Interleukin-1β mediates high glucose induced phenotypic transition in human aortic endothelial cells

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Objectives: *Previous studies have shown that high glucose (HG) induced endothelial cell (EC) damage via a phenotypic transition of EC. There is increasing evidence suggesting the role of inflammatory cytokines in mediated HG-induced EC damage. However, little is known about the potential role of interleukin-1β (IL-1β) in the process. The aim of present study was to investigate whether IL-1β mediated HG–induced phenotypic transition in human aortic endothelial cells (HAECs) and to determine the possible underlying mechanism.*

Methods: *Primary HAECs were exposed to normal glucose (NG, 5.5 nM), high glucose (HG,30 nM), IL-1β (10 ng/ml), HG + IL-1β (10 ng/ml) and HG + anti-IL-1β antibodies (1000 ng/ml) or HG + IL-1β small interfering RNA (siRNA). Pathological changes were investigated using confocal microscopy and electron microscopy. Confocal microscopy was performed to detect the co-expression of CD31 and fibroblast specific protein 1 (FSP1).*
To study the effect of protein kinase C-β (PKCβ) activation on IL-1β in HAECs, HAECs were stimulated with 30 nM PMA (PKCβ activator) and 0.3 uM PKCβ inhibition
(LY317615) for 48 h in the NG or HG group. The expressions of PKCβ and IL-1β were detected by RT-PCR and Western blot. And the concentration of IL-1β in the supernatant of HAECs was mesured by ELISA. The expressions of FSP1, a-SMA and CD31 were detected by Western blot.

Results:

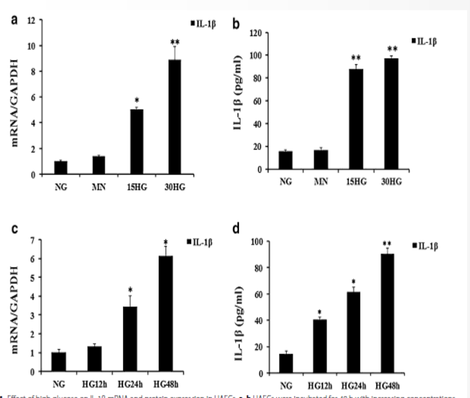

Fig. 1 Effect of high glucose on IL-1β mRNA and protein expression in HAECs. **a**, **b** HAECs were incubated for 48 h with increasing concentrations of glucose (5.5, 15, 30 mM). Mannitol was used as a control for hyperosmolarity. **c**, **d** HAECs were grown in a 30 mM glucose medium for 12–48 h. IL-1β protein level was measured in the supernatant using ELISA and IL-1β mRNA level was assayed by quantitative RT-PCR. The data are expressed as the mean ± SD. Experiments were repeated at least three times. *NG* normal glucose (5.5 mM), *HG* high glucose (30 mM), *MN* 5.5 mM glucose + 24.5 mM mannitol. *$P < 0.05$ vs. MN or NG, **$P < 0.01$ vs. MN or NG

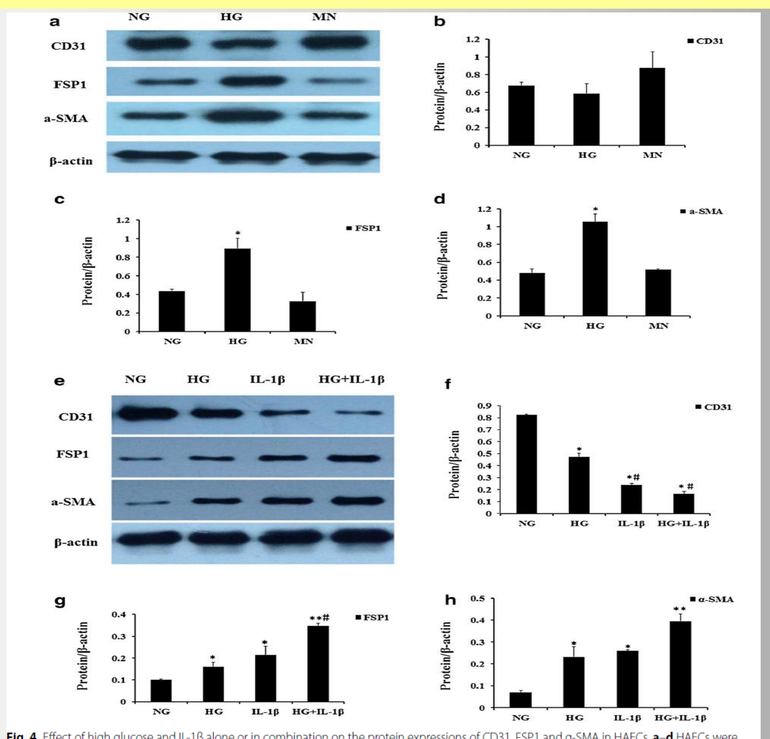

Fig. 4 Effect of high glucose and IL-1β alone or in combination on the protein expressions of CD31, FSP1 and α-SMA in HAECs. **a**–**d** HAECs were incubated for 48 h with NG and HG. Mannitol was used as a control for hyperosmolarity. Representative western blots (**a**) and quantitative determinations of CD31, FSP1 and α-SMA protein levels (**b**–**d**) are presented. **e**–**h** HAECs were treated for 48 h with NG, HG, IL-1β (10 ng/ml)and HG in the presence of the IL-1β (10 ng/ml). Representative western blots (E) and quantitative determinations of CD31, FSP1 and α-SMA protein levels (**f**–**h**) are presented. The data are expressed as the mean ± SD. Experiments were repeated at least three times. *NG* normal glucose (5.5 mM), *HG* high glucose (30 mM), *MN* 5.5 mM glucose + 24.5 mM mannitol, IL-1β (10 ng/ml), HG + IL-1β: high glucose (30 mM) + IL-1β (10 ng/ml) *$P < 0.05$ vs. MN or NG, **$P < 0.01$ vs. NG, #$P < 0.05$ vs. HG

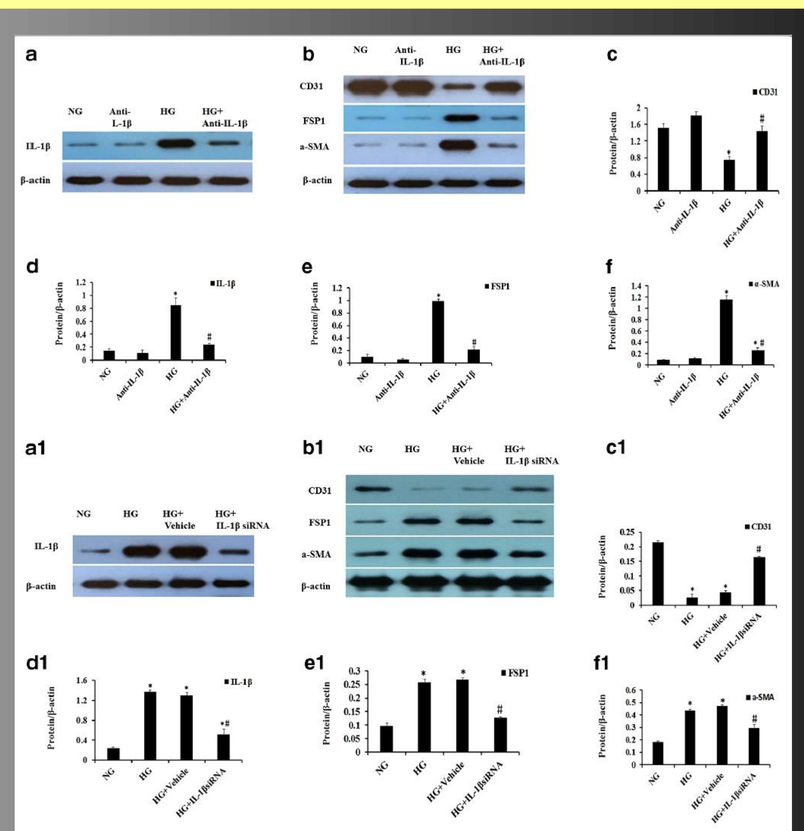

Fig. 6 The influence of blocking IL-1β treatment on the protein expressions of CD31, FSP1, a-SMA, and IL-1β. (**a**–**f**) HAECs were incubated for 48 h with anti-IL-1β antibodies (1000 ng/ml) in the presence of NG or HG. (**a1**–**f1**) We performed gene-silencing experiments using transfection with siRNA specific for IL-1β. The protein expressions of IL-1β, CD31, FSP1 and α-SMA were assessed by western blotting. The data are expressed as the mean ± SD. Experiments were repeated at least three times. *NG* normal glucose (5.5 mM), *HG* high glucose (30 mM). Anti-IL-1β: anti-IL-1β antibodies (1000 ng/ml). *$P < 0.05$ vs. NG or anti-IL-1β, #$P < 0.05$ vs. HG or HG +Vehicle

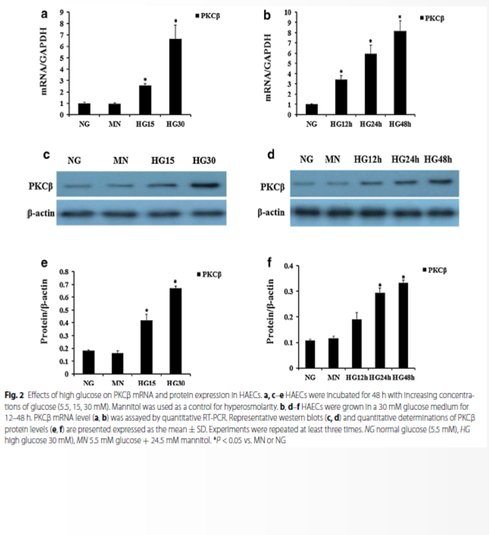

Fig. 2 Effects of high glucose on PKCβ mRNA and protein expression in HAECs. **a**, **c**–**e** HAECs were incubated for 48 h with increasing concentrations of glucose (5.5, 15, 30 mM). Mannitol was used as a control for hyperosmolarity. **b**, **d**–**f** HAECs were grown in a 30 mM glucose medium for 12–48 h. PKCβ mRNA level (**a**, **b**) was assayed by quantitative RT-PCR. Representative western blots (**c**, **d**) and quantitative determinations of PKCβ protein levels (**e**, **f**) are presented expressed as the mean ± SD. Experiments were repeated at least three times. *NG* normal glucose (5.5 mM), *HG* high glucose 30 mM), *MN* 5.5 mM glucose + 24.5 mM mannitol. *$P < 0.05$ vs. MN or NG

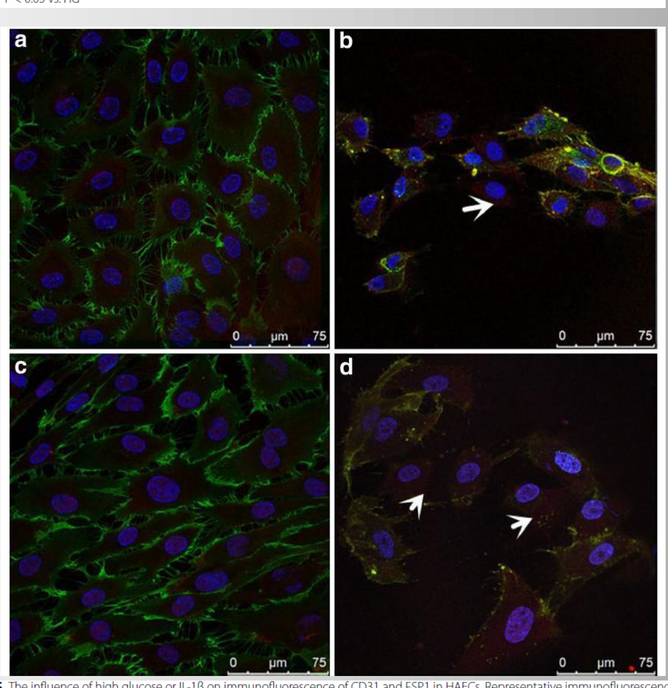

Fig. 5 The influence of high glucose or IL-1β on immunofluorescence of CD31 and FSP1 in HAECs. Representative immunofluorescence images showing CD31 (*green*), FSP1 (*red*) labeling and DAPI (*blue*) stains nuclei. **a** Normal ECs monolayers displayed a cobble stone morphology. **b** A merge of the three images revealed some cells populations that acquired a spindle-shaped morphology and lost CD31 expression (*white arrow*). **c** HAECs exposure to IL-1β alone for 48 h acquired a spindle-shaped morphology. **d** High glucose and IL-1β in combination resulted in decreased CD31 (*the left white arrow*) and increased FSP1staining (*the right arrow*). **a** normal glucose (5.5 mM) group, **b** high glucose (30 mM) group for 48 h; **c** treatment with a normal glucose (5.5 mM) + IL-1β (10 ng/ml) treatment for 48 h, **d** treatment with a high glucose (30 mM) + IL-1β (10 ng/ml) treatment for 48 h. *Scale bar*, 75 μm

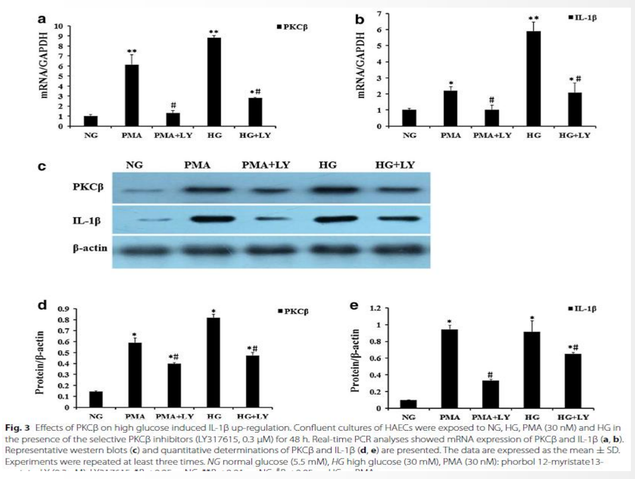

Fig. 3 Effects of PKCβ on high glucose induced IL-1β up-regulation. Confluent cultures of HAECs were exposed to NG, HG, PMA (30 nM) and HG in the presence of the selective PKCβ inhibitors (LY317615, 0.3 μM) for 48 h. Real-time PCR analyses showed mRNA expression of PKCβ and IL-1β (**a**, **b**). Representative western blots (**c**) and quantitative determinations of PKCβ and IL-1β (**d**, **e**) are presented. The data are expressed as the mean ± SD. Experiments were repeated at least three times. *NG* normal glucose (5.5 mM), *HG* high glucose (30 mM), PMA (30 nM): phorbol 12-myristate13-

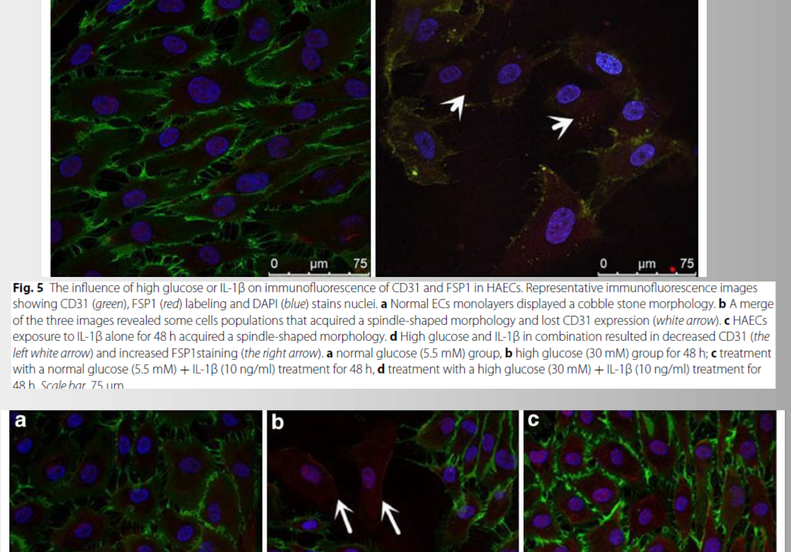

Fig. 7 Anti-IL-1β antibodies treatment inhibited high glucose-induced phenotypic transition of HAECs, asassessed by laser scanning confocal microscopy. Representative immunofluorescence images showing CD31 (*green*), FSP1 (*red*) labeling and DAPI (*blue*) stains nuclei. **a** Normal ECs monolayers displayed a cobble stone morphology. **b** A merge of the three images revealed some cells populations that acquired a *spindle-shaped* morphology and lost CD31 expression (*white arrow heads*). **c** The administration of anti-IL-1β antibodies treatment caused a reduction of these changes ($P < 0.05$). **a** normal glucose (5.5 mM) group, **b** high glucose (30 mM) group for 48 h; **c** treatment with a high glucose concentration (30 mM) + anti-IL-1β antibodies (1000 ng/ml) treatment for 48 h. Experiments were repeated three times. *Scale bar*, 75 μm.*$P < 0.05$ vs.HG

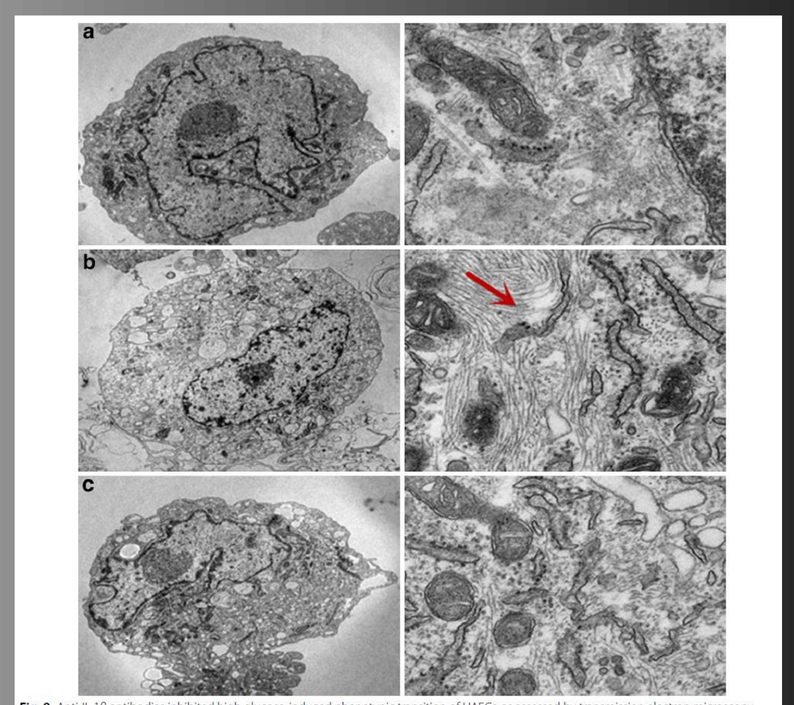

Fig. 8 Anti-IL-1β antibodies inhibited high glucose-induced phenotypic transition of HAECs, as assessed by transmission electron microscopy. Transmission electron microscopy depicts the change in cellular ultrastructure following HG (30 mM) exposure (left magnification ×10,000,vs right magnification ×40,000 in the same group). **a** It can be seen that normal HAECs present with few microfilaments and a rough endoplasmic reticulum. **b** After exposure to HG, microfilamentation and a swollen rough endoplasmic reticulum appeared in the cytoplasm. **c** These changes were attenuated by treatment with anti-IL-1β antibodies. **a** normal glucose (5.5 mM) group, **b** high glucose (30 mM) group for 48 h; **c** treatment with a high glucose concentration (30 mM) + anti-IL-1β antibodies (1000 ng/ml) treatment for 48 h

Conclusions: *Our fingdings suggested that HG-induced phenotypic transition of HAECs might require IL-β activation via the PKCβ pathway.*

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