Glutamine and 1 mM pyruvate at 37°C in an atmosphere that contained 5% CO_2.

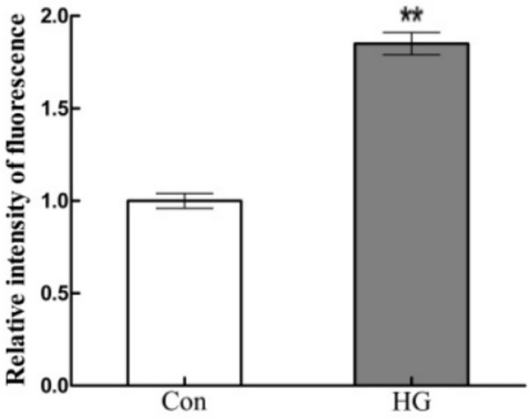

Figure 1. Glucose uptake after high glucose treatment. The uptake of glucose by THP1 cells was increased after high glucose treatment for 2 h. **P<0.01, vs control. N=4.

THP1 monocytes were treated by high glucose (30 mmol/L) for 20 h. The harvested MVs from cultured supernatants were used to treat HRMC for given time point (24 h, 48 h and 72 h).

Reagents and chemicals

Antibodies against IL-6, 8, MCP-1 was purchased from Tianjin SaierBio. Antibodies against VEGF and HIF-1α were purchased from Proteintech Group (Rosemont, IL, USA). Glucose Uptake Cell-Based Assay Kit was from Cayman, USA.

Glucose uptake assay

THP-1 cells were treated by high glucose (30 mmol/L) for 2 h. Glucose uptake cell-based assay kit was applied to directly detect the uptake of glucose by THP1 cells, using fluorescently-labeled deoxyglucose analog 2-NBDG as the probe, according to manufacturer's manual.

MV purification and quantification by ELISA

THP-1 cells were treated by high glucose (30 mmol/L) for 20 h. Culture supernatants were purified by differential centrifugation as previous description [3, 11]. Zymuphen MP-Activity ELISA kit was used to quantify total PS-positive MVs in culture supernatants prepared by low-speed centrifugation to remove cells. The assessment was performed according to the manufacturer's instruction with 20-fold dilutions of THP-1-conditioned media. Results are expressed as PS equivalents (nM PS eq).

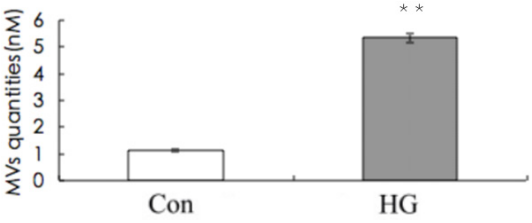

Figure 2. MV generation from THP1 cells by high glucose stimulation for 20 h. **P<0.01, vs control (n=4).

Immunoblots

Cells and MVs were extracted into a lysis buffer containing 20 mM Tris/HCl, Ph 7.4, 150 mM NaCl, 1 mM EGTA, 1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4. Samples containing the same amount of total protein were electrophoresed through a 10% SDS-polyacrylamide gel, followed by transfer to a nitrocellulose membrane (Bio-Rad). The membranes were then blocked with skim milk (5%, w:v) and probed with primary antibodies against human inflammatory cytokines in MVs (IL-8, IL-6 and MCP1), HIF1-α and VEGF in HRMC. Thereafter, detection was accomplished with horseradish peroxidaseconjugated secondary antibodies, to generate a chemiluminescent product. Signals were quantified using Image J densitometry software and normalized in each independent experiment to values from control cells.

Statistical analyses

All data were analyzed by SPSS 18.0. Normally distributed data are shown as mean ± SEM (n=4-6). Comparisons among three or more groups were performed by one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls (SNK) test, test, with P<0.05 considered significant. Comparisons between two groups used the Student's unpaired, two-tailed *t*-test. Comparisons between two groups of non-normally distributed data used Games-Howell test.

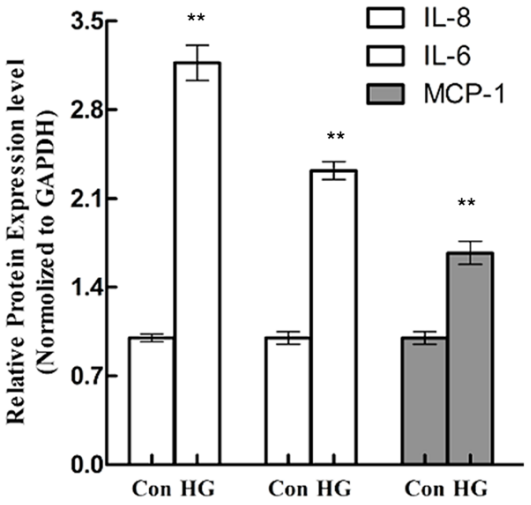

Figure 3. Inflammatory cytokine expression in MVs. Expression of IL-8, IL-6 and MCP-1 in MVs from high glucose-treated THP1 were increased significantly, compared to control group. **P<0.01, vs control (n=4).

Results

Glucose uptake

The uptake of glucose by THP1 cells was significantly increased by 1.85 times after high glucose treatment for 2 h (P<0.01), detected by Glucose Uptake Cell-Based Assay Kit, (**Figure 1**).

Quantities of MVs

We found that high glucose significantly increased total MV release from THP-1 monocytes after 20 h stimulation, a 4.6 fold increase, compared to the control (P<0.01), (**Figure 2**).

Expression of proinflammatory factors in MVs

Our findings showed that expression of IL-8, IL-6 and MCP-1 in MVs from high glucose-treated THP1 were increased significantly, compared to control group, examined by western blot. IL-8 expression in MVs was increased by 3 times, compared to control group (P<0.01) (**Figure 3**).

Expression of HIF-1A and VEGF in HRMC

We found that after MVs treatment for given timepoints, the expression of HIF-1α and VEGF in HRMC were significantly increased at 48 h and 72 h, compared to 0 h (P<0.01), (**Figure 4**).

Discussion

Microvesicles, sized 100 nm-1 μm, are members of the family of extracellular vesicles shed from the plasma membrane of activated or apoptotic cells. MVs are known to mediate important biological functions and to be increasingly involved in cell physiology and in many diseases, especially in oncology and cardiovascular diseases [10].

Diabetes is the primary cause for end-stage renal disease according to the United States Renal Data system. Mesangial expansion has been identified as a major factor contributing to glomerulosclerosis, which is a typical symptom of diabetic nephropathy. However, the role of MVs in kidney disease is largely unclear, and the mechanism underlying MV production and secretion remains elusive. Recently, Rodrigues et al. [7] found that type 2 diabetes patients with DKD presented with higher circulating microparticles-from platelets, leukocytes, endothelial cells and expressing tissue factor-which correlated with metabolic alterations. For the first time, we investigated the effect of MVs on the profibrotic progression of renal mesangial cells. Our findings show that high glucose induced-MVs from monocytes carried proinflammatory mediators (IL-6, IL-8 and MCP1), which in turn, significantly augmented the expression of HIF/VEGF in renal mesangial cells.

Hypoxia inducible factor (HIF) is a transcription factor consisting of α subunits and β subunits. During hypoxia, HIF forms a heterodimer complex, and translocates to the nucleus where it binds hypoxia response element (HRE) in the promoter sequences to numerous genes involved in maintaining cellular and tissue oxygen homeostasis including angiogenesis and cellular energy metabolism. HIF activities and downstream signaling are significantly altered in the diabetic kidney. HIF signaling is involved in profibrotic signaling [6]. Whether HIF is activated in the diabetic kidney and in what cell types is currently not fully elucidated. Our study indicated that HIF1α protein and downstream vascular endothelial growth factor signaling was increased in human renal mesangial cells, also seen in rat glomerular mesangial cells and type 2 diabetes patients [8, 9].

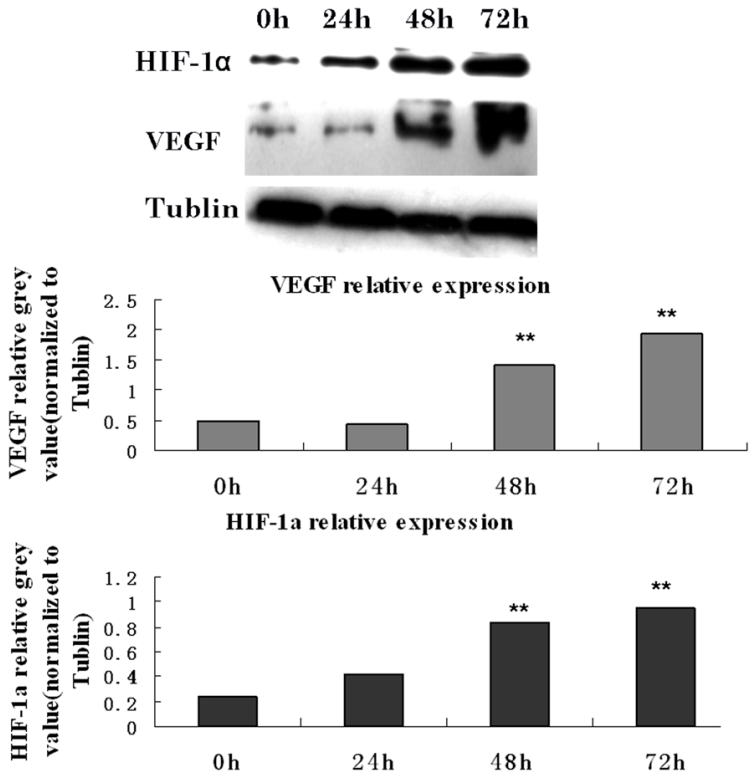

Figure 4. Expression of HIF-1α and VEGF in HRMC after MVs treatment. After MVs treatment for given timepoints, the expression of HIF-1α and VEGF in HRMC were significantly increased at 48 h and 72 h. **P<0.01 vs control (n=4).

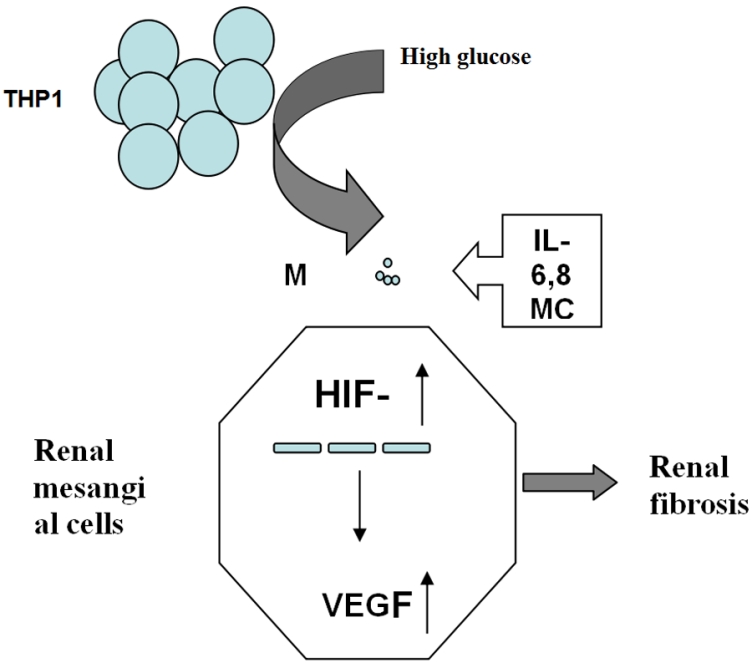

Figure 5. Schematic diagram. Our work indicates that high glucose can evoke microvesicle generation from THP1 cells, bearing proinflammatory factors (IL-6, IL-8 and MCP1). The MVs can enhance the activation of HIF/VEGF in renal mesangial cells, which is one of the key components in the progression of diabetic glomerulofibrosis.

HIF-1α regulate cellular homeostasis under hypoxic conditions, but it also has pleiotropic effects in response to cellular stresses at normoxia [2]. Persson et al [6] found that elevated glucose levels induce HIF activity by a hypoxia-independent mechanism. Our study found that expression HIF/VEGF in renal mesangial cells was enhanced by proinflammatory mediators from MVs at normoxia.

VEGF influences renal function through angiogenesis. In normal glomerulus, tight homeostatic balance is maintained between the levels of VEGF-A isoforms produced by podocyte cells, and VEGF receptors (VEGFRs) expressed by glomerular endothelium, mesangium, and podocytes. Upregulated VEGF autocrine effect on podocytes is exacerbated by hyperglycemia [4]. In the present study, MVs with proinflammatory factors could increase the VEGF expression leading to glomerulosclerosis.

Taken together, our study shows that renal mesangial HIF/VEGF expression is increased after the stimulation of monocyte-derived MVs with proinflammatory factors, which may play a potential role in glomerulosclerosis, illustrated in **Figure 5**. Further studies will be needed to investigate the detailed mechanism.

Acknowledgements

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Disclosure of conflict of interest

None.

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