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RESEARCH ARTICLE

The Role of Glutamate Dehydrogenase 1 in Congenital Obstructive Nephropathy: Insights from Neonatal Rodent Models and Implications for Veterinary Medicine

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ABSTRACT

Congenital obstructive nephropathy (CON) refers to kidney damage caused by impaired urine flow due to congenital malformations of the urinary tract. CON is a leading cause of chronic kidney disease (CKD) in young animals, particularly in dogs and cattle, characterized by congenital urinary tract obstruction-induced renal tissue inflammation, fibrosis, and progressive parenchymal damage. It represents a significant clinical challenge in veterinary medicine due to the lack of effective therapeutic options. Glutamate dehydrogenase 1 (GDH1), a key mitochondrial enzyme regulating amino acid metabolism, energy homeostasis, and redox balance, is linked to renal fibrosis via Reactive Oxygen Species (ROS) and profibrotic pathways, making it a plausible target for CON, while the role of GDH1 in animals with CON remains unclear. This study evaluates the role of GDH1 in obstructive renal injury via in vivo and in vitro models. The function of GDH1 was investigated in a neonatal rat model of partial unilateral ureteral obstruction (PUUO) and the potential mechanism was explored in an *in vitro* model. GDH1 expression was reduced in a neonatal rat model of PUUO. GDH1 overexpression alleviated renal fibrosis in PUUO-operated rats. TGF-β1-treated rat renal tubular epithelial cells (NRK-52E) were employed as an *in vitro* model. Besides, GDH1 overexpression conferred protection against TGF-β1-induced cellular damage, which was mediated by enhanced cell survival, reduced apoptosis, as well as the suppression of pro-fibrotic marker expression. In conclusion, our study confirms GDH1-mediated protection conferring resistance against CON, highlighting GDH1 as a promising therapeutic target.

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INTRODUCTION

Congenital obstructive nephropathy (CON) is one of the main contributors to chronic kidney disease (CKD) in young animals and represents an important challenge in veterinary practice (Hylton & Trent, 1987; Yoshida *et al.*, 2022). In most cases, CON results from congenital anomalies, namely ureteral ectopia, congenital urethral obstruction, and polycystic kidney disease, which particularly affect dogs and cattle. Epidemiologically, CON accounts for approximately 15-20% of all congenital renal anomalies in purebred dogs companion animals

affects about 2-3% of male bovine calves, leading to a mortality rate of over 40% within the first 6 months of life if left untreated (Hunt & Allen, 1989; Chiaramonte *et al.*, 2022). In large animal practice, conditions such as urorectal fistulas in foals or urethral obstruction in male calves can also lead to life-threatening renal compromise (Hunt & Allen, 1989). CON is pathologically characterized by progressive hydronephrosis, glomerulosclerosis, tubular/ vascular atrophy, and interstitial fibrosis. Treatment of affected veterinary patients is limited to surgical defect repair and supportive care due to the lack of disease-modifying therapies; thus, investigating CON's underlying mechanisms is critical to developing novel, effective therapeutic strategies.

Glutamate dehydrogenase 1 (GDH1) encodes a NADP(+)-dependent glutamate dehydrogenase isoform that mediates the deamination of glutamate to α-ketoglutarate, thereby furnishing the tricarboxylic acid (TCA) cycle with critical intermediates to sustain anaplerotic metabolic reactions (Yeh *et al.*, 2020). By virtue of its critical function in core carbon metabolism, GDH1 acts as a molecular link bridging amino acid catabolism and cellular energy generation, thereby emerging as a pivotal modulator of intracellular redox homeostasis and biosynthetic potential. (Cheng *et al.*, 2025; Zhou *et al.*, 2025) . It was previously assumed that GDH1 in eukaryotes was localized exclusively within mitochondria (Shao *et al.*, 2021). Nevertheless, several studies have demonstrated that this enzyme is also present in lysosomes, the endoplasmic reticulum, and the nucleus (Bunik *et al.*, 2016). This broad subcellular distribution suggests that GDH1 may fulfill distinct, compartment-specific functions beyond its classical metabolic role, potentially involving signal transduction, reactive oxygen species (ROS) modulation, and epigenetic regulation through nuclear metabolism. GDH1 is upregulated in several cancer types and is a contributing factor to the progression of cancer (Jin *et al.*, 2015; Hu *et al.*, 2023; Yang *et al.*, 2024). Regulation of GDH activity is required for the apoptosis of renal tubular epithelial cells induced by aristolochic acid (Romanov *et al.*, 2011). The targeting of glutamine metabolism via the regulation of GDH expression and activity in hepatic stellate cells has been demonstrated to be an effective method for alleviating liver fibrosis (Yin *et al.*, 2022), while these findings highlight GDH1 as a potential metabolic checkpoint in tissue fibrosis and cell survival, its role in veterinary CON, a condition with distinct epidemiological and pathological features compared to human obstructive nephropathy, remains entirely unexplored.

The present study aimed to investigate the role of GDH1 in a neonatal rat model of partial unilateral ureteral obstruction (PUUO), a well-established preclinical model that recapitulates key aspects of veterinary CON. Complementary in vitro studies were performed using TGF-β1-treated NRK-52E cells, a rat renal tubular epithelial cell line, to explore the underlying molecular mechanisms, thereby facilitating the development of mechanism-based treatments to improve renal outcomes in animals.

MATERIALS AND METHODS

Animal models: Pregnant Sprague-Dawley rats weighing between 230 and 300 grams were purchased from Beijing HFK Bio-technology Co., Ltd. (China). The rats were housed under specific pathogen-free conditions with controlled temperature (22±1°C), humidity (50±5%), and 12-h light/dark cycle. The surgical PUUO procedure was performed on the left ureter of neonates aged postnatal day 1-2 (Wang *et al.,* 2012). Neonatal rats (postnatal day 1) were randomly assigned to six experimental groups (n=6 per group). To control litter effects, no more than two neonatal rats from the same dam were assigned to any single group. To inhibit GDH1 expression *in vivo*, a continuous regimen of intraperitoneal injections of the GDH1 inhibitor R162 (2 mg/kg/day; MedChemExpress) or DMSO was initiated on the second day post-surgery and maintained for 14 days. To overexpress GDH1 *in vivo*, neonatal rats were intraperitoneally injected with an adenovirus overexpressing GDH1 (1×10^9 PFU, 50μL) or a negative control adenovirus (OBiO Technology, China) for three consecutive days, commencing on the second day post-surgery. The rats were euthanized on day 7 post-surgery. The neonatal rats that underwent the sham operations served as the controls. The animal experiments were approved by the Ethics Committee of Shengjing Hospital of China Medical University (2023PS1331K).

Cell treatment: NRK-52E cells were procured from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). NRK-52E cells were transfected with siRNAs targeting GDH1 (si-GDH1) using Lipofectamine 3000 reagent (Invitrogen, USA). si-GDH1 sense: 5'-GCAUCCUGCCGGAUCAUCAATT-3'; si-GDH1 antisense: 5'-UUGAUGAUCCGCAGGAUGCTT-3'. Meanwhile, NRK-52E cells were infected with adenovirus overexpressing GDH1 or a negative control adenovirus. Following transfection or virus infection, the cells were cultured with 10ng/mL recombinant TGF-β1 (Sino Biological Inc., China) or/and 10nM 2-Deoxy-D-glucose (2-DG; MedChemExpress, USA) for 48h.

Histological analyses: The abovementioned renal tissues were embedded in paraffin and subsequently sectioned at 2.5μm. Next, the kidney tissue samples were subjected to the standard immunohistochemical staining procedure using the antibody against GDH1 (1:200, No. 12793T, CST, USA).

Western blot analysis: Proteins were extracted from cell pellets or renal tissues, subjected to SDS-PAGE, and subsequently transferred to PVDF membranes. Then, the PVDF membranes were incubated in the following primary antibodies at 4℃ overnight and HRP-conjugated secondary antibody (Proteintech, USA) at 25℃ for 2h. Finally, bands were visualized by ECL (Abbkine, Wuhan, China) and quantified with ImageJ.

Primary antibodies were listed: GDH1 (1:1000, Proteintech, USA), E-cadherin (1:1000, Proteintech, USA), α-SMA (1:500, Proteintech, USA), collagen I (1:1000, Bioss, China), Bax (1:1000, Proteintech, USA) and Bcl-2 (1:1000, Proteintech, USA).

Real-time PCR: Total RNA was extracted from renal tissues or cells, and real-time PCR was performed using SYBR Green Premix Pro Taq HS qPCR Kit II (Accurate Biology).

The primer sequences are listed in Table

Table 1: Primer sequences for real-time PCR

Gene names	Primer sequence
GDH1 (rat)-F	5'-GCCTACACAATGGAGCGATCTGC-3'
GDH1 (rat)-R	5'-GGTCACGCCAGCCTCATTATACAC-3'
β-actin (rat)-F	5'-CCCATCTATGAGGGTTACGC-3'
β-actin (rat)-R	5'-TTTAATGTCACGCACGATTTC-3'

Flow cytometric analysis of apoptosis: Cells were detached with 0.25% EDTA-free trypsin, centrifuged, resuspended in binding buffer, stained with Annexin V-FITC/PI (APExBIO, USA) in the dark, and finally analyzed by flow cytometry.

CCK-8 assay: NRK-52E cells seeded into 96-well plates were incubated with 10μL CCK-8 reagent (MedChemExpress) at 37°C, and absorbance at 450 nm was measured using a microplate reader (Thermo, USA).

EdU assay: The cells were fixed with 4% paraformaldehyde, incubated with 10μM EdU (Beyotime), washed, permeabilized with 0.3% Triton X-100/PBS, reacted with Click Additive Solution (Beyotime) in the dark, stained with Hoechst 33342, and visualized via fluorescence microscopy (Olympus).

Statistical analysis: All data are expressed as mean ± SD. Comparisons between two groups used Student's t-test, while three or more groups used one-way ANOVA with Tukey's post-test. $P<0.05$ was considered statistically significant.

RESULTS

GDH1 expression is reduced in a neonatal rat model of PUUO: To validate the differential expression of GDH1 in rat exposed to PUUO surgery, we employed real-time PCR and western blotting to assess GDH1 mRNA and protein levels in renal tissues from neonatal rats at 5 and 7 days following PUUO surgery, there was a reduction in GDH1 mRNA levels in the renal tissues of neonatal rats that underwent PUUO surgery, in comparison to the sham group (Fig. 1A). A considerable reduction (approximately 40%) in GDH1 protein levels was observed in renal tissues of PUUO-operated rats at day 7 compared to the sham group (Fig. 1B), which is consistent with the immunohistochemical staining results showing a marked decrease in both staining intensity and positive area fraction of GDH1 (Fig. 1C).

GDH1 overexpression alleviates renal fibrosis in PUUO-operated rats: To ascertain the function of GDH1 in obstructive nephropathy, an adenovirus overexpressing GDH1 or a GDH1 inhibitor R162 was administered intraperitoneally to PUUO-operated neonatal rats. At the conclusion of the experiments, kidney tissues were obtained for further analysis. The kidney of the sham group exhibited a normal structural configuration with minimal collagen deposition. A markedly elevated level of collagen deposition was evident in renal tissues from the PUUO+oe-NC group. However, injection with the adenovirus overexpressing GDH1 resulted in a notable reduction in collagen deposition in PUUO-operated neonates. Additionally, the PUUO+DMSO group displayed a notable accumulation of collagen fibers within the renal interstitium and glomerular mesangial matrix. Following R162 administration, a further increase in collagen deposition was observed in the renal tissue of PUUO-operated neonatal rats (Fig. 2A). Furthermore, the PUUO+oe-NC group exhibited elevated collagen I (approximately 3-fold) and α-SMA (approximately 6-fold) in the kidneys of neonatal rats, while E-cadherin (approximately 50%) was observed to be diminished. Following injection with the adenovirus overexpressing GDH1, there was a notable downregulation of collagen I (approximately 40%) and α-SMA (approximately 35%) expression in renal tissues of PUUO-operated neonates, accompanied by a notable increase in the expression level of E-cadherin. Compared to the sham group, PUUO+DMSO elevated neonatal rat renal collagen I/α-SMA and reduced E-cadherin, with R162 further enhancing these changes (Fig. 2B).

GDH1 overexpression promotes proliferation and inhibits apoptosis *in vitro*: The findings demonstrated a decline in GDH1 expression at 24, 48 and 72 hours following TGF-β1 treatment (Fig. 3A). In comparison to the oe-NC-transfected cells, treatment with TGF-β1 resulted in a reduction in cell viability among the oe-NC-transfected population. The overexpression of GDH1 was found to elevate cell viability in comparison to the TGF-β1+oe-NC group. Furthermore, exposure to TGF-β1 led to a reduction in cell viability among the si-NC-transfected population. The knockdown of GDH1 led to an additional worsening of the TGF-β1-induced reduction in cell

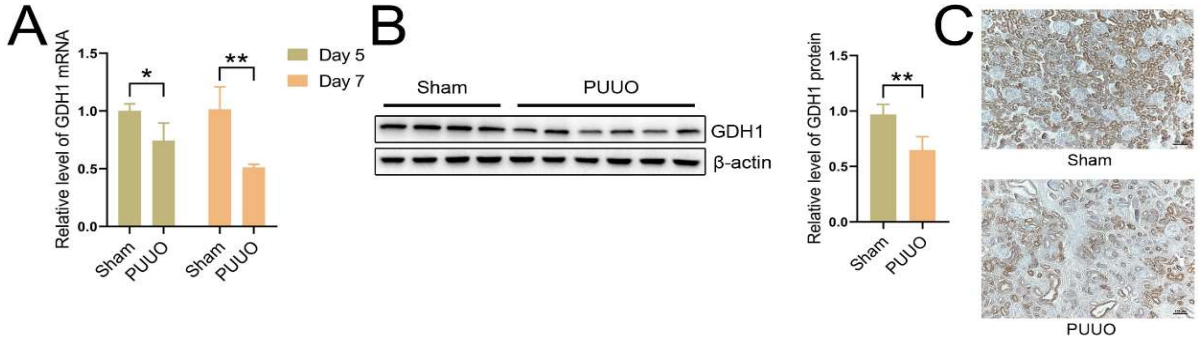

Fig. 1. GDH1 expression is reduced in a neonatal rat model of PUUO. (A) The neonatal rats were subjected to PUUO surgery and then renal tissues were excised from the rats at day 5 or 7 post-surgery. The transcription of GDH1 mRNA in the renal tissues was analyzed using real-time PCR. β-actin was used as an internal control. (B) Seven days following the surgical procedure, the renal tissues were excised from the rats and the level of GDH1 protein was quantified by western blotting. β-actin was used as an internal control. (C) The renal tissues were obtained from the rats at day 7 post-surgery. The level of GDH1 protein was quantified by immunohistochemical staining. * indicates $P< 0.05$ and ** indicates $P<0.01$.

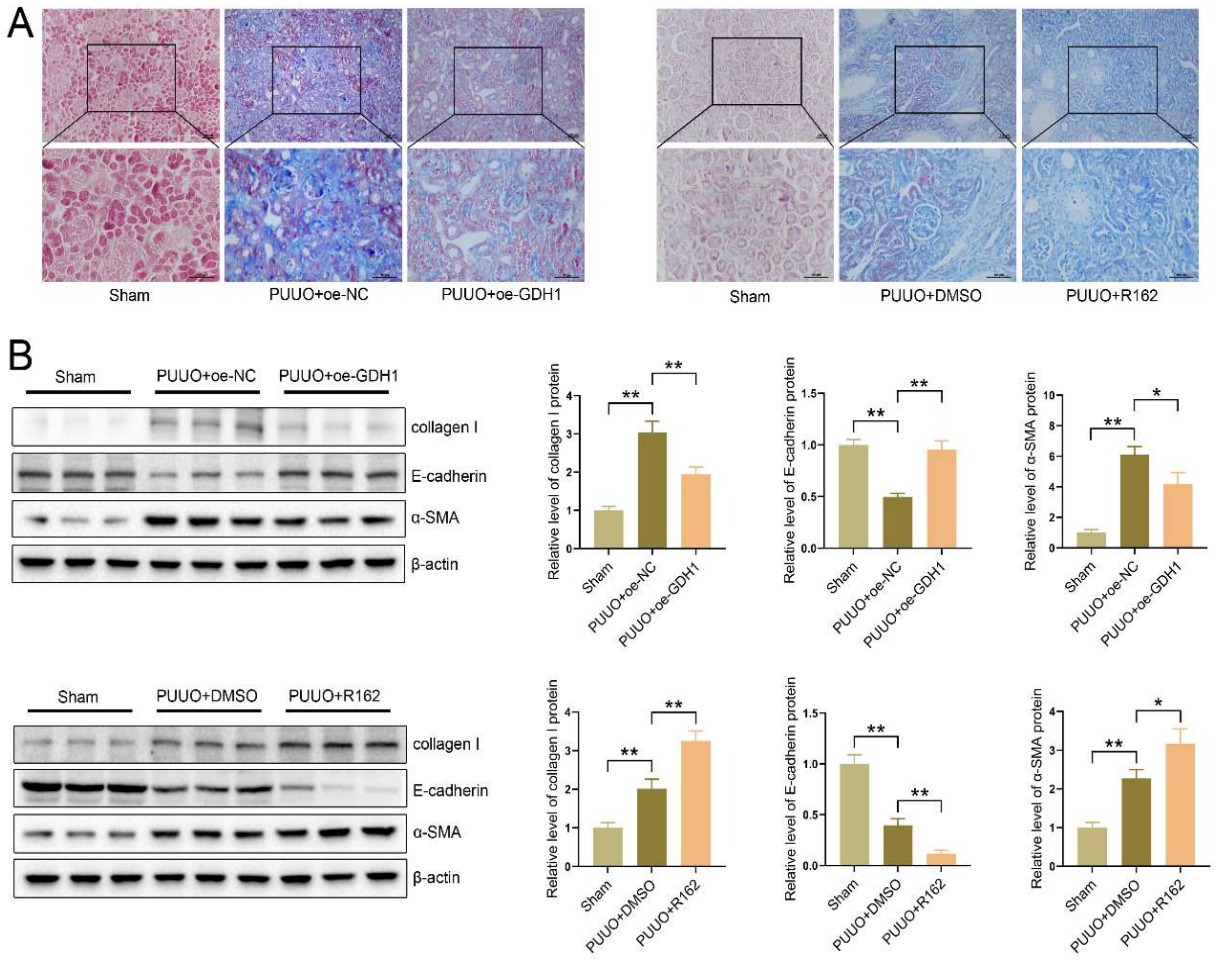

Fig. 2. GDH1 overexpression alleviates renal fibrosis in PUUO-operated rats. (A) An adenovirus overexpressing GDH1 or a GDH1 inhibitor R162 was administered intraperitoneally to PUUO-operated neonatal rats. At the conclusion of the experiments, renal tissues were obtained and subjected to Masson's trichrome staining for the purpose of analyzing fibrosis. (B) The expression levels of collagen I, α-SMA, and E-cadherin in the kidneys of neonatal rats were quantified by western blotting. β-actin was used as an internal control. * indicates P<0.05 and ** indicates P<0.01.

viability (Fig. 3B). The results of EdU assay demonstrated that TGF-β1 treatment resulted in a reduction in the proportion of EdU-positive cells among the oe-NC- or si-NC-transfected populations. The overexpression of GDH1 resulted in an elevated EdU-positive cell rate. Conversely, the knockdown of GDH1 resulted in a further exacerbation of the TGF-β1-induced decrease in EdU-positive cell rate (Fig. 3C). As illustrated in Fig. 3D, treatment with TGF-β1 resulted in an increase in the cell apoptosis rate among the oe-NC- or si-NC-transfected populations. The overexpression of GDH1 was observed to reduce the cell apoptosis rate. Conversely, GDH1 knockdown resulted in a further exacerbation of the TGF-β1-induced increase in cell apoptosis. Additionally, TGF-β1 incubation resulted in elevated Bax expression and decreased Bcl-2 expression in oe-NC- or si-NC-transfected cells. The overexpression of GDH1 was observed to downregulate Bax expression and upregulate Bcl-2 expression, while the knockdown of GDH1 exerted an opposite impact on the apoptosis-associated regulators (Fig. 3E).

GDH1 overexpression regulates the expression of multiple markers associated with fibrosis *in vitro*: TGF-β1 treatment resulted in the upregulation of collagen I, vimentin, and α-SMA expression levels, while E-cadherin was downregulated in oe-NC- or si-NC-transfected cells. The overexpression of GDH1 was observed to result in a downregulation of collagen I, vimentin, and α-SMA expression, while an upregulation of E-cadherin expression was noted. The knockdown of GDH1 was observed to further exacerbate the TGF-β1-induced upregulation of collagen I, vimentin, and α-SMA expression and downregulation of E-cadherin expression in comparison to the TGF-β1+si-NC group (Fig. 4).

GDH1 overexpression modulates glycolysis *in vitro:* To ascertain whether GDH1 exerts a regulatory effect on renal fibrosis through the modulation of glycolysis in renal tubular epithelial cells, western blot analysis was employed to quantify the levels of two glycolytic enzymes. The findings revealed that exposure to TGF-β1 resulted in elevated HK2 and PKM2 expression levels in oe-NC- or si-NC-transfected cells. The overexpression of GDH1 prevented the elevation of HK2 and PKM2 expression induced by TGF-β1. Conversely, the knockdown of GDH1 resulted in a more pronounced TGF-β1-induced upregulation of HK2 and PKM2 expression (Fig. 5).

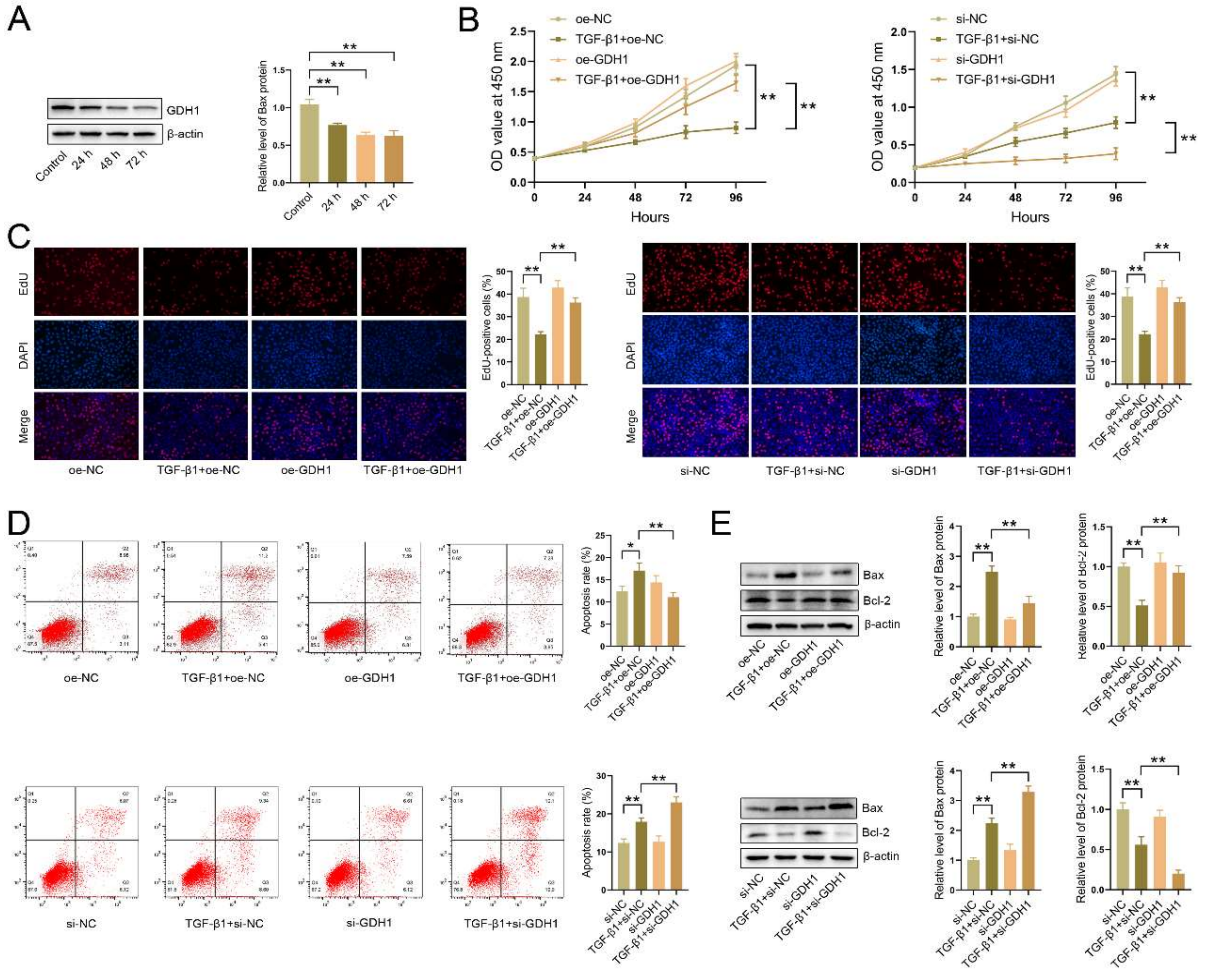

Fig. 3: GDH1 overexpression promotes proliferation and inhibits apoptosis *in vitro*. (A) NRK-52E cells (a rat renal tubular epithelial cell line) were incubated with TGF-β1 for 24, 48 and 72 hours. The expression level of GDH1 in NRK-52E cells was quantified by western blotting. β-actin was used as an internal control. (B) Cell proliferation was assessed by CCK-8 assay. (C) EdU assay was employed to evaluate cell proliferation. (D) Cell apoptosis was analyzed using an Annexin V-FITC/PI Apoptosis Detection Kit by flow cytometry. (E) The expression levels of Bax and Bcl-2 expression in NRK-52E cells was quantified by western blotting. β-actin was used as an internal control. * indicates P<0.05 and ** indicates P<0.01.

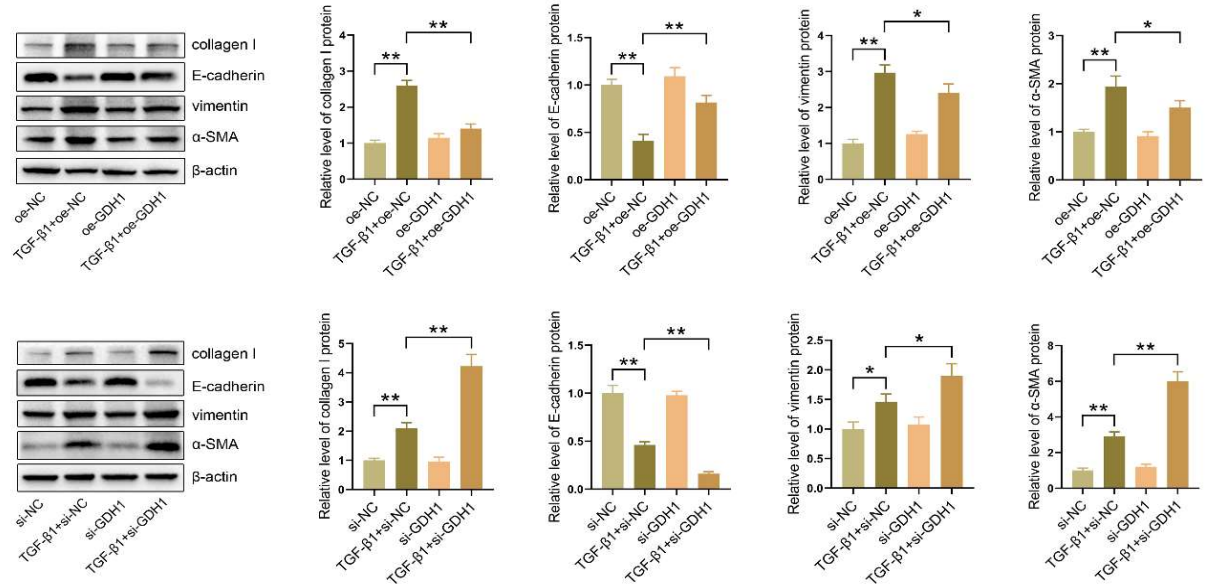

Fig. 4: GDH1 overexpression regulates the expression of multiple markers associated with fibrosis *in vitro*. Total protein was extracted from the NRK-52E cells treated accordingly and then subjected to western blot analysis. The levels of multiple markers associated with fibrosis, including collagen I, vimentin, α-SMA, and E-cadherin, were quantified by western blotting. β-actin was used as an internal control. * indicates *P* < 0.05 and ** indicates P<0.01.

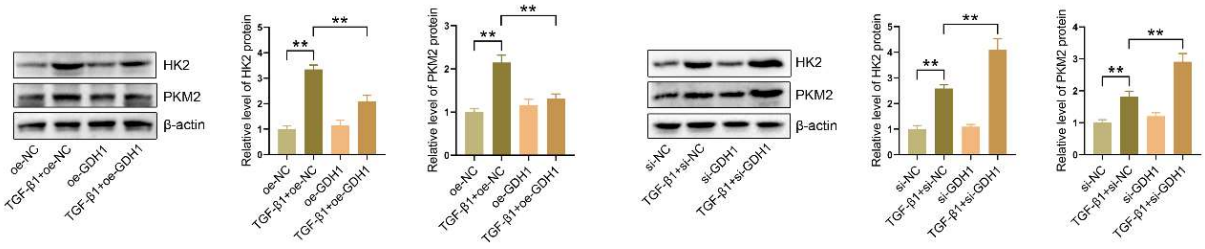

Fig. 5: GDH1 overexpression modulates glycolysis *in vitro*. Total protein was extracted from the NRK-52E cells treated accordingly and then subjected to western blot analysis. The levels of two glycolytic enzymes (HK2 and PKM2) were quantified by western blotting. β-actin was used as an internal control. ** indicates P<0.01.

DISCUSSION

CON represents the primary etiological factor in the development of CKD in young domestic animals, such as dogs and cattle. This study provides the first evidence, to our knowledge, implicating GDH1 as a protective factor in the pathogenesis of obstructive nephropathy, with direct translational relevance for animals with economic value. Notably, GDH1 protein sequences are highly conserved between cattle, dogs, and humans, supporting the translational potential of our rodent-derived findings. This study presents, for the first time, comprehensive evidence of the role and dual cellular mechanisms of GDH1 in rodent obstructive nephropathy, filling a critical gap in the literature where GDH1's function in CON had remained unclear despite its established roles in cancer progression and liver fibrosis regulation.

Our initial finding that GDH1 expression was significantly reduced in the renal tissue of a well-established neonatal rat PUUO model highlights that GDH1 downregulation is a fundamental event in the pathophysiology of obstructive renal injury, consistent with reduced GDH1 expression observed in TGF-β1-treated NRK-52E cells (an in vitro fibrosis model). These observations align with recent studies demonstrating GDH1's involvement in metabolic and fibrotic pathways, such as E2F1-transcriptional regulation of GDH1-mediated glycolysis in obstructive nephropathy, and extend this knowledge to CON-specific pathogenesis. GDH is overexpressed in a number of different types of cancer and plays a role in the progression of these cancers (Jin *et al.*, 2015; Hu *et al.*, 2023; Yang *et al.*, 2024). The regulation of GDH activity and expression in hepatic stellate cells represents an efficacious strategy for the alleviation of liver fibrosis (Yin *et al.*, 2022). Nevertheless, the role of GDH1 in CON pathogenesis remains undefined. We thus employed animal models to clarify its function in CON progression, and found that GDH1 overexpression markedly alleviated renal fibrosis in a neonatal rat model of PUUO, whereas the GDH1 inhibitor R162 exerted the opposite effect. These findings support GDH1 as a potential targeted anti-fibrotic agent, rather than a non-specific suppressor of renal injury. As progressive interstitial fibrosis represents the final common pathway to end-stage renal disease in CON and analogous conditions, anti-fibrotic strategies are a high priority in veterinary nephrology. Our data identify GDH1 as a key molecular mediator of this fibrotic cascade.

To elucidate the cellular mechanisms of GDH1-mediated renoprotection, we used TGF-β1-treated NRK-52E cells. As a canonical master profibrotic cytokine, TGF-β1 signaling drives renal fibrosis pathogenesis in humans and animals; moreover, the proliferation-apoptosis imbalance of renal tubular epithelial cells contributes to obstructive nephropathy. (Bascands & Schanstra, 2005; Liu *et al.*, 2018). To elucidate the underlying mechanism by which GDH1 restrains the development of CON in a neonatal rat model of PUUO, our initial focus was on the balance between cell proliferation and apoptosis. GDH1 overexpression of has been demonstrated to promote the proliferation of acute myeloid leukemia cells in vitro (Ma *et al.*, 2023). On the contrary, GDH1 knockdown inhibits the proliferation of multiple cancer cell lines (Jin *et al.*, 2015; Yang *et al.*, 2020). We found that GDH1 overexpression promoted cell proliferation while GDH1 knockdown inhibited it in TGF-β1-treated NRK-52E cells. The previous works have reported that GDH1 is also associated with the process of apoptosis (Jin *et al.*, 2018; Marsico *et al.*, 2021). Evidence indicates that GDH1 silencing or R162 exposure promotes apoptotic processes in lung cancer cells. *(Jin et al.*, 2018). Similarly, GDH1 knockdown has been shown to accelerate hepatocellular carcinoma cell apoptosis via the mitochondrial pathway. (Marsico *et al.*, 2021). Our findings revealed that GDH1 overexpression suppresses, while knockdown accelerates TGF-β1-induced apoptosis in NRK-52E cells through regulating Bax/Bcl-2 expression, thereby alleviating CON by restoring the proliferation-apoptosis balance of tubular epithelial cells.

CON, a pathological condition characterized by interstitial fibrosis (Silverstein *et al.*, 2003; Chevalier *et al.*, 2010), shows altered fibrotic progression with GDH1 modulation. In short, the overexpression of GDH1 was shown to have anti-fibrotic effect in neonatal rat model of PUUO, while GDH1 inhibitor R162 was demonstrated to induce a pro-fibrotic effect, which was consistent with the results of the in vitro studies.

Since the deregulation of mesenchymal markers and epithelial markers contributes to the formation of organ fibrosis (Song *et al.*, 2015), we conducted relevant experiments to investigate whether GDH1 modulates renal fibrosis by regulating the expression of these markers in tubular epithelial cells. Our results showed that GDH1 knockdown exerted a pronounced effect on TGF-β1-induced fibrosis by modulating the balance between these two classes of markers. Glycolysis, a pivotal energy-supplying glucose metabolic pathway (Zheng *et al.*, 2024), drives renal fibrosis progression, and its inhibition attenuates this pathology (Cui *et al.*, 2022; Xu *et al.*, 2022). We investigated GDH1-mediated regulation of renal fibrosis via glycolysis, showing GDH1 overexpression inhibited and knockdown enhanced TGF-β1-induced

upregulation of glycolytic regulators HK2 and PKM2 in tubular epithelial cells. These findings suggest GDH1 alleviates CON by modulating fibrosis- and glycolysis-related markers, providing a novel direction for metabolic therapies targeting renal fibrosis in companion and livestock animals. From a veterinary perspective, this study identifies GDH1 as a novel therapeutic target for CON and enhancing GDH1 activity pharmacologically may provide a needed anti-fibrotic strategy, supplementing current palliative or surgical care in dogs and cattle.

Conclusions: In conclusion, GDH1 emerges as a promising, mechanism-driven therapeutic target for the treatment of CON in rodents, with strong translational potential for dogs and cattle—supported by sequence conservation and shared fibrotic pathways. Its renoprotective effects are mediated through dual mechanisms: regulating tubular epithelial cell proliferation/apoptosis via the mitochondrial pathway and suppressing glycolysis-driven fibrosis. While species-specific structural differences in GDH1 warrant further validation in target animal models, our findings contextualize GDH1 within the existing literature on metabolic regulation of fibrosis and open a novel avenue for developing species-tailored metabolic therapies for renal fibrosis in veterinary nephrology.

Ethical approval: The animal experiments were approved by the Ethics Committee of Shengjing Hospital of China Medical University (2023PS1331K).

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Authors contribution: Rui Wang and Qian Zhao designed the study, performed most experiments, collected/analyzed data, and drafted the manuscript. Xu Fan and Xilin Gao assisted with animal experiments and sample processing. Jianjun Zhang participated in data analysis and provided molecular biology technical support. Xiaohan Yu assisted with literature retrieval and manuscript revision. Xiaofeng Gao and Jia You contributed to result discussion. Xin Liu and Yi Yang designed the study, conceived and supervised the project, critically revised the manuscript, and approved the final version.

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