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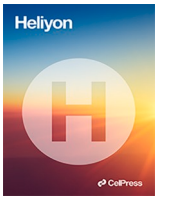

O-GlcNAcylation regulates HIF-1α and induces mesothelial-mesenchymal transition and fibrosis of human peritoneal mesothelial cells

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ABSTRACT

O-GlcNAcylation is a post-translational modification of proteins that regulates various biological processes. However, its involvement in peritoneal dialysis fibrosis remains unclear. This study aimed to investigate the impact of O-GlcNAcylation on human peritoneal mesothelial cells (HPMCs) cultured in control and high-glucose medium. To manipulate cellular conditions, we employed knockdown techniques targeting HIF-1α and OGT, along with the administration of pharmacological agents (PUGNAc, OSMI-1, MG-132, FG-4592, and HIF-1α inhibitor). Our findings revealed that elevated glucose levels increased global O-GlcNAcylation and the abundance of HIF-1α, α-SMA, fibronectin, and COL1A2. Conversely, the expression of E-Cadherin was decreased. Significantly, a positive correlation was observed between O-GlcNAcylation, HIF-1α, mesothelial-to-mesenchymal transition (MMT), and fibrosis in HPMCs. Notably, O-GlcNAcylation was found to regulate HIF-1α, thereby promoting MMT and fibrosis under high glucose conditions. Furthermore, we discovered that high glucose levels induced O-GlcNAcylation of HIF-1α, preventing its ubiquitination and proteasomal degradation. In summary, our study demonstrates the critical role of O-GlcNAcylation-mediated regulation of HIF-1α in MMT and fibrosis during peritoneal dialysis.

1. Introduction

Peritoneal dialysis (PD) serves as a crucial form of replacement therapy for individuals who are afflicted with end-stage renal disease. China, the USA, and Thailand are among the countries where the utilization of this therapy is experiencing a growth trend [1]. PD utilizes the semi-permeable membrane of the peritoneum for the elimination of toxins together with metabolic waste, as well as for maintaining equilibrium in terms of water, electrolytes, along with acid-base levels. Non-sugar-containing peritoneal dialysates still have many limitations in clinical application, including inadequate nutrition, unsuitability for patients with high metabolic rates,

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increased incidence of hypoglycemia, and metabolic disorders. Due to their safety and effectiveness, glucose-containing dialysates are most widely used. Nonetheless, continuous exposure to high-glucose dialysates can lead to mesothelial-mesenchymal transition (MMT), extracellular matrix accumulation, inflammation, and angiogenesis, resulting in peritoneal fibrosis, peritoneal dysfunction, and ultrafiltration failure [2,3].

O-GlcNAcylation, known as a form of post-translational modification, involves non-standard glycosylation, in which a singular O-linked N-acetylglucosamine (O-GlcNAc) is linked to the residues of serine/threonine amino acid present in proteins [4]. O-GlcNAcylation has been found to be essential for the control and modulation of gene expression, protein synthesis, metabolic reprogramming, as well as immune response [5]. High glucose levels can increase the global O-GlcNAcylation of human peritoneal mesothelial cells (HPMCs) [6], but the influence of O-GlcNAcylation on MMT as well as fibrosis of HPMCs is unclear. Previous reports have shown that Hypoxia-inducible factor-1α (HIF-1α) promotes the MMT of HPMCs as well as peritoneal fibrosis, and HIF-1α inhibition could effectively retard the advancement of peritoneal fibrosis and enhance peritoneal function [7–10]. Interestingly, it has been reported that O-GlcNAcylation can regulate HIF-1α degradation of breast cancer cells [11]. Accordingly, we speculate that the regulation of HIF-1α through O-GlcNAcylation may be related with the MMT of HPMCs as well as the fibrosis induced by high glucose.

2. Materials and methods

2.1. Antibodies & chemicals

The antibodies against O-linked N-acetylglucosamine (O-GlcNAc) (RL2, ab2739) were purchased from Abcam. Anti-OGT (F-12,sc-74546), anti- O-GlcNAcase (OGA) (G-12,sc-376429), anti–HIF–1α (28b,sc-13515), as well as anti-ubiquitin (P4D1 , sc-8017) were obtained from Santa Cruz Biotechnology. Anti-fibronectin (A12932), anti-COL1A2 (A5786), anti-E-Cadherin (A11509), anti-α-SMA (A17910), as well as anti-β-Actin (AC038) were purchased from Abclonal (China). Horseradish peroxidase (HRP)-conjugated secondary goat anti-rabbit (AK1007) and goat anti-mouse (AK801X) antibodies, as well as TRITC Goat anti-mouse antibody (GM200G-39C) were purchased from SUNGENE BIOTECH (China). The 4′-6-diamidino-2-phenylindole (DAPI) (C0065) was obtained from Solarbio (China) and PUGNAc (ab144670) was obtained from Abcam. OGT inhibitor OSMI-1 (T16409), MG-132 (T2154) and FG-4592 (T2515) were obtained from TargetMol. HIF-1α inhibitor (sc-205346) was purchased from Santa Cruz Biotechnology.

2.2. Cell culture

The HMrSV5 cell line, derived from HPMCs, was placed in Roswell Park Memorial Institute (RPMI) 1640 tissue culture medium (0.2 % glucose) with the supplementation of fetal bovine serum (10 %) as well as penicillin/streptomycin (1 %) in an environment containing 5 % CO_2 at the temperature of 37 °C. A low-glucose (LG) medium was used as the control medium. Upon reaching confluence, the cells were subsequently exposed to LG medium, high-glucose (HG) medium (1.5 % or 2.5 % glucose, corresponding to the glucose concentration of 1.5 % or 2.5 % peritoneal dialysate) or the same osmotic pressure mannitol control medium as high-glucose medium. As described in figure legends, different concentrations of pharmacological agents (PUGNAc, OSMI-1, MG-132, FG-4592 and HIF-1α inhibitor) were added to LG medium or HG (2.5 %) medium for 48 or 72 h.

2.3. HIF-1α and OGT knockdown based on siRNA transfection

When the HPMCs reached a confluence of 70–90 %, transfection of negative control (NC) siRNA (sense: UUCUCCGAACGUGUCACGUTT, antisense: ACGUGACACGUUCGGAGAATT), a HIF-1α siRNA (sense: GCCGCUCAAUUUAUGAAUATT, antisense: UAUUCAUAAAUUGAGCG GCTT), or a OGT siRNA (sense: GGAGGCAAUUGAGCAUUAUTT, antisense: AUAAUGCUCAAUUGCCUCCTT) (GenePharma, China) was performed utilizing Lipofectamine 2000 (Invitrogen). Following a 24-h transfection period, the culture medium was substituted, and then cells underwent a 48-h incubation in LG/HG medium or PUGNAc treatment (50 μM).

2.4. mRNA extraction as well as real-time quantitative PCR (RT-qPCR)

The TRIzol (10296028, ThermoFisher) was utilized for extracting RNA complying with the instructions of the manufacturer. Another Kit (R233, Vazyme, China) was utilized to perform reverse transcription of each sample into cDNA. RT-qPCR was performed with applied biosystems purchased from Thermo Fisher Scientific. For each sample, β-actin served as the internal control to standardize the expression levels of target genes. The primers included: HIF-1α, F: ACTGCACAGGCCACATTCACG, R: AATCAGCACCAAGCAGGTCATAGG; OGT, F: CAGGAAGGCTATTGCTGAGAGG; R: CGGAACTCACATATCCTACACGC; Fibronectin, F: ACAACACCGAGGTGACTGAGAC, R: GGACACAACGATGCTTCCTGAG; COL1A2, F: CCTGGTGCTAAAGGAGAAAGAGG, R: ATCACCACGACTTCCAGCAGGA; E-Cadherin, F: GCTCTTCCAGGAACCTCTGTGATG, R: TGTAAGCGATGGCGGCATTGTAG; a-SMA, F: CTCTGGACGCACAACTGGCATC, R: CACGCTCAGCAGTAGTAACGAAGG; β-Actin, F: GCTCACCATGGATGATGATATCGC; R: CACATAGGAATCCTTCTGACCCAT.

2.5. Protein extraction and western blots

Proteins were extracted from cells lysed in radioimmunoprecipitation assay (RIPA) buffer (R0010, Solarbio, China) containing the inhibitors of protease and phosphatase on ice. After a centrifugation of 14,000g at 4 °C for 5 min, the lysates were obtained and the

supernatants were treated with a loading buffer of sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), heated at the temperature of 100 °C for 10 min and kept in storage at −20 °C. Equal quantities of proteins were subjected to gradient gels and subsequently transferred to alternative membranes (nitrocellulose, Merck Millipore Ltd). After blocked by milk (5 %, nonfat), membranes underwent an overnight incubation (4 °C) with the presence of primary antibodies, followed by another 1-h incubation at ambient temperature with the presence of secondary antibodies. Protein visualization was performed using an electrochemiluminescence substrate with GelView 6000 Pro (BLT, China).

2.6. Fluorescent imaging

Cells were subjected to fixation using a 4 % paraformaldehyde solution for a duration of 15 min, and then underwent permeabilization by exposure to a Triton X-100 solution (0.5 %) in phosphate-buffered saline (PBS) at ambient temperature for a period of 15 min. Next, after blocked with bovine serum albumin (BSA, 1 %)/PBS, cells underwent an overnight incubation at the temperature of 4 °C with the presence of a primary antibody against HIF-1α. Subsequently, the samples were subjected to a 1-h incubation at 37 °C with the presence of a fluorescent secondary antibody at the temperature of 37 °C, after which the nuclei were stained using DAPI. Cell visualization was conducted utilizing a fluorescence microscope (OLYMPUS IX83).

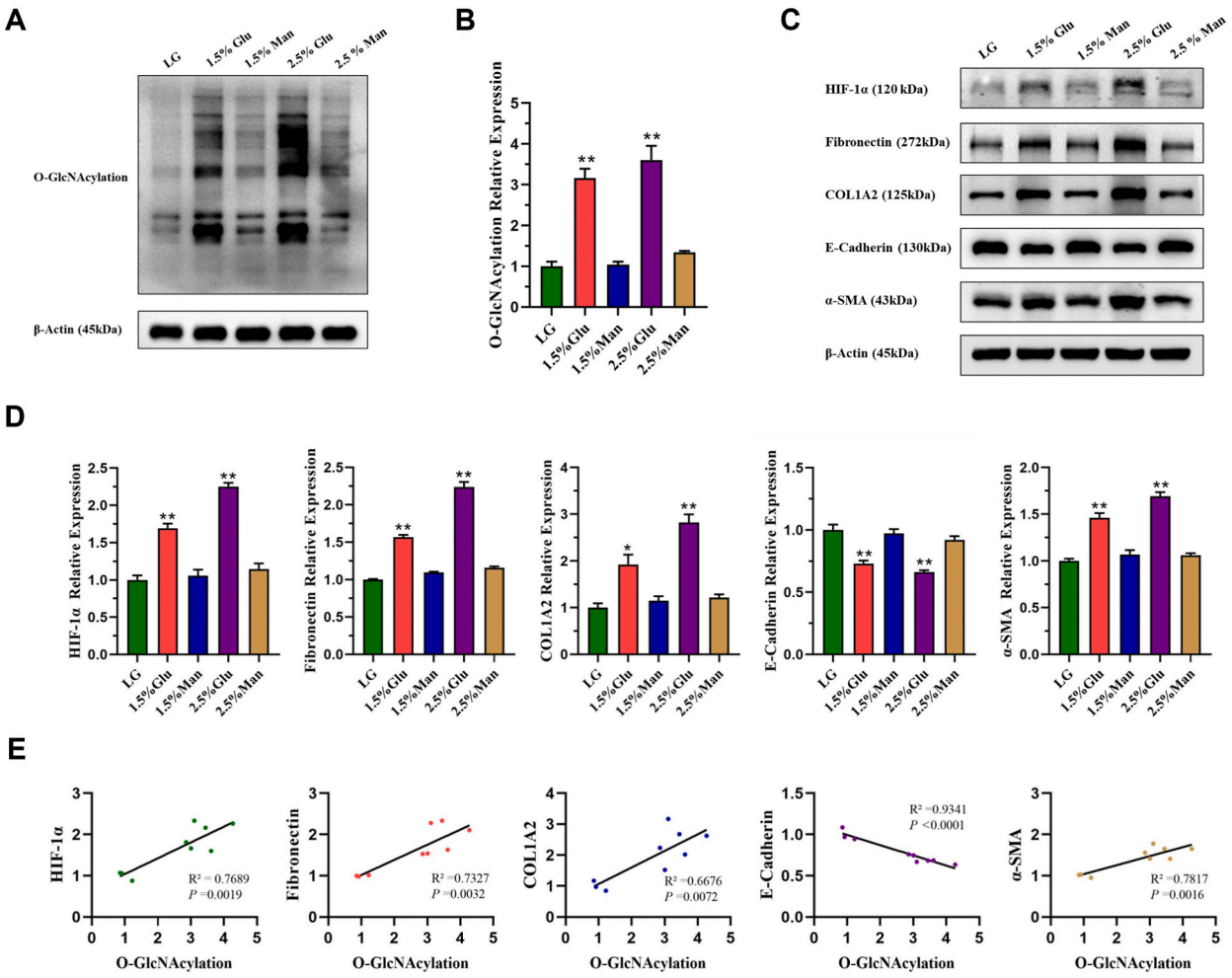

Fig. 1. High glucose increases the abundance of O-GlcNAcylation and HIF-1α, promotes MMT and fibrosis of HPMCs. (A and B) Representative immunoblots and quantification of global O-GlcNAcylation in cells exposed to different glucose or mannitol concentration mediums for 48 h. **(C and D)** Immunoblots as well as quantification of the abundance of HIF-1α and proteins relevant to MMT and fibrosis. **(E)** Correlation between O-GlcNAcylation and each protein in combined data from groups of LG, 1.5%Glu and 2.5%Glu. *, $P < 0.05$; **, $P < 0.001$ versus LG group. LG: control medium; 1.5%Glu: 1.5 % glucose medium; 1.5%Man: 1.5 % mannitol medium; 2.5%Glu: 2.5 % glucose medium; 2.5%Man: 2.5 % mannitol medium.

2.7. Immunoprecipitation (IP)

RIPA buffer supplemented with the inhibitors of protease & phosphatase were adopted for lysing the cells on ice. Following a centrifugation (14,000 g, 4 °C, 5 min), the lysates were collected. A primary anti–HIF–1α or anti-*O*-Linked N-Acetylglucosamine antibody was subjected to the incubation with Magnetic Beads (MBs, Protein A/protein G) (HY–K0202, MCE) for a duration of 30 min on a rotary shaker. After washed using PBS with 0.5 % Tween-20, MBs were subjected to a 30-min incubation with supernatants on a rotary shaker. After another round of washing, the MBs were eluted with the loading buffer of SDS-PAGE at the temperature of 100 °C for 10 min. At last, the MBs were boiled, and the resulting supernatant collected for SDS-PAGE detection.

2.8. Statistical analysis

GraphPad Prism 8 was adopted for statistical analysis. Data were described with means ± the standard error of the mean (SEM) based on no less than three independent experiments. The difference between groups was evaluated utilizing Student's t-test, while a one-way analysis of variance (ANOVA) was utilized to assess the disparity in more than 2 groups, with Tukey's test for further multiple group comparisons. $P < 0.05$ was indicative to be statistically significant.

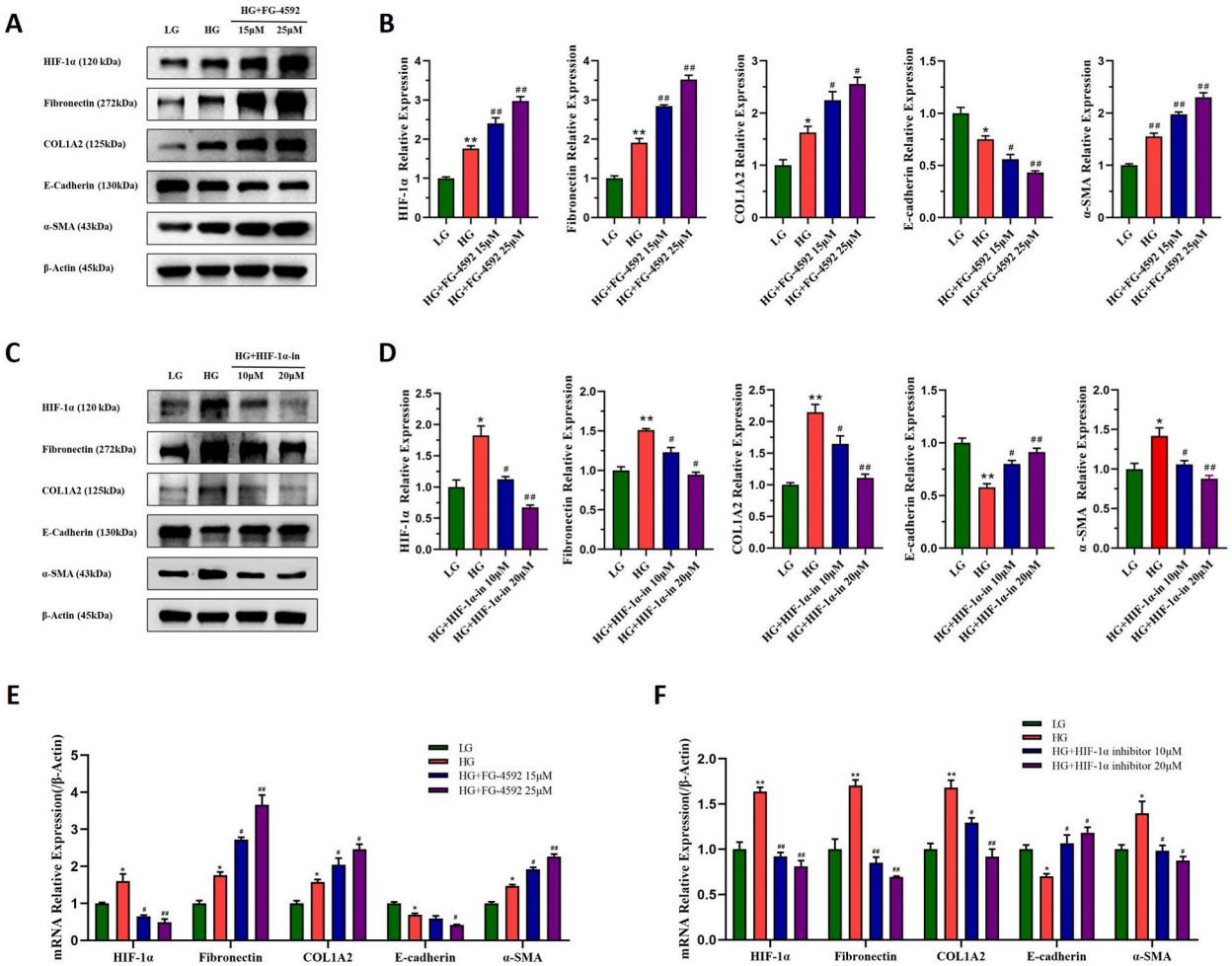

Fig. 2. High glucose promotes MMT and fibrosis of HPMCs via HIF-1α. (A and B) Representative immunoblots and quantification of the abundance of HIF-1α and proteins related to MMT and fibrosis in cells exposed to different concentrations of FG-4592 for 48 h. **(C and D)** Representative immunoblots and quantification of the abundance of HIF-1α and proteins related to MMT and fibrosis in cells exposed to different concentrations of a HIF-1α inhibitor for 48 h. **(E and F)** Real-time quantitative PCR detection of mRNA levels of HIF-1α and genes related to MMT and fibrosis in cells exposed to FG-4592 or HIF-1α inhibitor for 48 h. *, $P < 0.05$; **, $P < 0.001$versus LG group. $^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.001$ versus HG group. LG: control medium; HG: 2.5 % glucose medium; HIF-1α-in: HIF-1α inhibitor.

3. Results

3.1. HG resulted in an elevated abundance of O-GlcNAcylation and HIF-1α , promoting MMT and fibrosis of HPMCs

O-GlcNAcylation levels are elevated in HPMCs in a HG peritoneal dialysate, while the relationships between O-GlcNAcylation and HIF-1α, MMT, and fibrosis of HPMCs induced by HG are not clear. Western blotting quantification showed that global O-GlcNAcylation was elevated in cells cultured in a 1.5 % or 2.5 % glucose medium (Fig. 1A and B). HG exerted a concentration-dependent effect on promoting the protein abundance of HIF-1α, α-SMA, Fibronectin, and COL1A2, while decreasing that of E-Cadherin, as shown in Fig. 1C and D, resulting in the MMT of the HPMCs and fibrosis. Linear regression analysis showed an elevated level of O-GlcNAcylation

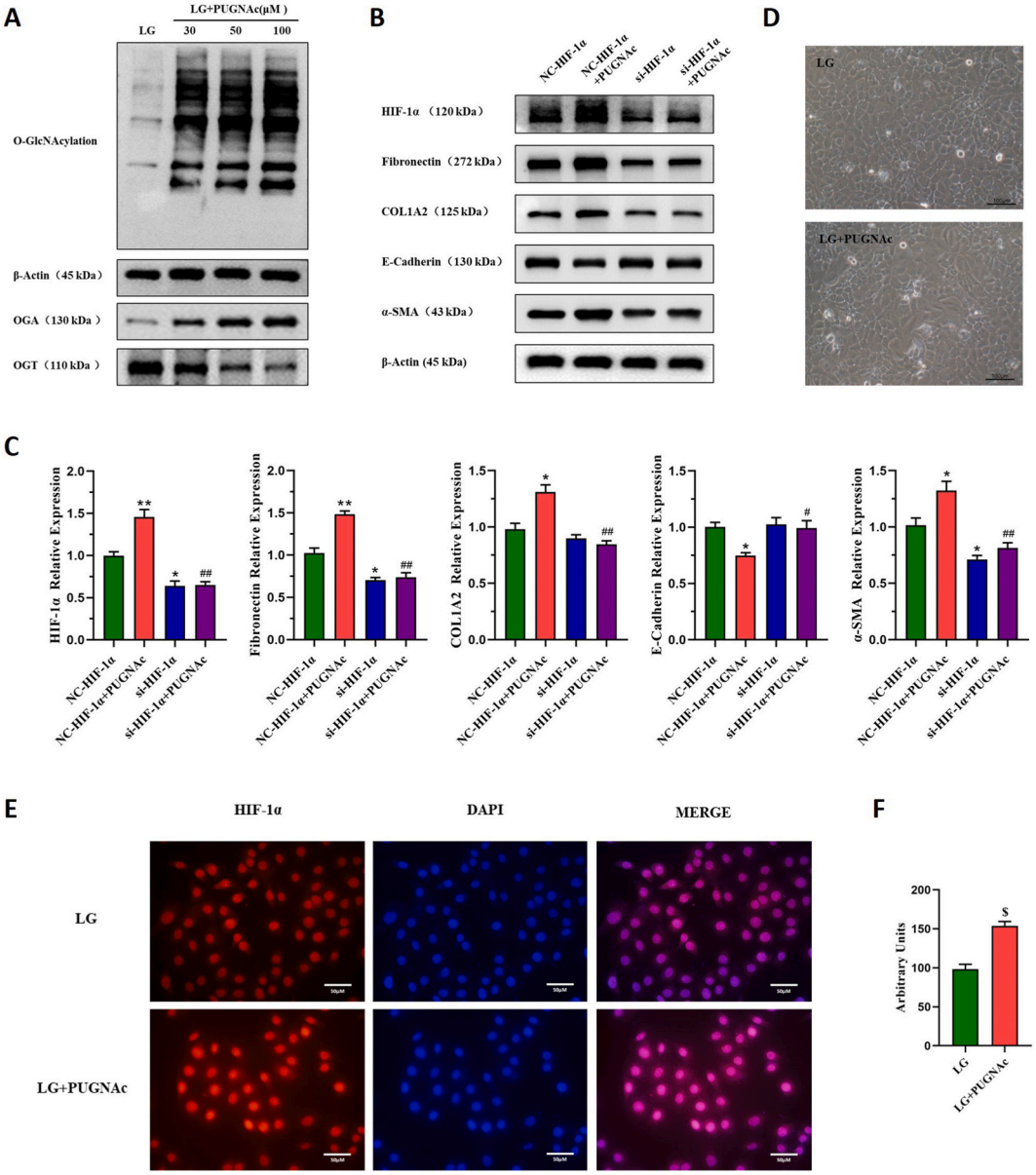

Fig. 3. O-GlcNAcylation regulates HIF-1α, promotes MMT and the fibrosis of HPMCs (A) Abundance of global O-GlcNAcylation and O-GlcNAcylation enzymes upon the addition of PUGNAc for 48 h. **(B and C)** Representative immunoblots and quantification of HIF-1α and proteins related to MMT and fibrosis in HIF-1α knockdown cells exposed to LG medium with or without 50 μM PUGNAc for 48 h. **(D)** Representative morphological images of the HPMCs cultivated in LG medium with or without 50 μM PUGNAc for 72 h. **(E and F)** Representative immunofluorescence and quantification of HIF-1α expression in cells exposed to LG medium with or without 50 μM PUGNAc for 48 h. LG: control medium; HG: 2.5 % glucose medium. *, $P < 0.05$; **, $P < 0.001$ versus the NC–HIF–1α group. $^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.001$ versus the NC–HIF–1α+PUGNAc group. $^{\$}$, $P < 0.05$ versus LG group.

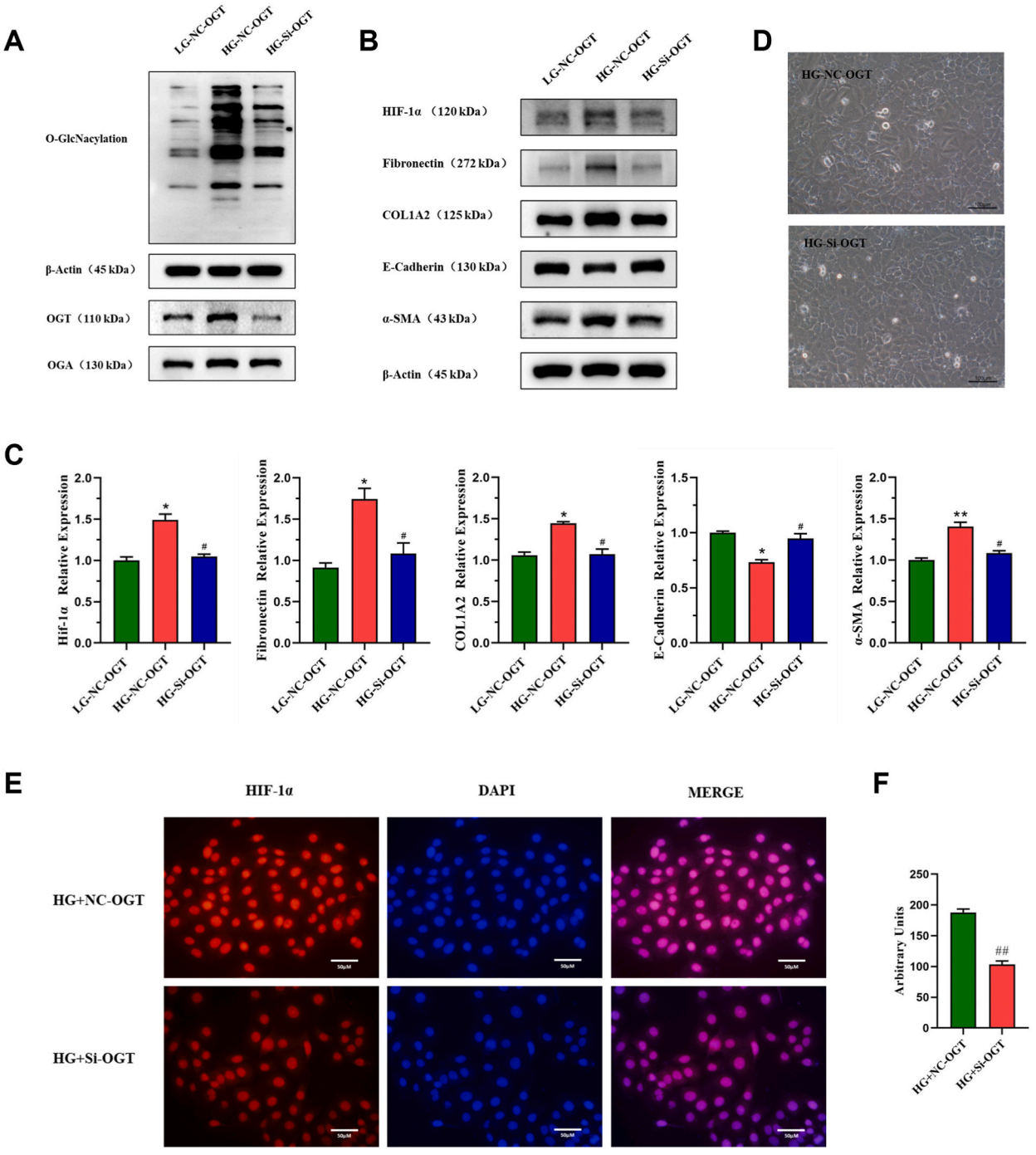

Fig. 4. High glucose promotes HIF-1α expression, MMT and fibrosis of HPMCs via O-GlcNAcylation (A) Abundance of global O-GlcNAcylation and O-GlcNAcylation enzymes upon OGT knockdown. **(B and C)** Representative immunoblots and quantification of HIF-1α and proteins related to MMT and fibrosis in cells with OGT knockdown. **(D)** Representative morphological images of the HPMCs under HG medium with or without OGT knockdown for 72 h. **(E and F)** Representative immunofluorescence and quantification of HIF-1α expression in cells exposed to HG medium with or without OGT knockdown for 48 h. LG: control medium; HG: 2.5 % glucose medium. *, $P < 0.05$; **, $P < 0.001$ versus LG–NC–OGT group. $^{\#}$, $P < 0.05$ versus HG–NC–OGT group.

was closely related to the higher HIF-1α abundance and the MMT and fibrosis of HPMCs (Fig. 1E). Mannitol groups had a slight effect on global O-GlcNAcylation, HIF-1α abundance, MMT and fibrosis of HPMCs with no statistical difference (Fig. 1A–D).

3.2. HG promoted MMT and fibrosis of HPMCs via HIF-1α

HIF-1α is reported to be increased in peritoneum during peritoneal dialysis. An agonist (FG-4592) and inhibitor of HIF-1α were applied to intervene HPMCs for 48 h to validate if high glucose promotes MMT and fibrosis via HIF-1α. Western blot experiments

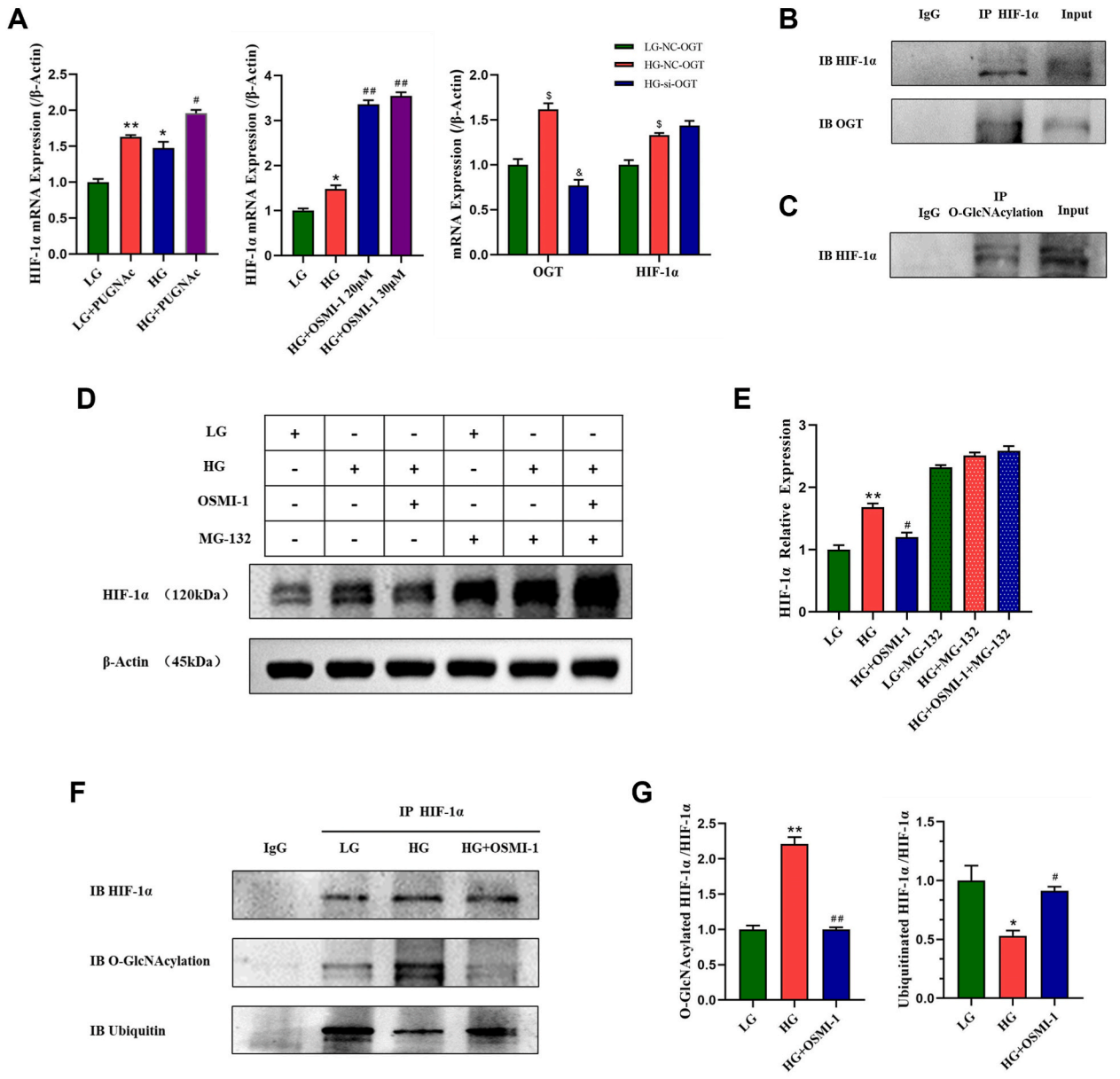

Fig. 5. High glucose increases HIF-1α O-GlcNAcylation, prevents its ubiquitination along with proteasomal degradation in HPMCs. (A) Changes in HIF-1α expression at mRNA level after incubation with PUGNAc (50 μM) or OSMI-1, or OGT knockdown for 48 h. **(B)** Immunoblots of HIF-1α and OGT following the immunoprecipitation (IP) of HIF-1α in the HG medium. **(C)** Immunoblots of HIF-1α following the IP of O-GlcNAcylated proteins in the HG medium. **(D and E)** Representative immunoblots as well as quantification of HIF-1α expression under LG, HG, or HG + 30 μM OSMI-1 conditions for 48 h. Some cells were also incubated with 0.1 μM MG-132 in the latter 24 h. **(F and G)** Immunoprecipitated HIF-1α, immunoblots as well as quantification of total, O-GlcNAcylated and ubiquitinated HIF-1α. *, $P < 0.05$; **, $P < 0.001$ versus LG group. $^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.001$ versus HG group. $^{\$}$, $P < 0.001$ versus LG–NC–OGT and $^{\&}$, $P < 0.001$ versus HG–NC–OGT. LG: control medium; HG: 2.5 % glucose medium.

showed that FG-4592 concentration-dependently increased the abundance of HIF-1α, and fibrotic proteins α-SMA, fibronectin and COL1A2, while it decreased E-cadherin abundance (Fig. 2A and B). RT-qPCR showed that the mRNA levels of α-SMA, fibronectin, COL1A2 and E-cadherin were in agreement with Western blot data, however there was a decrease in HIF-1α mRNA (Fig. 2 E). HIF-1α inhibitor could suppress HIF-1α and prevent MMT and fibrosis of HPMCs induced by high glucose (Fig. 2C, D, F). These results indicated HIF-1α played a critical role in MMT and fibrosis of HPMCs treated with high glucose.

3.3. O-GlcNAcylation regulated HIF-1α, promotes MMT and fibrosis of HPMCs

Global O-GlcNAcylation is positively correlated with HIF-1α, MMT and fibrosis of HPMCs (Fig. 1E), PUGNAc (acting as an inhibitor of OGA activity to upregulate the level of O-GlcNAcylation in cells) together with HIF-1α knockdown were used to explore whether O-GlcNAcylation can regulate the HIF-1α, MMT and fibrosis of HPMCs. PUGNAc was shown to increase the global O-GlcNAcylation of the HPMCs, while OGA increased and OGT decreased (Fig. 3A). Moreover, PUGNAc resulted in an increased abundance of HIF-1α, α-SMA, fibronectin, and COL1A2, while concomitantly decreasing that of E-cadherin (Fig. 3B and C, E and F). These changes induced by PUGNAc were abolished by HIF-1α knockdown (Fig. 3B and C), indicating that MMT and fibrosis induced by O-GlcNAcylation mainly function through HIF-1α. As shown in Fig. 3D, PUGNAc promotes the transformation of HPMCs from polygon to a spindle-shape, further confirming that O-GlcNAcylation regulates the process of MMT and fibrosis in HPMCs.

3.4. HG promoted HIF-1α expression, MMT and fibrosis of HPMCs via O-GlcNAcylation

To investigate whether HG regulates HIF-1α expression, MMT and fibrosis of HPMCs through O-GlcNAcylation, OGT knockdown and OSMI-1 (an inhibitor of OGT) were applied to interfere with cells. OGT knockdown by siRNA or inhibition using OSMI-1 resulted in a reduced level of O-GlcNAcylation and HIF-1α protein abundance as well as suppressed MMT and fibrosis induced by HG conditions (Fig. 4A–C, E and F , Supplementary Figs. S1A–C). OGT knockdown reduced OGT protein levels, while OSMI-1 inhibited OGT activity, with no significant effect on its expression (Fig. 4A and Supplementary Fig. S1A). As global O-GlcNAcylation levels decreased, OGA tended to decrease (Fig. 4A and Supplementary Fig. S1A). Light microscopy showed that partial HPMCs were spindle-shaped under HG conditions, and the spindle-shaped HPMCs were obvious reduced after OGT knockdown, indicating that HG promotes MMT and fibrosis of HPMCs via O-GlcNAcylation (Fig. 4D).

3.5. HG increased HIF-1α O-GlcNAcylation, prevented its ubiquitination and proteasomal degradation in HPMCs

The increase in O-GlcNAcylation induced by PUGNAc stimulates the expression of HIF-1α at the mRNA level, while the decrease in O-GlcNAcylation induced by OGT knockdown or OGT inhibitor did not reduce HIF-1α mRNA transcription and rather caused an increase of it (Fig. 5A), indicating that O-GlcNAcylation may mainly regulate the protein level expression of HIF-1α. HIF-1α degradation primarily occurs via the ubiquitin-proteasome pathway. To examine whether O-GlcNAcylation has a direct effect on HIF-1α degradation, MG-132, knowns as a cell-permeable 20S proteasome inhibitor, was used to observe this effect. The addition of MG-132 brought the HIF-1α levels of LG group and HG + OSMI-1 group closer to that of the HG group (Fig. 5D and E). These findings suggest that HG-induced O-GlcNAcylation prevents HIF-1α from being degraded by proteasomes. We immunoprecipitated HIF-1α and found that it can combine with OGT and be directly O-GlcNAcylated (Fig. 5B and F). The detection of HIF-1α in the immunoprecipitated proteins of O-GlcNAcylation also indicates that HIF-1α can be O-GlcNAcylated (Fig. 5C). HG increases the O-GlcNAcylation level of HIF-1α with a low level of ubiquitination, OSMI-1 suppressed HIF-1α O-GlcNAcylation while increased its ubiquitination (Fig. 5F and G). The above results suggest that HG increases HIF-1α O-GlcNAcylation, prevents its ubiquitination and the proteasomal degradation in HPMCs.

4. Discussion

O-GlcNAcylation is related with protein stability, subcellular trafficking, enzymatic activity, signaling pathways, gene expression, and cell-cell communication [12]. Increased cardiac protein O-GlcNAcylation levels could improve cardiac function and mitigate tissue damage in the isolated heart during reperfusion [13]. O-GlcNAcylation has also been shown to improve the functions of various organs and reduce inflammation in a trauma hemorrhage model [14,15]. Elevating O-GlcNAcylation is a protective effect observed in acute injury models, while the persistent elevation of O-GlcNAcylation level contributes to the advancement of hypertension [16], cardiac failure [17], hyperglycemic toxicity [18], nephropathy [19], as well as retinopathy induced by diabetes [20]. O-GlcNAcylation is thought to exert a pivotal effect on the fibrosis of glomerular mesangial cells [21]. However, it is unclear if O-GlcNAcylation modulates the MMT as well as the fibrosis of HPMCs. Our results show that OGT knockdown or inhibition by OSMI-1 could prevent the MMT and fibrosis of HPMCs induced by HG conditions (Fig. 4B and C, Supplementary Figs. S1B and C), which confirmed that HG conditions increase the global O-GlcNAcylation of HPMCs by increasing OGT expression to further mediate MMT and fibrosis. OSMI-1 suppresses the activity of OGT without influencing protein levels and decreasesing OGA expression (Supplementary Fig. S1A), which is consistent with results reported by Ortiz-Meoz [22]. PUGNAc was shown to raise levels of global O-GlcNAcylation, along with an upregulation of OGA expression and a downregulation of OGT expression (Fig. 4A).

Chronic hypoxia and elevated expression of HIF-1α were found in cells of the peritoneum after long-term dialysis [7,8]. Our results showed that both HIF-1α and O-GlcNAcylation could induce MMT and the fibrosis of HPMCs and that O-GlcNAcylation regulated HIF-1α abundance. Therefore, it was speculated that O-GlcNAcylation induced MMT and the fibrosis of HPMCs through the regulation

of HIF-1α expression. HIF-1α knockdown abolished the elevation of fibrotic proteins and decreased E-cadherin expression induced by PUGNAc (Fig. 3B and C), which verifying our hypothesis. The increase in O-GlcNAcylation by PUGNAc promoted the levels of both HIF-1α protein and its corresponding mRNA, while the decrease in O-GlcNAcylation by OGT knockdown or the OGT inhibitor decreased HIF-1α protein abundance while simultaneously enhancing the transcription of HIF-1α mRNA (Fig. 5A). The reduced levels of O-GlcNAcylation only decreased the protein abundance of HIF-1α, but not at mRNA level, which is in accordance with the results from Ferrer et al. [11], indicating that O-GlcNAcylation primarily regulates HIF-1α expression at the protein level, while the transcriptional regulation of HIF-1α mRNA is primarily regulated by processes other than O-GlcNAcylation.

The precise mechanisms by which O-GlcNAcylation influences the regulation of HIF-1α expression remain elusive, leading to significant uncertainty in our current understanding. According to Ferrer et al., O-GlcNAcylation was found to diminish alpha-ketoglutarate levels and HIF-1α hydroxylation. As a result, HIF-1α degradation was suppressed among breast cancer cells [11]. Another study on breast cancer cells revealed that HIF-1α can be O-GlcNAcylated and that O-GlcNAcylation can regulate HIF-1α abundance [23]. Wu et al. reported that HIF-1α can be O-GlcNAcylated by OGT, which was detected using a novel method of tandem glycan labeling [24]. Our IP experiments showed that HIF-1α can bind to OGT and be directly O-GlcNAcylated (Fig. 5B, C and F). The O-GlcNAcylation of HIF-1α induced by HG conditions decreased its ubiquitination levels and prevented HIF-1α from being degraded by proteasomes in the HPMCs (Fig. 5D–G).

In conclusion, our study showed that HG conditions induced MMT and the fibrosis of HPMCs by modulating the O-GlcNAcylation of HIF-1α. O-GlcNAcylation has a potential in serving as a therapeutic target for peritoneal fibrosis.

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Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Jian Wang: Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation. **Xin lv:** Writing – original draft, Project administration, Data curation. **Ashanjiang Aniwan:** Software, Resources, Formal analysis, Data curation. **Hongyan Liu:** Supervision, Software, Methodology, Formal analysis, Data curation. **Yao Lin:** Validation, Supervision, Resources, Methodology, Formal analysis, Data curation. **Xian Shao:** Formal analysis, Data curation. **Saijun Zhou:** Writing – review & editing, Project administration, Conceptualization. **Pei Yu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22916.

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