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Original article

Hydrogen sulfide-producing cystathionine γ-lyase is critical in the progression of kidney fibrosis

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ABSTRACT

Cystathionine γ-lyase (CSE), the last key enzyme of the transsulfuration pathway, is involved in the production of hydrogen sulfide (H_2S) and glutathione (GSH), which regulate redox balance and act as important antioxidant molecules. Impairment of the H_2S- and GSH-mediated antioxidant system is associated with the progression of chronic kidney disease (CKD), characterized by kidney fibrosis and dysfunction. Here, we evaluated the role of CSE in the progression of kidney fibrosis after unilateral ureteral obstruction (UUO) using mice deficient in the *Cse* gene. UUO of wild-type mice reduced the expression of H_2S-producing enzymes, CSE, cystathionine β-synthase, and 3-mercaptopyruvate sulfurtransferase in the obstructed kidneys, resulting in decreased H_2S and GSH levels. *Cse* gene deletion lowered H_2S and GSH levels in the kidneys. Deleting the *Cse* gene exacerbated the decrease in H_2S and GSH levels and increase in superoxide formation and oxidative damage to proteins, lipids, and DNA in the kidneys after UUO, which were accompanied by greater kidney fibrosis, deposition of extracellular matrixes, expression of α-smooth muscle actin, tubular damage, and infiltration of inflammatory cells. Furthermore, *Cse* gene deletion exacerbated mitochondrial fragmentation and apoptosis of renal tubule cells after UUO. The data provided herein constitute in vivo evidence that *Cse* deficiency impairs renal the H_2S- and GSH-producing activity and exacerbates UUO-induced kidney fibrosis. These data propose a novel therapeutic approach against CKD by regulating CSE and the transsulfuration pathway.

1. Introduction

Pyridoxal-5′-phosphate (P5P)-dependent cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and P5P-independent 3-mercaptopyruvate sulfurtransferase (3-MST) produce hydrogen sulfide (H_2S) [1]. CSE and CBS are involved in the synthesis of glutathione (GSH) by suppling endogenous cysteine, a component of GSH. These two molecules, H_2S and GSH, produced by those enzymes, play important roles in the progression and development of various fibrogenic diseases, which are associated with oxidative tissue damage [1]. H_2S controls cellular redox status by regulating NF–E2-related factor 2 (Nrf2)/antioxidant responsive element (ARE) signaling and directly scavenging free radicals [2,3]. GSH is the most abundant antioxidant molecule and plays a critical role in maintaining cellular redox balance [4,5]. Emerging evidence has demonstrated that H_2S and GSH are associated with the progression of fibrosis in various organs [6,7]. However, the roles of these enzymes in kidney fibrosis remain to be defined.

Kidney fibrosis is a major cause in the development and progression of chronic kidney disease (CKD), which evokes severe clinical problems [8]. Kidney fibrosis is characterized by increased numbers of myofibroblasts, infiltration and accumulation of inflammatory cells, and excessive accumulation of extracellular matrix components such as collagen and fibronectin [9,10]. Recently, it was reported that

List of abbreviations: CSE, Cystathionine γ-lyase; H_2S, Hydrogen sulfide; GSH, Glutathione; CKD, Chronic kidney disease; UUO, Unilateral ureteral obstruction; P5P, Pyridoxal-5′-phosphate; CBS, Cystathionine β-synthase; 3-MST, 3-mercaptopyruvate sulfurtransferase; Nrf2, NF–E2-related factor 2; ARE, Antioxidant responsive element; ESRD, End-stage renal disease; α-SMA, α-smooth muscle actin; Gpx, Glutathione peroxide; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; Fis1, Fission 1 protein; PAG, Propargylglycine; NAC, N-acetylcysteine; TGF-β1, Transforming growth factor β1; 4-HNE, 4-hydroxynonenal; MnSOD, Manganese superoxide dismutase; CuZnSOD, Copper-zinc superoxide dismutase; TNF-α, Tumor necrosis factor α; Opa1, Optic atrophy 1; XIAP, X-linked inhibitor-of-apoptosis protein

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impairments in H_2S production and GSH-associated antioxidant system play a critical role in the development and progression of kidney fibrosis; plasma H_2S and CSE mRNA levels in end-stage renal disease (ESRD) patients are lower than levels observed in healthy people. Furthermore, levels of plasma homocysteine and cysteine, precursors of H_2S and GSH, respectively, are higher in ESRD patients than in healthy people [11,12]. Plasma GSH level is lower in patients with moderate and severe CKD, and the GSH level is positively correlated with creatinine clearance [13]. These clinical studies suggest that H_2S, GSH, and the CSE-transsulfuration pathway are deeply associated with the progression of kidney fibrosis. Supporting this, many investigators, including us, have recently reported that kidney fibrosis induced by 5/6 of nephrectomy, unilateral ureteral obstruction (UUO), or streptozotocin injection impair the functions of all three H_2S-producing enzymes in the kidneys, and exogenous supplements of H_2S attenuate kidney fibrosis and GSH reduction, while inhibition of H_2S production worsens them [2,14–19]. These studies have suggested that the antifibrotic effects of H_2S are mediated by inhibition of oxidative stress, inflammation, and fibrogenic pathways including TGF-β1/SMAD3, MAPK, and NF-κB [2,14,19–23].

Mitochondria are most susceptible organelle to ROS/oxidative damage and mitochondrial oxidative damage is critical for the progression of fibrosis by inducing apoptosis and inflammation [10,24]. It was recently reported that CSE regulates mitochondrial H_2S and GSH levels [25,26]. We also recently found that a reduction in mitochondrial GSH level exacerbates kidney fibrosis by enhancing mitochondrial oxidative stress, thereby inducing mitochondrial damage and apoptosis of tubule cells [27]. Exogenous supplementation of H_2S ameliorates renal fibrosis by downregulating ROS, oxidative damage, and the inflammatory responses, and upregulating GSH in kidneys after UUO or ischemic injuries [2,14]. I n the kidney, CSE is abundantly expressed, 20-fold more than the CBS expression, and is mainly responsible for H_2S production in combination with CBS [6,21,22]. Therefore, we have investigated the role of CSE in fibrotic changes of kidneys after UUO and its underlying mechanisms using *Cse* knockout ($Cse^{-/-}$) mice.

2. Result

2.1. Cse gene deletion exacerbates UUO-induced fibrosis in the kidney

First, we determined the effect of the systemic *Cse* gene deletion on kidney fibrosis after UUO. UUO induced tubular damage and expansion of the interstitial area; these phenotypes were much more apparent in $Cse^{-/-}$ mice than in $Cse^{+/+}$ mice (Fig. 1A [top panel], B). Collagen deposition, as evaluated by Sirius red staining, also increased in the interstitium after UUO and this increase was significantly greater in $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys (Fig. 1A [middle panel], C). Furthermore, UUO-induced increases in F4/80-positive cells, monocytes/macrophages, were greater in the interstitium of $Cse^{-/-}$ kidneys than $Cse^{+/+}$ kidneys. (Fig. 1A [bottom panel], D). In contrast, there were no significant differences in tubular damage, collagen deposition, and number of F4/80-positive cells between sham-operated $Cse^{-/-}$ and $Cse^{+/+}$ mouse kidneys (Fig. 1A-D). Expressions of TNF-α, a pro-inflammatory cytokine, and Ly6G, a neutrophil marker, increased after UUO and these increases were significantly greater in $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys (Fig. 1E-G). There were no significant differences in TNF-α or Ly6G expression between sham-operated $Cse^{-/-}$ kidneys and $Cse^{+/+}$ kidneys (Fig. 1E-G).

TGF-β1 acts as a main mediator in fibrosis development and progression in CKD via Smad-3 phosphorylation (p-Smad3) and the subsequent activation of collagen-producing myofibroblasts [9]. Post-UUO increases of TGF-β1, p-Smad3, and α-smooth muscle actin (α-SMA; a myofibroblast marker) expression were all significantly greater in $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys (Fig. 1H-K). Taken together, the systemic *Cse* gene deletion in mice exacerbates kidney fibrosis, tubular damage, and inflammation by promoting greater activation of TGF- β1 signals and myofibroblast activation after UUO.

2.2. Cse gene deletion impairs H_2S and GSH production in the kidney

To investigate whether increased fibrosis in *Cse* gene-deleted mice after UUO is associated with impaired transsulfuration pathways, which are linked to H_2S and glutathione (GSH) production, [4,25] we examined expressions of CSE, CBS, and 3-MST proteins, and levels of H_2S and GSH in the kidneys. After UUO of $Cse^{+/+}$ mice, kidney levels of H_2S decreased in a time-dependent manner (Fig. 2A), which were accompanied by time-dependent decreases in renal CSE, CBS, and 3-MST expression (Fig. 2B-E). Similarly, renal GSH levels were decreased a time-time-dependent manner after UUO (Fig. 2F), accompanied by time-dependent decreases in renal glutathione peroxide 1 (GPx1) and glutathione reductase (GR) expression (Fig. 2G-I). Levels of H_2S and GSH and these enzymes (CSE, CBS, and 3-MST) were negatively correlated with α-SMA expression and collagen deposition (Fig. 2A-L). These results indicate that CSE/CBS/3-MST-mediated H_2S/GSH production is intimately linked with progression of kidney fibrosis.

Next, we examined whether the systemic *Cse* gene deletion in mice affects H_2S, GSH production, the ratio of GSSG to tGSH, and CBS and 3-MST expression. After sham-operation, H_2S and GSH levels in the $Cse^{-/-}$ mouse kidneys were significantly lower than those in $Cse^{+/+}$ kidneys, about 60% and 70%, respectively (Fig. 3A, B). These data indicate that *Cse* gene deletion impairs H_2S and GSH production in the kidney. At 5 days after UUO, H_2S and GSH levels significantly decreased in both $Cse^{-/-}$ and $Cse^{+/+}$ kidneys but both decreases were significantly greater in the $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys (Fig. 3A, B). In addition the ratio of GSSG to tGSH was significantly greater in the $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys after UUO (Fig. 3C). Consistent with the levels of H_2S and GSH, expressions of CSE, CBS, and 3-MST decreased after UUO (Fig. 3D-G). In contrast, renal CBS and 3-MST expression was comparable between $Cse^{+/+}$ and $Cse^{-/-}$ mice at either pre- or post-UUO (Fig. 3D, E, G). These results suggest that CSE plays a pivotal role in the maintenance of renal H_2S/GSH levels, thereby protecting kidney against fibrosis via their anti-oxidative activities.

2.3. Cse gene deletion exacerbates ROS production and oxidative stress after UUO

H_2S and GSH play important roles in maintaining redox balance, which is critical for the progression of renal fibrosis and tubular damage [6]. Expression of 4-HNE, oxidized peroxiredoxin (Prx-SO_3), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), indicators of lipid peroxidation, peroxiredoxin oxidation, and DNA oxidation, [28,29] respectively, significantly increased in both $Cse^{-/-}$ and $Cse^{+/+}$ mouse kidneys after UUO and those increases were greater in $Cse^{-/-}$ kidneys (Fig. 4A-E). There were no significant differences in expression of 4-HNE, Prx-SO_3, or 8-OHdG between sham-operated $Cse^{-/-}$ and $Cse^{+/+}$ mouse kidneys (Fig. 4A-E). These results indicate that *Cse* gene deletion in mice exacerbates oxidative stress in the ureteral obstructed kidneys after UUO, and this increased oxidative stress may be associated with a decreased antioxidative effect in kidneys due to reduced levels of H_2S and GSH by *Cse* gene deletion.

GSH is required for the GSH-mediated antioxidative system, which contains enzymes such as glutathione peroxidase 1 (GPx1) and glutathione reductase (GR). Since GPx1 is a major enzyme for removal of H_2O_2 using reduced GSH, which consequently produces oxidized glutathione (GSSG) [30], we determined GPx1 levels. UUO significantly decreased GPx1 levels in the kidneys and this decrease was greater in the $Cse^{-/-}$ kidneys than in $Cse^{+/+}$ kidneys (Fig. 5A, B). Furthermore, the expression of GR, which reduces GSSG [30] to GSH, was lowered in the kidneys from both groups after UUO, and this reduction was greater in the $Cse^{-/-}$ kidneys than in the $Cse^{+/+}$ kidneys (Fig. 5A, C). There was no significant difference in GPx1 or GR expression between sham-operated $Cse^{-/-}$ and $Cse^{+/+}$ mouse kidneys (Fig. 5A-C). These data