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Heme Oxygenase-1 as a Target for TGF- β in Kidney Disease

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Abstract

Transforming growth factor- β (TGF- β) is a multifunctional regulatory cytokine that is implicated in a variety of kidney diseases, including diabetic nephropathy and chronic transplant rejection where it promotes stimulation of the extracellular matrix deposition, cell proliferation and migration. TGF- β exerts its biological functions largely via its downstream complex signaling molecules, Smad proteins. Paradoxically, TGF- β is also essential for normal homeostasis and suppression of inflammation through mechanisms that are yet to be fully elucidated. One feasible mechanism by which TGF- β may exert its beneficial properties is through induction of heme oxygenase-1 (HO-1). Induction of this redox sensitive enzyme is known to be cytoprotective through its potent antioxidant, anti-inflammatory and anti-apoptotic properties in different conditions including several kidney diseases. In this overview, recent advances in our understanding of the role of TGF- β in kidney disease, its molecular regulation of HO-1 expression and the potential role of HO-1 induction as a therapeutic modality in TGF- β mediated kidney diseases are highlighted.

Keywords

Transforming growth factor- β ; Heme Oxygenase-1; Kidney Injury; Fibrosis

Introduction

Transforming growth factor-beta (TGF- β) is the founding member of a large group of structurally related polypeptides that have pleiotropic functions in a wide variety of organisms ranging from insects to humans.¹ The TGF- β superfamily is generally subdivided into four major families: the Mullerian inhibitory factor family, the inhibin/activin family, the bone morphogenetic protein (BMP) family, and the TGF- β family.² Compared to many other growth factors, the TGF- β family is unique in the sense that it signals through an initial serine-threonine kinase, while activation of tyrosine kinase is the preferred mode of signal transduction for most other growth factors.³ In addition, mammalian TGF- β is synthesized and secreted as biologically inactive aggregates consisting of mature TGF- β ,

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latency associated peptide and a latent TGF- β binding protein.⁴⁻⁶ Different mechanisms such as proteases (plasmin and cathepsin D), low pH and reactive oxygen species have been proposed for the activation of TGF- β but the exact molecular pathways still need to be clarified.⁴⁻⁶ Irrespective of the nature of TGF- β activation, such latency is critical due to ubiquitous expression of TGF- β receptors.⁶

The intracellular signaling of TGF- β is mediated by a group of proteins called Smads.⁷ The Smad second messengers were initially described by two groups of investigators studying embryologic patterns in *Drosophila*⁸ and *Caenorhabditis elegans*.⁹ They termed their newly discovered proteins “mothers against decapentaplegic” (MAD)⁸ and “Sma”⁹, respectively. Ultimately the terms were combined to Smad proteins and it is now clear that the Smad proteins are key elements in TGF- β signaling.^{7, 10} Vertebrate Smads are subdivided based on structural and functional considerations into receptor-regulated Smads, common mediator Smads and inhibitory Smads.^{7, 10} The cellular events of TGF- β are initiated following its binding to TGF- β receptor II. Following ligand binding, the TGF- β receptor II triggers the activation of the TGF- β receptor I via its phosphorylation. The receptor-regulated Smads (Smad 1, 2, 3, 5 and 8) which are pathway specific are then phosphorylated by interaction with the receptors.^{7, 10} This allows the receptor-regulated Smads to form oligomeric complexes with the common Smad protein (Smad 4) that subsequently translocate to the nucleus, wherein transcriptional regulation of the target genes is initiated.^{7, 10} The presence of one or more Smad binding elements, a β hairpin with the major groove of the sequence GTCT and CAGA, is required for multiple genes to respond to members of the TGF- β family.¹¹⁻¹⁴ Furthermore, Smads have been shown to interact with several different transcription factors such as FAST-1, CEBP/P300 and based on the availability of cofactors within a cell type, TGF- β has the potential to elicit diverse gene responses.¹¹ Another class of Smad proteins is responsible for antagonizing the activity of the receptor-regulated Smads (Smad 6 and 7).^{7, 10} It is important to note that the TGF- β is also involved in biological activities that follow Smad independent pathways. These include, but are not limited to, activation of mitogen-activated protein Kinase, Rho family, phosphoinositol-3 kinase and protein phosphatase.¹⁵

TGF- β in physiology and disease

Three of five distinct members of TGF- β family (TGF- β 1, 2, 3) are expressed in mammals and have been extensively studied.¹⁶ Although members of the TGF- β family were initially distinguished for their ability to inhibit the growth of epithelial and hematopoietic cells, their versatile roles in development, acute and chronic inflammation, cell cycle regulation, wound repair and mediating extracellular matrix (ECM) production has been increasingly recognized.¹⁷⁻²⁰ These disparate homeostatic functions attributed to TGF- β are further highlighted by TGF- β knockout animals. TGF- β 1 null mice die of wasting and widespread inflammation within 3–4 weeks of age²¹, TGF- β 2 null mice have defective mesoderm formation and exhibit a wide range of developmental defects in major organs including heart and kidneys and die near the time of birth^{22, 23}. TGF- β 3 null mice exhibit cleft palates and major defects in pulmonary development and die within the first day of birth.²⁴ In contrast, TGF- β 1 overexpressing mice (under the control of an albumin promoter) exhibit nephrotic syndrome and progressive glomerulosclerosis and interstitial fibrosis and die by 15 weeks of age.²⁵

In response to injury, TGF- β s and other growth factors are released via autocrine and/or paracrine mechanisms to maintain cellular homeostasis. They enhance wound repair by stimulating the synthesis of connective tissue matrix proteins, structural proteins (collagen and fibronectin) and by inducing proliferative arrest and promoting differentiation.²⁶ Moreover, mounting evidence suggests that TGF- β s play important function in maintaining

immune tolerance²⁰ and controlling the initiation and resolution of inflammatory conditions such as multiple sclerosis²⁷, autoimmune encephalomyelitis²⁸, inflammatory bowel disease²⁹, erosive arthritis³⁰ and others, via regulation of chemotaxis and activation of leukocytes in the periphery. Therefore, it is evident that while TGF- β has essential physiological properties, its deranged expression has detrimental consequences acting as a “double edged sword” nature. One prominent example of such nature of TGF- β is in cancer. On the one hand it can act as a tumor suppressor by attenuating cell proliferation through attenuation of c-Myc expression and some cyclin-dependent kinase inhibitors. It has been demonstrated that when exposed to TGF- β some cancer cells secrete an anti-angiogenic factor, thrombospondin-1.³¹ On the other hand, TGF- β can also function as a pro-oncogenic agent via stimulation of matrix deposition and derangements in normal immune function.³² The dual nature of TGF- β in renal injury and disease is also explicitly documented. For instance, while neutralization of TGF- β has been demonstrated to mitigate renal fibrosis, it also results in aggravation of proteinuria and albuminuria.^{33, 34}

TGF- β and heme oxygenase-1 (HO-1) interaction in kidney disease

An exhaustive list of diseases and insults lead to progressive loss of differentiated glomerular, tubular, and vascular cells that constitute the normal nephron and eventually lead to their replacement by interstitial fibrosis³⁵, a hallmark of chronic kidney disease (CKD). Due to exceptionally high incidence and prevalence, CKD is a major challenge for patients, clinicians and the society as a whole.³⁶ The molecular events that lead to renal tubulointerstitial fibrosis are diverse and have been a subject of much debate. Nonetheless, there is consensus that irrespective of etiology, TGF- β has a central role in initiating and modulating tissue repair and its aberrant expression is directly involved in the pathogenesis of progressive CKD.³⁷

In the context of kidney disease, TGF- β 1 is implicated in a number of pathological conditions such as IgA nephropathy, cyclosporin-induced nephrotoxicity, focal segmental glomerulosclerosis, crescentic glomerulonephritis, lupus nephritis, diabetic nephropathy, obstructive nephropathy, light chain deposition disease and chronic transplant rejection where it promotes stimulation of the ECM components, cell proliferation, migration and inflammation.³⁷⁻⁴⁰ In experimental and human disease, TGF- β 1 has been implicated in the pathogenesis of renal fibrosis not only by inducing apoptosis and promoting ECM accumulation but also by decreasing the synthesis of proteases and increasing the levels of protease inhibitors such as tissue inhibitor of metalloproteinases and integrins.^{41, 42} TGF- β 1 expression is associated with apoptotic tubular cells that are thought to underlie the mechanism of tubular atrophy seen in CKD.⁴³ More importantly, TGF- β blockade reduces tubular epithelial apoptosis and in turn attenuates the extent of tubular atrophy in models of obstructive nephropathy and diabetic kidneys.^{44, 45} These encouraging results in pre-clinical models have led to ongoing clinical trials with a monoclonal TGF- β antibody (fresolimumab) in patients with focal and segmental glomerulosclerosis.⁴⁶ TGF- β has also been implicated in the pathogenesis of proteinuria by inducing podocyte apoptosis and depletion.⁴⁷ Similarly, TGF- β is shown to induce apoptosis in endothelial cells that may explain the loss of peritubular capillaries associated with tubulointerstitial fibrosis and tubular atrophy.⁴⁸ Additionally, disruption of TGF- β Smad signaling mitigates renal tubulointerstitial fibrosis in obstructive nephropathy.⁴⁹

In diabetes, advanced glycation end products induce TGF- β overexpression in proximal tubular cells, leading to tubulointerstitial fibrosis.⁵⁰ The role of TGF- β in the pathogenesis of diabetic nephropathy is further highlighted by increased TGF- β expression in various renal cell types cultured in the presence of high glucose.^{51, 52} Additionally, there is marked increase in the renal levels of TGF- β in both experimental animals and humans with

diabetes.^{53, 54} Moreover, overexpression of TGF- β in the glomerulus causes renal dysfunction characterized by proteinuria and fibrosis⁵⁵ and the early manifestations of diabetic renal disease in mice is attenuated by administration of anti-TGF- β antibody.⁵⁶ Transient hyperglycemia in healthy individuals is associated with increased urinary levels of TGF- β 1 along with F2-isoprostanes, a marker for oxidative stress.⁵⁷ Overall, increased TGF- β is the final common pathway in the pathogenesis of renal injury by several factors including high glucose, angiotensin II, hypoxia, immune complexes, advanced glycosylation end products, and platelet derived growth factor.^{37–40} Hence, TGF- β plays a central role in the pathogenesis of renal inflammation, fibrosis and ultimately CKD.

Chronic elevation of TGF- β 1 plays an important pathogenic role in the progression of renal diseases. However, TGF- β 1 also stabilizes and attenuates tissue injury. Therefore, in order to fully comprehend the mechanism of TGF- β mediated renal injury and to explore prospects of therapeutic modalities, the “good” side of TGF- β should not be neglected. One mechanism by which the beneficial effects of TGF- β may be mediated and its harmful effects counteracted, is through induction of cytoprotective proteins such as heme oxygenase-1 (HO-1).^{58–61} HO-1 catalyzes the degradation of heme into equimolar amounts of iron, carbon monoxide (CO) and biliverdin (Fig. 1).^{62–64} Biliverdin is subsequently converted to bilirubin via biliverdin reductase, while iron induces the expression of ferritin. Two isoforms of heme oxygenase (HO-1, HO-2) have been identified.^{62–64} They are the products of two different genes sharing about 40% amino acid homology. HO-1 is a microsomal enzyme that is induced by heme products as well as a wide variety of non-heme stimuli, including multiple noxious agents as well as growth factors such as platelet derived growth factor and TGF- β 1.^{62–64} In contrast, HO-2 is a constitutive enzyme and functions as a physiologic regulator of cellular function. The cytoprotective effects of HO-1 induction are diverse. It catalyzes degradation of the heme moiety (a pro-oxidant molecule) and generates beneficial products that have been thoroughly investigated. Bilirubin, one of the byproducts, possesses antioxidant properties via scavenging peroxy radicals and inhibiting lipid peroxidation.⁶⁵ Ferritin, an intracellular iron repository, is co-induced with HO-1 allowing safe sequestration of unbound iron liberated from heme degradation that would otherwise lead to increased reactive oxygen species formation.⁶⁶ Moreover, CO has vasodilatory effects mediated via cGMP and potassium channels⁶⁷, as well as anti-apoptotic and immunomodulatory functions.⁶⁸ Finally, the protective effects of HO-1 overexpression have also been attributed to the upregulation of the cell-cycle regulatory protein, p21.⁶⁹

Numerous studies have demonstrated the protective effects of HO-1 in both *in vitro* and *in vivo* models of injury and disease (reviewed in ^{62–64}). In the context of kidney injury and disease, HO-1 has been shown to be protective against rhabdomyolysis, ischemia-reperfusion injury, acute nephrotoxicity from chemotherapeutic agents, diabetes, sepsis, obstructive nephropathy and transplant rejection (reviewed in ^{62–64}). Repeated exposure of HO-1^{-/-} mice to heme proteins leads to intense interstitial cellular inflammation with significant increase in monocyte chemotactic protein-1 expression and activation of nuclear factor- κ B.⁷⁰ In addition to protecting against acute cytotoxicity, HO-1 down-regulates the inflammatory response in both renal and non-renal tissues.⁷¹ The phenotype of the HO-1^{-/-} mouse (at ages beyond 20–24 weeks) is characterized by chronic renal and hepatic inflammation, tissue iron deposition, anemia, splenomegaly and increased susceptibility to cardiovascular diseases which highlights the functional and biological significance of HO-1.⁷² These *in vitro* and animal model findings are also corroborated in human case reports. Two patients with HO-1 deficiency have so far been described who presented with several phenotypic similarities with the HO-1^{-/-} mouse, and had extensive atherosclerosis and marked renal tubulointerstitial injury associated with tubular dilation and atrophy, inflammatory cell infiltration and interstitial fibrosis.^{73, 74} The level of HO-1 expression can be variable within the human population since the promoter of human HO-1 gene is highly

polymorphic and contains a (GT) repeat region. Evidence suggests that patients with lower (GT)_n repeats have higher HO-1 expression and thereby are associated with better patient outcome in a number of clinical conditions such as renal graft survival⁷⁵, vascular stenosis⁷⁶, arteriovenous fistula patency in hemodialysis patients⁷⁷ polycystic kidney disease and IgA nephropathy.⁷⁸ Furthermore, there are now ongoing clinical trials that are examining the beneficial effects of HO-1 byproducts including CO in kidney transplantation (clinicaltrials.gov, NCT 00531856) and bilirubin in endotoxemia (clinicaltrials.gov, NCT 00916448).

The anti-fibrogenic properties of HO-1 and its products, particularly CO, in different pathological conditions and tissues have been extensively studied. Fujita et al. demonstrated that inhaled CO increased survival of HO-1^{-/-} mice with lethal ischemic lung injury by inhibiting a key profibrotic agent, plasminogen activator inhibitor-1.⁷⁹ In a rat hypoxia model, chemical inhibition of HO-1 increased collagen (type I, type III) and TGF- β 3 expression, an effect attributed to a decrease in CO levels.⁸⁰ Morse et al. demonstrated a protective effect of inhaled CO in a model of bleomycin-induced pulmonary fibrosis.⁸¹ Exogenous CO administration has also been shown to decrease proliferation of human fibroblasts.⁸¹ Increased HO activity in human hepatic myofibroblasts correlates with decreased proliferation and procollagen I mRNA expression, which was attributed to bilirubin.⁸² Bilirubin has also been shown to attenuate bleomycin-induced pulmonary fibrosis, partly by inhibiting inflammation and TGF- β 1 production.⁸³

Role of HO-1 in obstructive nephropathy

Unilateral ureteral obstruction (UO) is a well characterized experimental model of renal injury and tubulointerstitial fibrosis.^{84, 85} Multiple investigations have demonstrated the central role of TGF- β in the pathogenesis of UO. The mechanism by which renal fibrosis occurs following UO is postulated to be mediated through an epithelial mesenchymal transition (EMT) pathway. This pathway has been under intense research using both *in vitro* and *in vivo* models by different laboratories and investigators and substantial progress has been made in our understanding of TGF- β mediated renal inflammation and fibrosis. However, the precise nature of cells that contribute to renal fibrosis and EMT are still being debated and remain controversial.⁸⁶ Irrespective of the EMT pathway, it is well accepted that oxidative stress is a principal regulator of UO and TGF- β -mediated renal fibrosis.⁸⁷ Therefore, HO-1 which possesses potent anti-oxidant properties ought to play a beneficial role in suppression of TGF- β mediated renal injury and fibrosis (Fig. 2). In fact, recent studies corroborate this hypothesis. Following injury, TGF- β is induced in renal epithelial cells which is associated with robust induction of HO-1 in these cells (Fig. 3, 4).⁸⁴ Using human renal epithelial cells, *in vitro* studies revealed that HO-1 induction suppresses the profibrotic effects of TGF- β (Fig. 5).⁸⁸ *In vivo*, it was demonstrated that HO-1 mRNA is induced as early as 12 hours following UO.⁸⁷ Additionally, preinduction of HO-1 using hemin significantly alleviates renal levels of TGF- β and tubulointerstitial fibrosis in the obstructed kidney that was mediated via antiapoptotic pathway involving Bcl-2.⁸⁵ HO-1^{-/-} mice have exacerbated levels of TGF- β expression, inflammation and ECM deposition following UO.⁸⁴ Intriguingly, it was also demonstrated that hemin (a potent HO-1 inducer) not only prevents but also has the ability to reverse renal tubulointerstitial fibrosis.⁸⁹ More recently, the antifibrotic properties of HO-1 activation have extended to other organs such as the heart and liver.⁹⁰⁻⁹²

Similar to TGF- β , HO-1 may also have a dual role in tissue pathology and is not cytoprotective in all circumstances.⁹³ It has been suggested that an appropriate level of HO-1 induction is beneficial, whereas too much HO-1 may in fact be deleterious. In these studies, HO-1 activity was regulated between 3- and 17-fold *in vitro* in a model of hyperoxia

mediated cell injury.⁹³ Whereas, HO-1 overexpressing cells exposed to hyperoxia for 24h were protected against oxygen toxicity with low (<5 fold) HO-1 activity, significant oxygen cytotoxicity was observed at high levels (>15 fold) of HO-1 activity presumed due to increased iron release.⁹³ However, *in vivo* studies in transgenic hearts overexpressing different levels of HO-1 have demonstrated a dose-dependent protective effect in cardiac ischemia-reperfusion injury.⁹⁴ It is important to note that each of the products of the HO-1 reaction has potential toxic effects as well. For example, CO activates mitochondrial generation of free radicals and can poison heme proteins⁹⁵ and high levels of bilirubin can cause brain damage (kernicterus) in neonates.⁹⁶

Molecular regulation of HO-1 by TGF- β

Regulation of HO-1 expression by TGF- β 1 is complex and cell specific. TGF- β 1 increases the expression of HO-1 in human retinal pigment epithelial cells, human renal proximal tubular epithelial cells, human pulmonary epithelial cells derived from a lung cell carcinoma (A549 cells), HaCaT human keratinocytes, and bovine choroids fibroblasts.^{59, 60, 97, 98} However, TGF- β 1 does not induce HO-1 in all cell types, including HeLa or bovine corneal fibroblasts⁶⁰ and more recently, it was also shown that TGF- β suppresses endotoxin induced HO-1 in macrophages.⁹⁹ Interestingly, in an LPS-induced rat model of endotoxemia, as well as in IL-1 β treated cultured rat vascular smooth muscle cells, wherein HO-1 is pre-induced, the HO-1 mRNA and protein expression were reduced by TGF- β 1 in the heart and lung.¹⁰⁰ TGF- β 1 downregulated HO-1 mRNA after its induction by IL-1 β in vascular smooth muscle cells. TGF- β conferred a negative effect on HO-1 which contributed to the beneficial effects of TGF- β in endotoxic shock.¹⁰⁰ Conversely, overexpression of HO-1, using retrovirus gene transfer, markedly inhibited TGF- β mRNA and protein in a rat lung microvessel endothelial cell line.¹⁰¹ Thus, TGF- β -mediated HO-1 induction may counteract the negative effects of TGF- β 1 by blocking further TGF- β 1 production and/or affecting cell proliferation, apoptosis and deposition of ECM.

The mechanism by which TGF- β 1 enhances HO-1 expression is through increased gene transcription and does not involve increased mRNA stability.⁵⁹ Additionally, it is also important to note that while all three isoforms of TGF- β (1, 2 and 3) induce HO-1 protein in human renal proximal tubular epithelial cells, another member of the TGF- β superfamily, BMP-7 does not.⁵⁹ Whereas inhibitors of the MAPK pathway have no effect on TGF- β 1-stimulated HO-1 mRNA production in primary human renal epithelial cells, it appears that p38 MAPK is a mediator in A549 cells as well as human retinal pigment epithelial cells.^{59, 98} The Smad pathway is involved in TGF- β 1-mediated HO-1 induction in human renal epithelial cells. Expression of Smad7, but not Smad6, inhibits TGF- β 1-mediated induction of HO-1 in human renal proximal tubular cells.⁵⁹ Moreover, inhibitors of oxidative stress such as N-acetylcysteine prevent induction of HO-1 by cytokines (TNF- α) in endothelial cells¹⁰² and deferoxamine (an iron chelator) inhibits HO-1 induction by oxidized LDL and hyperoxia in endothelial cells.¹⁰³ However, TGF- β 1-mediated induction of HO-1 mRNA in human renal proximal tubular epithelial cells is not modulated by co-treatment with N-acetylcysteine or deferoxamine. These observations suggest that different signal transduction pathways are involved in the regulation of HO-1 expression by TGF- β .⁵⁹

HO-1 induction by TGF- β 1 is also different than that of hemin (heme chloride) a potent inducer of HO-1. TGF- β 1-mediated induction of HO-1 protein is not as persistent as that of hemin-induced, which may be explained by a simultaneous induction of the inhibitory Smads. TGF- β 1 has been shown to upregulate the inhibitory Smad (Smad 7) and overexpression of Smad7 inhibits the induction of the endogenous HO-1 gene by TGF- β 1 in human renal epithelial cells.⁵⁹ Furthermore, hemin requires the -4.5kb promoter region and an enhancer, internal to the HO-1 gene, for maximal induction and neither of these regions

are responsive to TGF- β 1.¹⁰⁴ In another study, a cis-acting region between -9.1 and -9.4 kb of the human HO-1 promoter was found to be responsible for TGF- β 1-mediated HO-1 upregulation. Following transient transfection experiments in HK-2 cells using 4.5-, 8.5-, 9.1-, 9.4-, 9.7-, and 11.6-kb promoter fragments of the HO-1 gene cloned into a promoterless luciferase vector, it was demonstrated that the 4.5- and 8.5-kb promoter fragments were not responsive to TGF- β 1, while 9.4-, 9.7-, and 11.6-kb fragments each demonstrated increased luciferase activity. Further studies revealed the requirement of a Smad binding element and consensus specificity protein 1-binding sites within the region between -9.1 and -9.4 kb for TGF- β mediated HO-1 induction.¹⁰⁵ Another recent study revealed that the anti-oxidant properties of TGF- β 1 in aortic smooth muscle cells are exerted via Nrf2-mediated HO-1 expression.¹⁰⁶

Induction of HO-1 is an adaptive response to provide a balance for some of the effects of TGF- β 1, mediated through its reaction products. In addition, HO-1 upregulation and its byproducts have antifibrotic effects which may also offset the profibrotic effects of TGF- β . It is plausible that persistently elevated TGF- β 1 overwhelms this response, leading to fibrosis and progression of renal disease. HO-1 induction by TGF- β and/or the downstream mediators of HO-1 expression (CO, bilirubin, biliverdin, and/or biliverdin reductase) may be dysregulated in pathophysiological states. Finally, late stage metastatic disease is typically characterized by increased TGF- β levels and a concomitant reduction in responsiveness of tumor cells to its suppressor functions.¹⁰⁷ The role of HO-1 in this setting is unclear. Upregulation of HO-1 has been associated with tumor growth and perhaps HO-1 induction is responsible for the loss of tumor suppressor functions of TGF- β .¹⁰⁸ An appropriate level of HO-1 induction may be beneficial, whereas in the setting of cancer, its proangiogenic effects may in fact potentiate progression of tumor growth.

Concluding remarks

We herein discussed some of the important aspects of TGF- β signaling, its role in physiology and disease as well as its interaction with HO-1 in renal disease. Over the past several years immense progress has been accomplished in the field of understanding TGF- β signaling and biological functions. The central role of TGF- β in renal fibrosis and CKD is widely accepted and the TGF- β /smad signaling pathway is now a viable candidate for anti-fibrotic therapeutic strategies. It is also evident that HO-1 is a crucial cytoprotective enzyme and a strong relationship between TGF- β and HO-1 expression exists. Understanding the cellular effects and molecular mechanisms of HO-1 gene expression in response to TGF- β will be important in designing interventional strategies in TGF- β -mediated diseases. This is particularly important since TGF- β may have a key role in mediating renal inflammation and fibrosis. Therefore, interventions aimed for example at manipulating the Smad7 pathway would aid in regulating the expression of HO-1, thereby exploiting its cytoprotective effects in TGF- β mediated kidney diseases.

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Abbreviations used

TGF-β	transforming growth factor
HO-1	heme oxygenase-1
BMP	bone morphogenetic protein

ECM	extracellular matrix
CKD	chronic kidney disease
CO	carbon monoxide
UUO	unilateral ureteral obstruction
EMT	epithelial mesenchymal transition

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Figure 1. The heme oxygenase (HO) pathway

HO catalyzes degradation of heme to produce equimolar amounts of iron, biliverdin, carbon monoxide (CO) and bilirubin. Acute and chronic kidney diseases are associated with renal tubular cell injury and interstitial fibrosis. HO-1 and one or more of its byproducts can attenuate cell injury.

Figure 2. The pathogenic pathway of TGF-β and its interaction with heme oxygenase-1 (HO-1) in kidney disease

Overexpression of TGF-β is associated with increased renal injury and extracellular matrix deposition that eventually lead to chronic kidney disease and renal failure. TGF-β also activates HO-1 expression and one or more products of the HO-1 reaction mitigate TGF-β mediated pathogenic and fibrogenic effects.

Figure 3. Both heme oxygenase-1 (HO-1) and TGF- β 1 are robustly induced in tubular cells subsequent to unilateral ureteral obstruction (UUO)

UUO, a well characterized model of obstructive nephropathy, results in prompt and strong induction of TGF- β and HO-1 in tubular cells. Upregulation of HO-1 is an adaptive response to counteract the injurious and profibrogenic properties of TGF- β 1 overexpression. (Modified from⁸⁴, with permission).

Figure 4. Induction of TGF- β 1 is predominately localized to proximal tubular cells
Serial section staining of kidneys that underwent unilateral ureteral obstruction demonstrated that TGF- β 1 is mainly localized to tubules that are positive for lotus lectin (proximal tubule marker)(arrows) and negative for peanut agglutinin (distal tubule marker) (asterisk). (Reprinted from⁸⁴, with permission).

Figure 5. Heme oxygenase-1 (HO-1) upregulation is associated with decreased fibronectin expression

Following TGF- β 1 treatment resulted in induction of HO-1 in several cells. Cells with HO-1 overexpression revealed decreased levels of fibronectin as compared to cells not expressing HO-1. This inverse correlation between HO-1 and fibronectin expressions suggests that HO-1 induction may regulate the expression of fibronectin and the development of fibrosis. (Reprinted from⁸⁸, with permission)