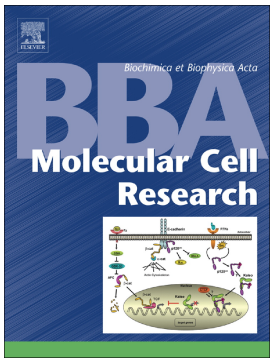

MDM2 controls NRF2 antioxidant activity in prevention of diabetic kidney disease

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MDM2 Controls NRF2 Antioxidant Activity in Prevention of Diabetic Kidney Disease

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Abstract

Oxidative stress and P53 contribute to the pathogenesis of diabetic kidney disease (DKD). Nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of cellular antioxidant defense system, is negatively regulated by P53 and prevents DKD. Recent findings revealed an important role of mouse double minute 2 (MDM2) in protection against DKD. However, the mechanism remained unclear. We hypothesized that MDM2 enhances NRF2 antioxidant signaling in DKD given that MDM2 is a key negative regulator of P53. The MDM2 inhibitor nutlin3a elevated renal P53, inhibited NRF2 signaling and induced oxidative stress, inflammation, fibrosis, DKD-like renal pathology and albuminuria in the wild-type (WT) non-diabetic mice. These effects exhibited more prominently in nutlin3a-treated WT diabetic mice. Interestingly, nutlin3a failed to induce greater renal injuries in the *Nrf2* knockout (KO) mice under both the diabetic and non-diabetic conditions, indicating that NRF2 predominantly mediates MDM2's action. On the contrary, P53 inhibition by pifithrin-α activated renal NRF2 signaling and the expression of *Mdm2*, and attenuated DKD in the WT diabetic mice, but not in the *Nrf2* KO diabetic mice. In high glucose-treated mouse mesangial cells, *P53* gene silencing completely abolished nutlin3a's inhibitory effect on NRF2 signaling. The present study demonstrates for the first time that MDM2 controls renal NRF2 antioxidant activity in DKD via inhibition of P53, providing MDM2 activation and P53 inhibition as novel strategies in the management of DKD.

Keywords: diabetes; diabetic nephropathy; Nrf2; oxidative stress; P53

1. Introduction

As a long-term complication of diabetes mellitus (DM), diabetic kidney disease (DKD) is the leading cause of end stage renal disease and results in high morbidity and mortality [1]. It is therefore essential to identify novel targets and develop more effective medicines to prevent the progression of DKD.

Oxidative stress is a major mechanism for the pathogenesis of DKD [2, 3]. Nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of cellular antioxidant activity [4, 5] and activates the transcription of antioxidant genes, such as heme oxygenase-1 (*Ho1*) and NAD(P)H dehydrogenase quinone 1 (*Nqo1*) [6]. The elevated antioxidants scavenge upon the excessive DM-induced free radicals. NRF2 plays a key role in protection against DKD, since *Nrf2* knockout (KO) diabetic mice exhibit more severe renal injury as compared with wild-type (WT) diabetic mice [7-10], and NRF2 activation improves DKD in mice [5, 8, 11, 12]. Hence, NRF2 is a viable target in the management of DKD.

Multiple functions of P53 to date have been identified [13], in addition to its critical role in coordinating cellular responses to genotoxic stress [14, 15]. Accumulating evidence has demonstrated that P53 contributes to the pathogenesis of several kidney diseases, and inhibition of P53 produces protective effects [16-20]. Notably, increased P53 was found in renal biopsy tissue from patients with DKD [21] and in renal cortex of diabetic rats [22] and mice [23], and accounted for the DM-induced glomerular hypertrophy and accumulation of fibrosis [23]. Importantly, P53 inhibits the expression [24] and function [25, 26] of *Nrf2*, the effect of which may result in enhanced renal oxidative stress, leading to the pathogenesis of DKD.

By using gas chromatography-mass spectrometry, 13 urine metabolites were identified to be robustly altered in the patients with DKD, providing these metabolites as reliable sources for

biomarkers of DKD [27]. More recently, the metabolomic data of human protein-protein interaction has uncovered 5 key bridge proteins that connect these pivotal metabolites, with mouse double minute 2 (MDM2) having the highest number of the connections [21]. Notably, *Mdm2* gene expression was significantly reduced in the glomerular and tubulointerstitial compartments of renal biopsy tissue harvested from patients with DKD [21]. Inhibition of MDM2 by nutlin3a impaired renal function in both the non-diabetic and diabetic mice [21]. Not surprisingly, *Mdm2* KO mice exhibited severe glomerular and tubular dysfunction [21]. Therefore, MDM2 shows a great potential for the treatment of DKD. However, the mechanism by which MDM2 improves DKD remained unclear. It is noted that MDM2 negatively regulates P53 [28] by sequestering P53 from exerting biological functions [13] and facilitating proteasomal degradation of P53 [29]. Hence, we hypothesized that an MDM2/P53 axis may control NRF2 antioxidant signaling in prevention of DKD.

In summary, the present study aimed to explore: (1) whether MDM2 controls NRF2 antioxidant activity in DKD; (2) whether or to what extent NRF2 is required for MDM2's action; (3) whether the effect of MDM2 is mediated by P53; (4) whether inhibition of P53 produces protection against DKD and (5) whether this action is mediated by NRF2. To these ends, C57BL/6 WT and *Nrf2* KO mice were induced to DM by streptozotocin (STZ), in the presence or absence of the MDM2 inhibitor nutlin3a or the P53 inhibitor pifithrin-α (PFT-α) [30]. The effect of MDM2 on P53 and NRF2 was further tested in high glucose (HG)-treated mouse mesangial cells (MMCs), by using nutlin3a or *Mdm2* siRNA. The role of P53 in mediating MDM2's action was further evaluated in HG-treated MMCs by using *P53* siRNA (si*P53*).

2. Methods

2.1 Acquisition and housing of the animals

C57BL/6 WT (*Nrf2* +/+) and *Nrf2* KO (*Nrf2* −/−) mice were obtained through breeding of heterozygotes (*Nrf2* +/−) [5, 11, 12]. All mice were housed in the Animal Center of Jilin University at 22°C, on a 12:12-h light-dark cycle with free access to rodent feed and tap water. All the experimental procedures were approved by the Institutional Animal Care and Use Committee at Jilin University and were in line with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2 DM induction and animal treatment

Eight-week-old male mice received intraperitoneal injections of either STZ (50 mg/kg daily, dissolved in 0.1 M sodium citrate, pH 4.5; Sigma-Aldrich, Shanghai, China) or sodium citrate for 5 consecutive days [5, 9, 12, 31]. Fasting glucose levels (4-hour fast) were determined one week after the last injection. DM was confirmed if a fasting glucose level was above 13.89 mM.

To test the role of MDM2/P53 in DKD and whether MDM2's action is mediated by NRF2, nutlin3a (10 mg/kg; MedChem Express, Shanghai, China) or vehicle (50% DMSO; Sigma-Aldrich, Shanghai, China) were intraperitoneally delivered to the C57BL/6 WT and *Nrf2* KO diabetic mice and the respective non-diabetic control mice every other day, for a period of 4 weeks, initiating from the 141st day post DM onset [21, 32]. The dose of nutlin3a at 10 mg/kg was a slight modification of the previously studied dose [21, 32], with the aim of avoiding the severe glomerular sclerosis and high mortality [21]. In order to determine the effect of P53 inhibition on DKD and further confirm whether or not NRF2 mediates P53's action, PFT-α (1.1 mg/kg, 3 times weekly [33]; MedChem Express) was intraperitoneally administered to the WT and *Nrf2* KO diabetic and non-diabetic mice right after DM was confirmed, for a period of 24 weeks.

Blood glucose, urinary albumin and creatinine were recorded on days 0, 140, 147, 154, 161 and 168, post DM onset. At the end of the procedures, all the mice were euthanized and their kidneys harvested for analysis.

2.3 Analysis of albuminuria

Urinary albumin and creatinine levels were determined on spot urine samples, using a mouse albumin kit (Bethyl Laboratories, Montgomery, TX) and a QuantiChrom™ Creatinine Assay Kit (BioAssay Systems, Hayward, CA), respectively. Urinary albumin to creatinine ratio (UACR) was then calculated to reflect kidney dysfunction.

2.4 Morphological analysis

The freshly harvested kidney tissues were fixed immediately into 10% buffered formalin solution and were embedded in paraffin, followed by sectioning into 5-μm-thick sections onto glass slides. Periodic acid-Schiff (PAS) staining and Masson's trichrome staining were performed to evaluate the renal pathological features, which were quantified by using Image-Pro Plus 6.0 software (Media Cybernetics Inc, Bethesda, MD). Selection of areas to photograph and scoring was done by people blind to the identity of the samples.

2.5 Mesangial cell culture and experiments

Primary MMCs were isolated from the kidneys of 8-week-old WT male mice, and were cultured under normal glucose (5.5 mM) condition, as previously described [12]. Passages 5-7 were used for experiments [12, 31]. To test the effect of MDM2 inhibition and P53 inhibition under HG (25 mM) condition, MMCs were treated with HG, in combination with nutlin3a (1 μM

[32]), *Mdm2* siRNA (25 nM [34], GenePharma, Suzhou, Jiangsu, China) or PFT-α (20 μM [35], MedChem Express), for 48 h. In order to test whether MDM2 activates *Nrf2* through P53 under HG condition, HG-challenged MMCs were treated with nutlin3a or si*P53* (20nM [36], GenePharma) or their combination, for 48 hours. The transfection reagent RFect^{PM} was provided by Changzhou Bio-generating Biotechnologies, Changzhou, China.

2.6 Real-time PCR

Kidney cortex or cell lysates of MMCs were used for quantitative real-time PCR, as previously described [37]. The primers for *Gapdh*, *Ho1*, *Mdm2*, *Nqo1*, *Nrf2* and *P53* were provided by Life technologies (Shanghai, China).

2.7 Western blot analysis

Western blot analysis was performed using kidney cortex or cell lysates of MMCs as described in our previous studies [37, 38]. The primary antibodies used were anti-connective tissue growth factor (CTGF, Abcam, Shanghai, China, 1:1000), anti-GAPDH (Santa Cruz Biotechnology, Dallas, TX, USA, 1:3000), anti-HO1 (Santa Cruz Biotechnology, 1:1000), anti-intercellular adhesion molecule-1 (ICAM-1, Santa Cruz Biotechnology, 1:500), anti-inducible nitric oxide synthase (iNOS, Cell signaling, Beverly, MA, USA, 1:1000), anti-MDM2 (Abcam, 1:1000), anti-NQO1 (Santa Cruz Biotechnology, 1:1000), anti-NRF2 (Santa Cruz Biotechnology, 1:1000), anti-TGF-β1, Cell signaling, 1:1000), anti-vascular cell adhesion molecule-1 (VCAM-1, Santa Cruz Biotechnology, 1:1000).

2.8 Quantitative analysis of reactive oxygen species (ROS) and lipid peroxides

ROS and malondialdehyde (MDA) levels were measured using kidney cortex or cell lysates, by a ROS assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) and a lipid peroxidation assay kit (Sigma-Aldrich) respectively, following the manufacturers' instructions.

2.9 Statistical analysis

Eight mice per group were studied. Cell experiments were performed in triplicate. The measurements for each group were summarized as means ± SD. Western blots were analyzed by Image Quant 5.2 software (GE Healthcare Bio-Sciences, Pittsburgh, PA). Two-way ANOVA was performed for the comparisons among different groups, followed by post hoc pairwise comparisons using Tukey's test with Origin 8.6 data analysis and graphing software Lab (OriginLab, Northampton, MA). A test is significant if $p < 0.05$.

3. Results

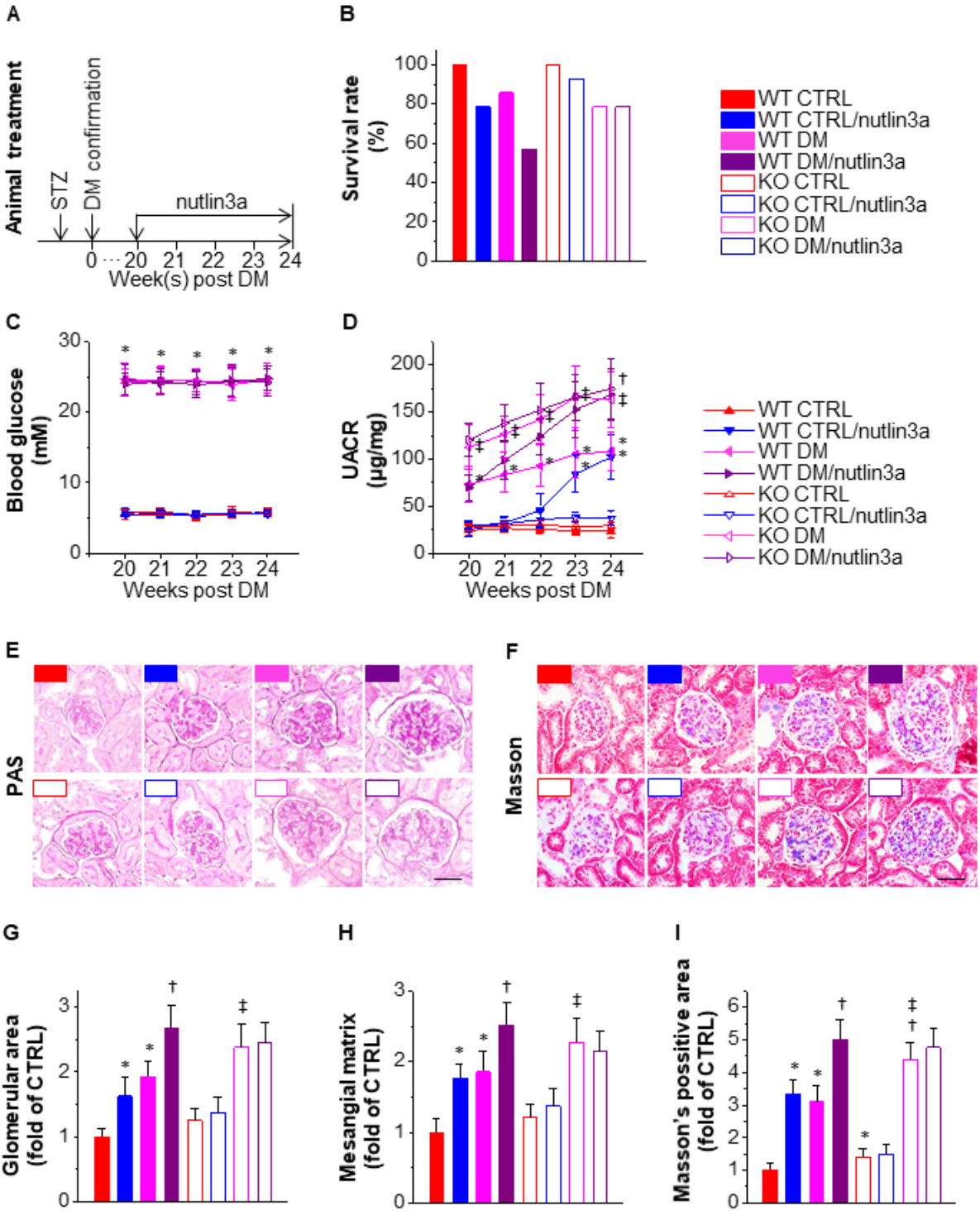
3.1 Inhibition of MDM2 by nutlin3a induced albuminuria and aggravated renal pathological features in the WT, but not the *Nrf2* KO, mice.

To test the role of MDM2/P53 in DKD and whether NRF2 mediates MDM2's effect on DKD, C57BL/6 WT and *Nrf2* KO diabetic mice and the respective non-diabetic control mice were treated with nutlin3a or vehicle for a period of 4 weeks, initiating from the 21th week post DM onset (Fig. 1A). The survival rates of the WT mice were 100% (14 in 14, WT CTRL), 78.57% (11 in 14, WT CTRL/nutlin3a), 85.71% (12 in 14, WT DM) and 57.14% (8 in 14, WT DM/nutlin3a) (Fig. 1B, solid bars). The *Nrf2* KO mice had survival rates of 100% (14 in 14, KO CTRL), 92.86% (13 in 14, KO CTRL/nutlin3a), 78.57% (11 in 14, KO DM) and 78.57% (11 in 14, KO DM/nutlin3a)

(Fig. 1B, hollow bars). Eight mice were randomly chosen from the remaining mice in each group. Consequently, for the following studies, there were 8 mice in each group.

The WT and *Nrf2* KO diabetic mice developed significantly higher blood glucose levels compared to their respective controls (Fig. 1C). Nutlin3a did not alter blood glucose levels in either type of the mice, under either the diabetic or the non-diabetic conditions (Fig. 1C). UACR, which reflects renal dysfunction, exhibited more prominently in the *Nrf2* KO diabetic mice, as compared with the WT diabetic mice (Fig. 1D). Nutlin3a significantly increased UACR in the WT diabetic (24 weeks post DM onset) and non-diabetic mice (23 and 24 weeks post DM onset), but not in the *Nrf2* KO mice (Fig. 1D). PAS (Fig. 1E) and Masson's trichrome staining (Fig. 1F) revealed significant increase in glomerular area (Fig. 1G), mesangial matrix expansion (Fig. 1H) and Masson's positive area (Fig. 1I) in the WT and *Nrf2* KO diabetic mice. Nutlin3a generated these DKD-like pathological features in the WT non-diabetic mice and exacerbated these features in the WT diabetic mice (Fig. 1G-I, solid bars). However, these actions of nutlin3a were completely lost in the absence of NRF2 (Fig. 1E-I, hollow bars). These results suggest that NRF2 predominantly mediates nutlin3a's effect on albuminuria and renal pathological change.

Fig. 1.

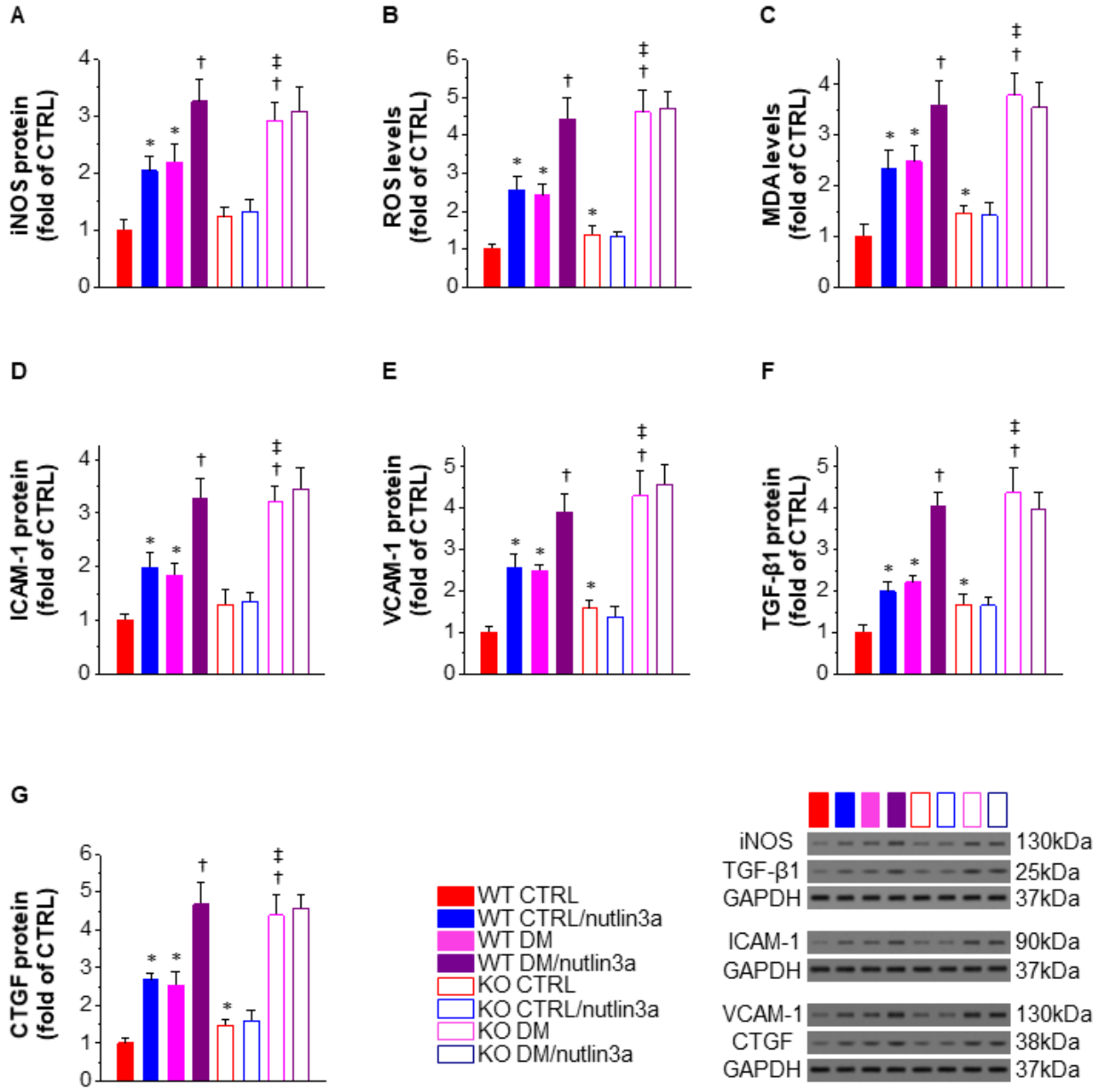


Inhibition of MDM2 by nutlin3a induced albuminuria and aggravated renal pathological features in the WT, but not the *Nrf2* KO, mice.

3.2 NRF2 was required for nutlin3a-enhanced renal oxidative stress and the expression of inflammatory and fibrotic factors.

To further evaluate the role of NRF2 in mediating MDM2's effect on renal injury, we measured indicators of oxidative stress (iNOS, ROS and MDA, Fig. 2A-C), inflammation (ICAM-1 and VCAM-1, Fig. 2D, E) and fibrosis (TGF-β1 and CTGF, Fig. 2F, G), all of which were elevated in the WT diabetic mice, and were even higher in the *Nrf2* KO diabetic mice (Fig. 2A-G). Nutlin3a increased these indices under both the non-diabetic and diabetic conditions in the WT, but not the *Nrf2* KO, mice (Fig. 2A-G).

Fig. 2.



NRF2 was required for nutlin3a-enhanced renal oxidative stress and the expression of inflammatory and fibrotic factors.

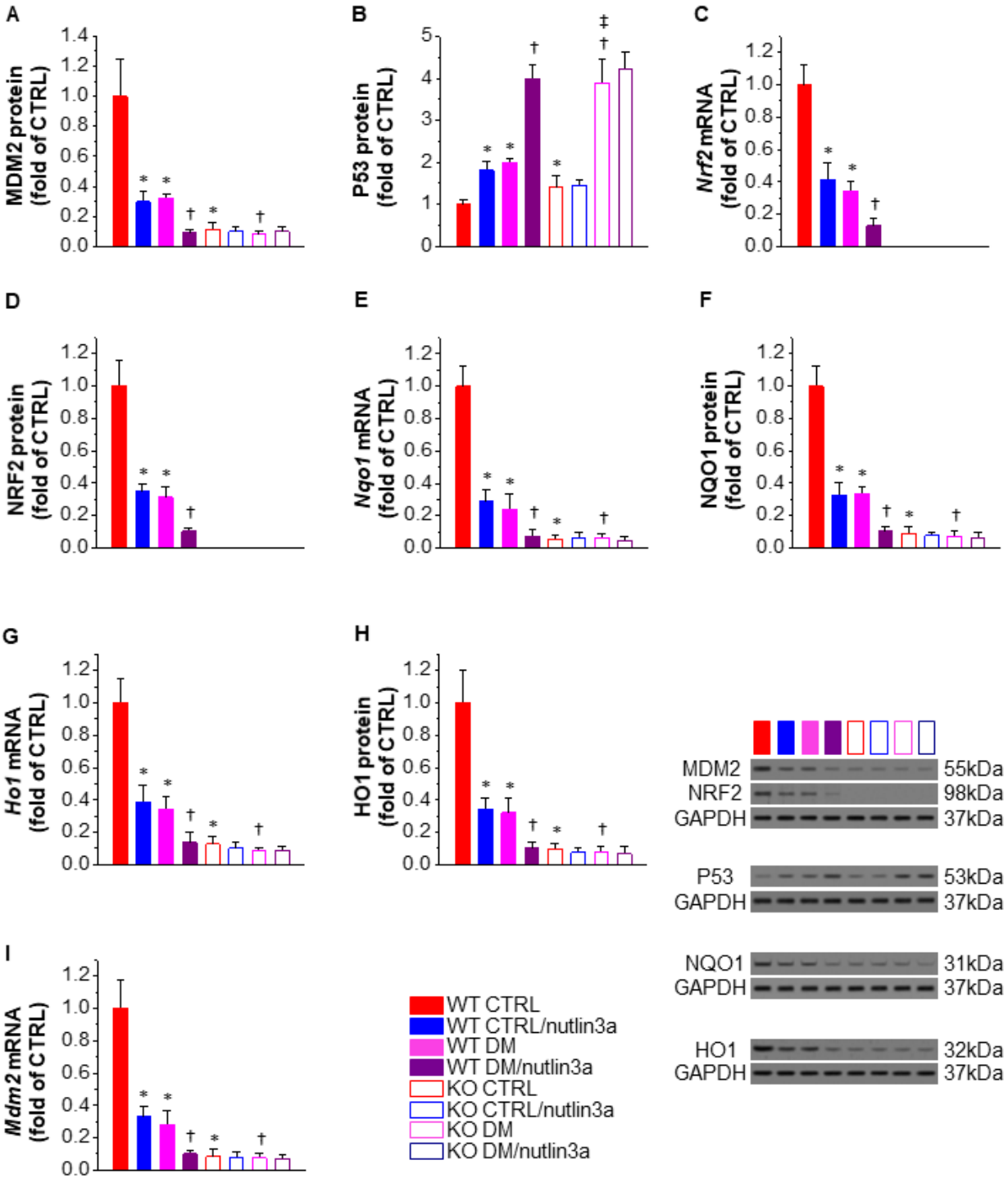
3.3 Nutlin3a decreased renal antioxidant activity via inhibition of *Nrf2* expression.

The following study aimed to test the effect of MDM2/P53 on *Nrf2* expression and function. Additionally, the role of NRF2 in mediating MDM2/P53's action was determined by using *Nrf2* KO mice. Decreased renal MDM2 protein level was detected in the WT diabetic compared to the WT non-diabetic CTRL (Fig. 3A, solid bars). Under both the diabetic and the non-diabetic conditions, nutlin3a decreased renal MDM2 protein in the WT mice (Fig. 3A, solid bars), but not in the *Nrf2* KO mice (Fig. 3A, hollow bars). These effects of nutlin3a on MDM2 led to increased P53 protein in the WT diabetic and non-diabetic mice (Fig. 3B, solid bars), and unaltered P53 levels in the *Nrf2* KO diabetic and non-diabetic mice (Fig. 3B, hollow bars).

Decreased renal *Nrf2* expression (Fig. 3C, D, solid bars) and function (Fig. 3E-H, solid bars) were found in the nutlin3a-treated WT diabetic and non-diabetic mice. In the KO mice, renal *Nrf2* expression was not detectable (Fig. 3C, D, hollow bars). This resulted in low expression of *Nqo1*, *Ho1* (Fig. 3E-H, hollow bars) and *Mdm2* mRNA (Fig. 3I, hollow bars), the levels of which were not altered by nutlin3a.

In summary, these results demonstrate that nutlin3a decreases renal antioxidant activity through inhibition of *Nrf2* expression. Additionally, the findings that the *Nrf2* KO mice developed lower basal *Mdm2* expression (Fig. 3A, I) and higher P53 level (Fig. 3B) supports the previous establishment of *Mdm2* as a downstream gene of NRF2 [39-42].

Fig. 3.

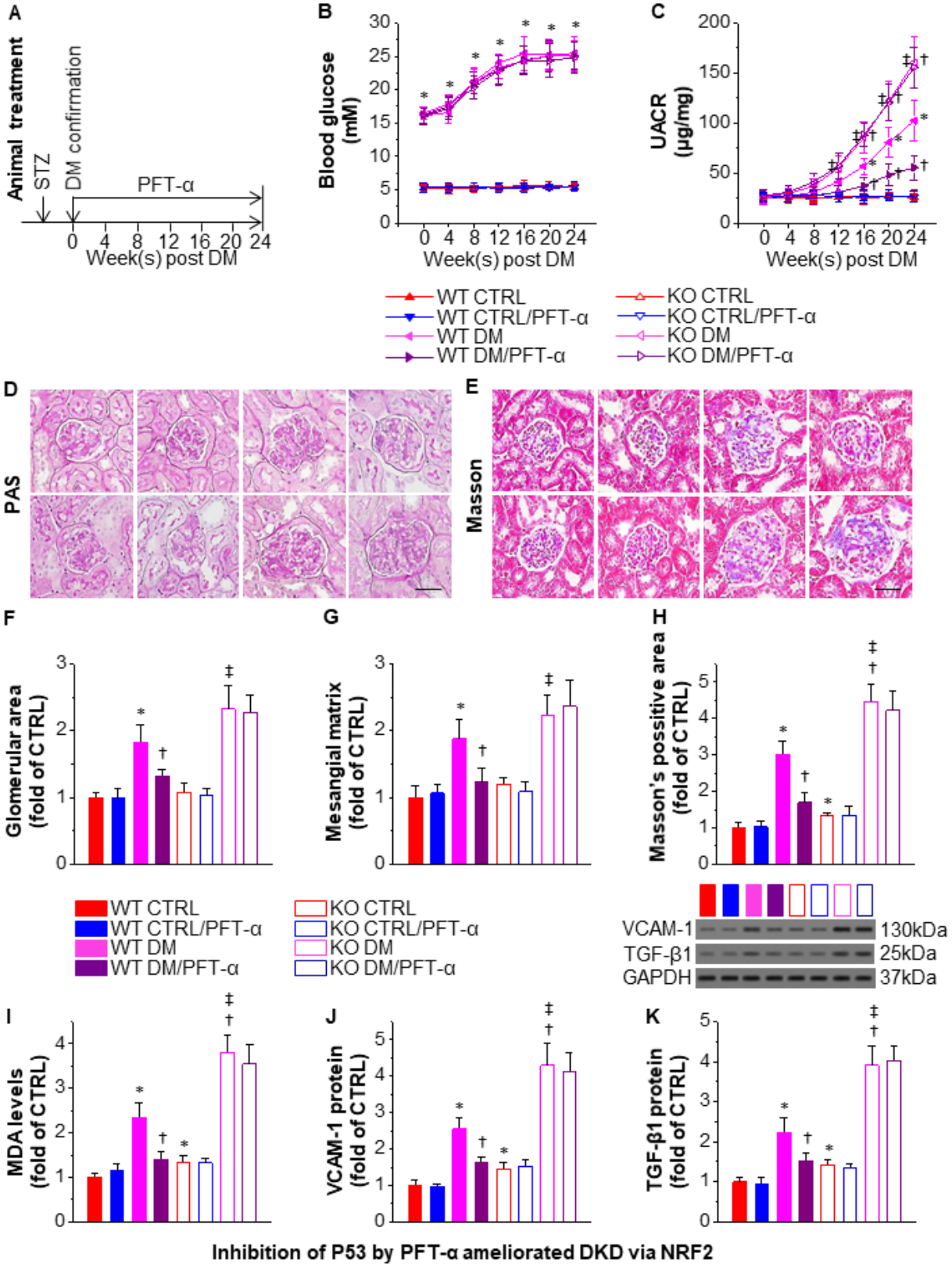


Nutlin3a decreased renal antioxidant activity via inhibition of *Nrf2* expression.

3.4 Inhibition of P53 by PFT-α ameliorated DKD via NRF2.

PFT-α was studied for the effect of P53 inhibition on DKD (Fig. 4A). PFT-α treatment did not change blood glucose levels (Fig. 4B). The DM-elevated UACR was significantly decreased by PFT-α in the WT, but not the *Nrf2* KO, mice (Fig. 4C). PAS (Fig. 4D) and Masson's trichrome (Fig. 4E) staining quantified remarkable amelioration of DM-induced renal pathological changes by PFT-α in the WT, rather than the *Nrf2* KO, mice (Fig. 4F-H). PFT-α decreased the levels of MDA, VCAM-1and TGF-β1 (Fig. 4I-K, solid bars) in the WT diabetic mice. However, PFT-α completely lost these efficacies in the absence of NRF2 (Fig. 4I-K, hollow bars). These results confirm the role of NRF2 in mediating the effects of P53, supporting the findings in nutlin3a-treated mice (Figs. 1-3).

Fig. 4.

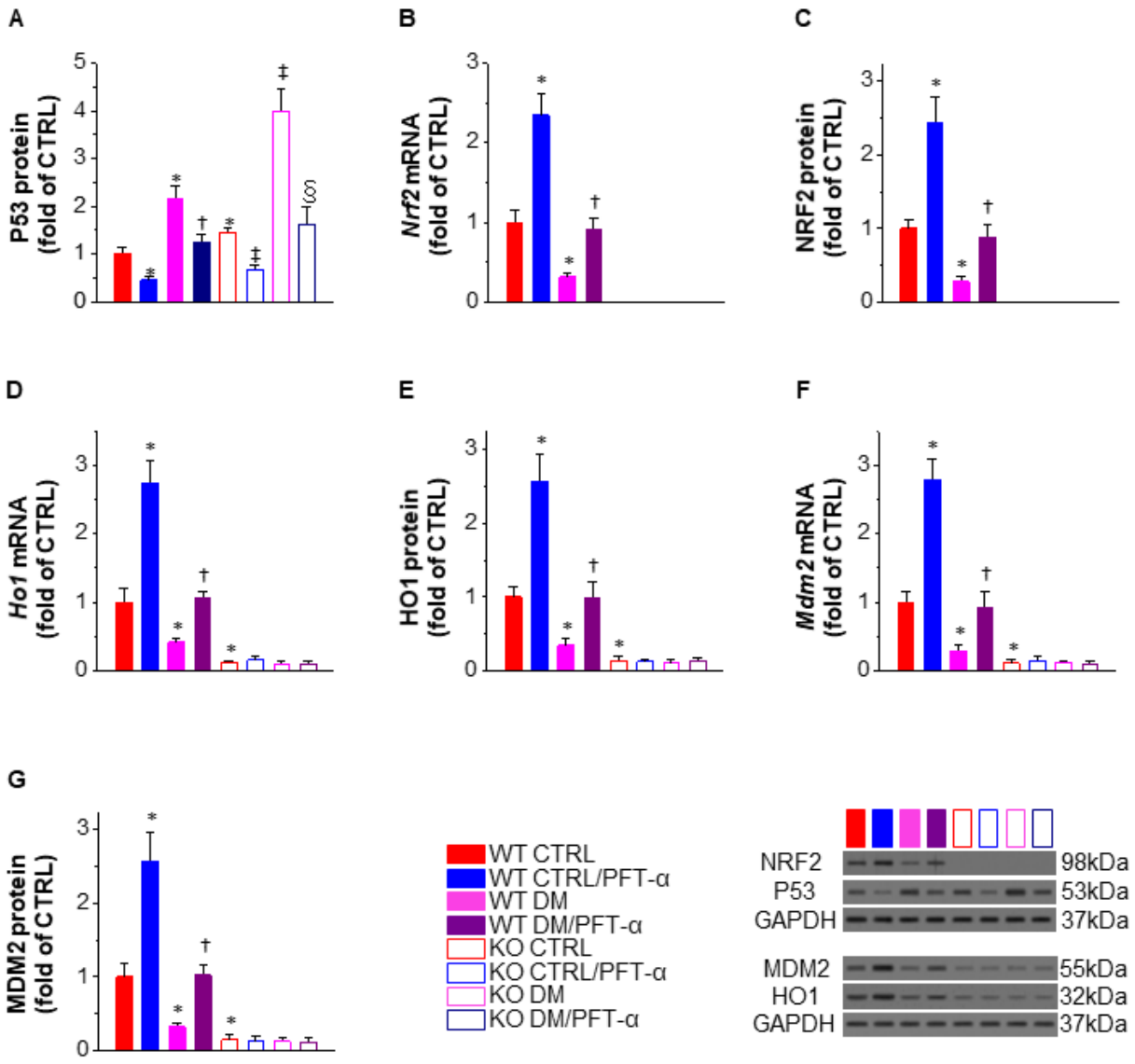


Inhibition of P53 by PFT-α ameliorated DKD via NRF2

3.5 NRF2 was indispensable for PFT-α-enhanced renal antioxidant activity.

We next determined the effect of P53 inhibition on renal antioxidant activity. PFT-α potently inhibited renal P53 protein levels in the WT and *Nrf2* KO mice, under both the diabetic and non-diabetic conditions (Fig. 5A). Confirming the inhibitory effect of P53 on NRF2 signaling (Fig. 3), PFT-α led to significantly enhanced *Nrf2* expression (Fig. 5B, C, solid bars) and function (Fig. 5D-G, solid bars) in the WT mice. These effects of PFT-α were completely lost in the *Nrf2* KO mice (Fig. 5B-G, hollow bars), indicating that NRF2 is required for PFT-α-enhanced renal antioxidant activity.

Fig. 5.

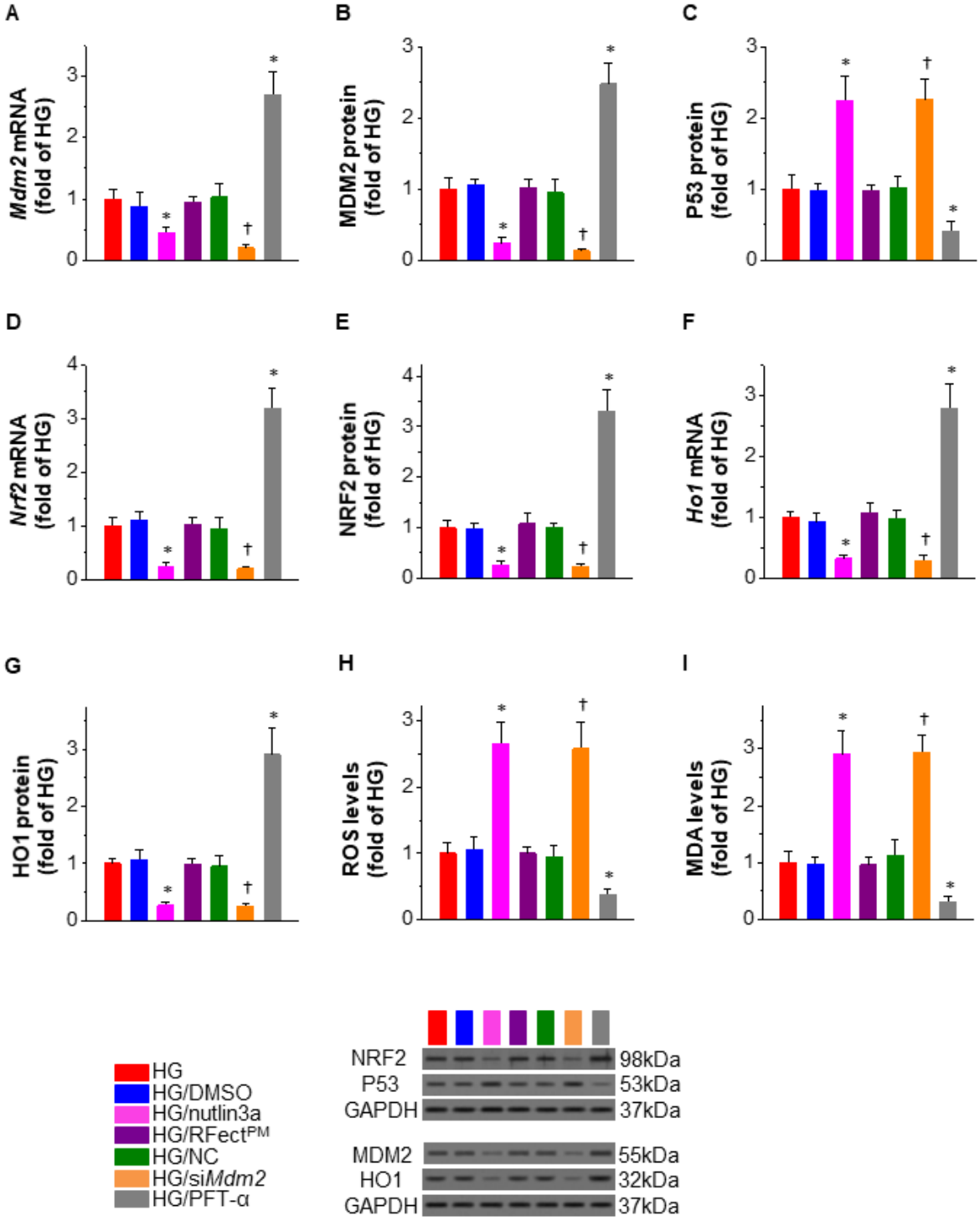


NRF2 was indispensable for PFT-α-enhanced renal antioxidant activity

3.6 NRF2 signaling was controlled by MDM2/P53 in HG-treated MMCs.

The following studies aimed to further confirm the MDM2/P53/NRF2 axis, by using gene silencing and small molecule-induced inhibition of *Mdm2* or *P53* in HG-treated MMCs (Figs. 6, 7). MMCs were chosen for the test because DM, nutlin3a and PFT-α induced significant effects on glomerular area, mesangial matrix and glomerular fibrosis as shown in Fig. 1E-I and Fig. 4D-H. Inhibition of MDM2 by either nutlin3a or *Mdm2* siRNA (Fig. 6A, B) led to elevation of P53 protein (Fig. 6C), inhibition of *Nrf2* (Fig. 6D, E) and *Ho1* (Fig. 6F, G) expression, and increased ROS (Fig. 6H) and MDA (Fig. 6I) levels. On the contrary, inhibition of P53 by PFT-α (Fig. 6C) resulted in enhanced expression of *Mdm2* (Fig. 6A, B), *Nrf2* (Fig. 6D, E) and *Ho1* (Fig. 6F, G), as well as decreased ROS (Fig. 6H) and MDA (Fig. 6I) levels. These results confirm that *Nrf2* expression and function are positively controlled by MDM2, but negatively regulated by P53.

Fig. 6.

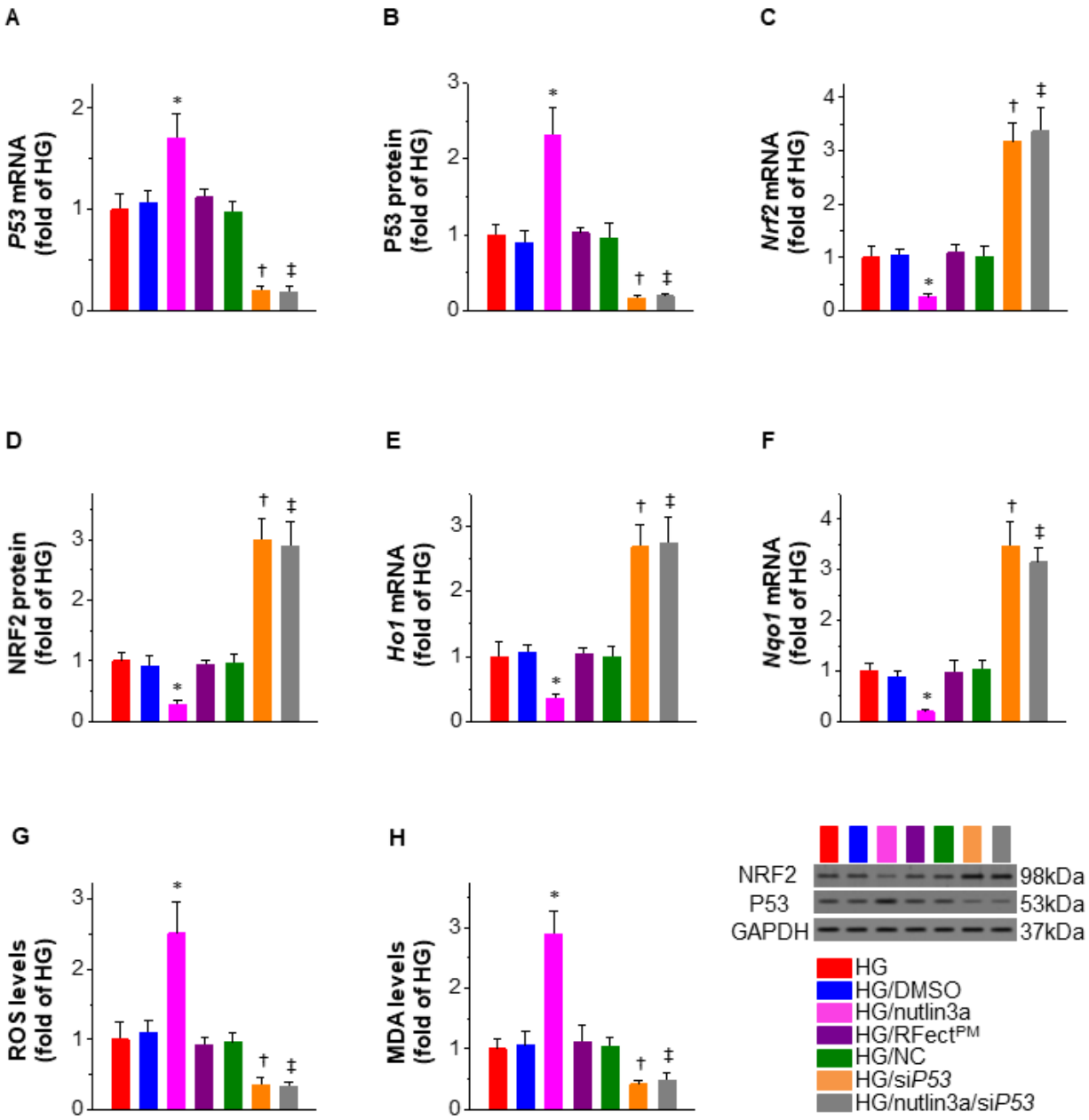


NRF2 signaling was controlled by MDM2/P53 in HG-treated MMCs

3.7 P53 mediated MDM2's effect on *Nrf2* expression and function in HG-treated MMCs.

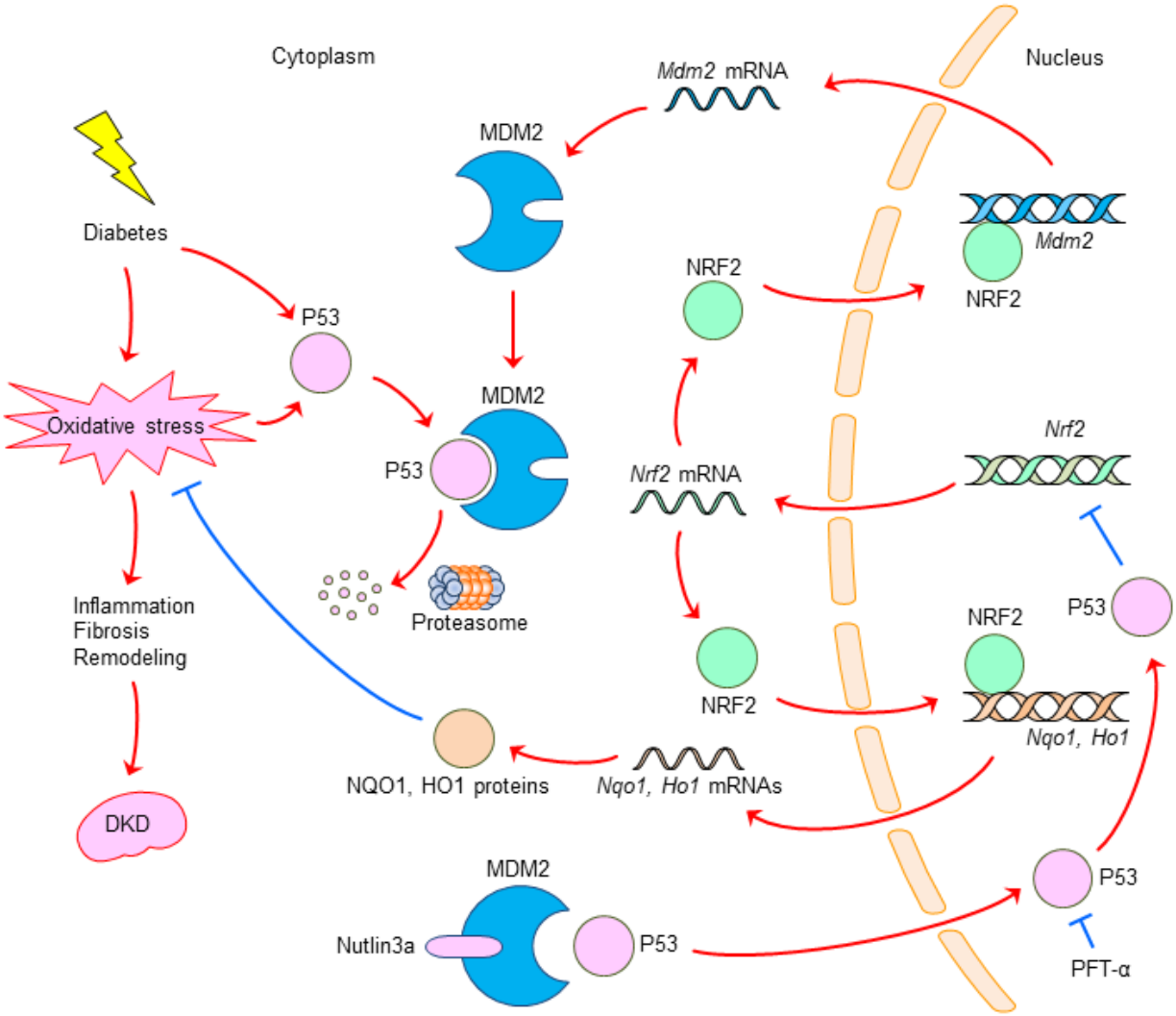
To define the role of P53 in mediating MDM2's effect on *Nrf2* expression and function, we tested the effect of si*P53* in HG-treated MMCs. *P53* gene silencing (Fig. 7A, B) led to a complete abolishment of nutlin3a's inhibitory effect on *Nrf2* expression (Fig. 7C, D) and function (Fig. 7E, F). In addition, Nutlin3a lost the ability to enhance oxidative stress in the presence of si*P53* (Fig. 7G, H). Hence, P53 mediated the control of *Nrf2* expression and function by MDM2 in HG-treated MMCs.

Fig. 7.



P53 mediated MDM2's effect on *Nrf2* expression and function in HG-treated MMCs.

Fig. 8.



Possible role of MDM2/P53/NRF2 network in control of antioxidant activity in DKD.

4. Discussion

The development of computational medicine and bioinformatics has brought to light discoveries of novel targets for therapeutic intervention [43], where MDM2 has recently gained attention in DKD. By using approaches including MDM2 inhibition/silencing, P53 activation/inhibition/silencing and *Nrf2* gene KO, the present study has discovered a MDM2/P53/NRF2 regulatory network which plays a critical role in control of renal antioxidant activity in DKD (Fig. 8). This conclusion is in line with the previous finding that MDM2 prevents P53-induced podocyte death [34, 44] which indicates MDM2's protective effect in DKD, given that podocyte loss is one of the hall marks of DKD.

It is noted that P53 not only induces podocyte loss, but also has a significant influence on mesangial cell injury [23, 45, 46] which contributes to the pathogenesis of DKD. In MMCs, P53 induces hypertrophy and fibrosis [23], both of which are key pathological features of DKD. In STZ-induced diabetic rats, P53 expression was more abundantly expressed in the more hypertrophic glomeruli, as compared with the less hypertrophic glomeruli [47]. In addition, higher expression of glomerular P53 and more severe glomerular hypertrophy were observed in the 6-month-period diabetic rats, as compared with the 4-month-period diabetic rats [48]. Supporting these reports, the effect of P53 on glomerular hypertrophy and fibrosis is confirmed by the present study (Fig. 1E-I).

P53 functions through miR-192 [23] that amplifies TGF-β1 [49] to induce DKD. However, in the present study, NRF2 was found to be the key factor that mediated P53's effect on DKD (Figs. 4C-G, 5B-G). Hence, a crosstalk may exist between miR-192/TGF-β1 and NRF2. Supporting this view, the *Nrf2* KO diabetic mice had higher renal TGF-β1 expression, as compared with the WT diabetic mice [7, 9], whereas activation of NRF2 by SFN [5, 8], epigallocatechin

gallate [50] or sodium butyrate [11] significantly reduced renal TGF-β1 in the WT diabetic mice. Moreover, the finding that NRF2 repressed *Tgf-β1* gene transcription via interacting with c-Jun and SP1 [51] provides a more direct evidence for the inhibitory effect of NRF2 on TGF-β1.

Although nutlin3a was reported to attenuate features of DM [52] and myocardial infarction [53], the present study has proven the pathogenic role of nutlin3a in renal injury under both non-diabetic and diabetic conditions, supporting the findings in the previous report by Saito et al. [21]. Moreover, nutlin3a was found to promote aortic inflammation and oxidative stress in healthy mice via activation of P53 [38]. The discrepancies between these studies may be caused by the differences between tissues, models of diseases and stages of DM. Under diabetic condition, the expression and function of numerous factors are altered in the kidney. MDM2, P53 and NRF2 are major factors in DKD, given that fact, in the present and previous studies [7, 12, 21, 23], alteration of these factors has significant impact on DKD. The important roles and close correlations of these major factors, as well as the findings of overexpressed P53 and the impaired MDM2/NRF2 expression in human biopsies, have granted P53 inhibition or MDM2/NRF2 activation a unique advantage in the future management of DKD. However, to date, few P53 inhibitors and no MDM2 activators have been developed. The pathogenic role of P53 in diabetic complications, such as nephropathy [21, 23], cardiomyopathy [54] and aortic endothelial dysfunction [38], should urge the development of more specific P53 inhibitors and MDM2 activators.

Despite the termination of bardoxolone methyl in the treatment of patients with DKD [55], NRF2 remains a viable drug target in disease, as evidenced by the approval of dimethyl fumarate for use in the treatment of multiple sclerosis [56]. Application of bardoxolone methyl at the end stage of DKD and the lack in specificity led to the failure of the trial [57, 58]. NRF2 activation at a much earlier stage would have increased the chance of therapeutic efficacy [50, 58]. The

preventive effect of NRF2 on experimental DKD has proven successful [5, 8, 11, 50, 51, 59-62] and may shed more light upon the clinical management of DKD.

One of the most innovative findings of the present study is that MDM2 controls NRF2 antioxidant activity in DKD. This positive regulatory effect of MDM2 on *Nrf2* expression and function (Figs. 3C-H; 6D-G) has suggested MDM2 to be a master regulator of the master of the cellular antioxidant system. Although MDM2 is not an antioxidant that directly scavenges upon excessive DM-induced free radicals, it makes NRF2 produce the antioxidants (Figs. 3E-H; 6F, G). To date, this has been the first report of MDM2's antioxidant-inducing activity. The finding that NRF2 predominantly mediated MDM2's action (Figs. 1D-I; 2A-G) suggests an unhindered effect passing from MDM2 to NRF2, with P53 as the key mediator (Fig. 7).

One concern for manipulation of MDM2/P53 would be the possibility of cancer generation, given the essential protective role of P53 in carcinogenesis. Therefore, a basal P53 level should be retained during MDM2 activation or P53 inhibition, allowing sufficient P53 functioning as a guardian for carcinogenesis. In the present study, PFT-α, which decreased renal P53 level by 54.31% in the healthy mice and maintained P53 level in the diabetic mice at a similar level compared to the healthy mice, did not induce cancer, providing information for cancer-related safety of this P53-inhibiting approach. Furthermore, activation of NRF2 has proven non-cancer-inducing in animal models [5, 8, 11, 50, 51, 59-62], clinical trials [55, 63] and clinical treatment of multiple sclerosis [56], suggesting the safety of manipulation of the MDM2/P53/NRF2 network. However, special attention should be paid to basal P53 level in future animal studies and clinical trials.

In summary, the present study established a MDM2/P53/NRF2 regulatory network in the control of renal antioxidant activity in DKD. This work may bring MDM2 activation and P53 inhibition as strategies to the management of DKD.

5. Declaration of Interest

The authors declare that there is no conflict of interest in this work.

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7. Author contribution

Hao Wu conceived the idea. Hao Wu, Weixia Sun and Weiying Guo designed the research. Weiying Guo, Dan Tian, Ye Jia, Wenlin Huang, Mengnan Jiang, Junnan Wang, Weixia Sun and Hao Wu researched and interpreted the data. Weiying Guo, Dan Tian, Ye Jia, Wenlin Huang, Mengnan Jiang, Junnan Wang, Weixia Sun and Hao Wu contributed to discussion. Hao Wu, Weiying Guo and Weixia Sun wrote the manuscript. Hao Wu, Weixia Sun, Weiying Guo, Dan Tian, Ye Jia, Wenlin Huang, Mengnan Jiang and Junnan Wang reviewed and revised the manuscript. Hao Wu, Weixia Sun, Junnan Wang and Ye Jia provided funding. All the authors approve the version to be published.

References

1. Dronavalli, S., I. Duka, and G.L. Bakris, *The pathogenesis of diabetic nephropathy.* Nat Clin Pract Endocrinol Metab, 2008. **4**(8): p. 444-52.
2. Zhang, M.Z., et al., *Intrarenal dopamine inhibits progression of diabetic nephropathy.* Diabetes, 2012. **61**(10): p. 2575-84.
3. Keshari, K.R., et al., *Non-invasive in vivo imaging of diabetes-induced renal oxidative stress and response to therapy using hyperpolarized 13C dehydroascorbate magnetic resonance.* Diabetes, 2014.
4. Kaspar, J.W., S.K. Niture, and A.K. Jaiswal, *Nrf2:INrf2 (Keap1) signaling in oxidative stress.* Free Radic Biol Med, 2009. **47**(9): p. 1304-9.
5. Zheng, H., et al., *Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy.* Diabetes, 2011. **60**(11): p. 3055-66.
6. Ruiz, S., et al., *Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease.* Kidney Int, 2013. **83**(6): p. 1029-41.
7. Jiang, T., et al., *The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy.* Diabetes, 2010. **59**(4): p. 850-60.
8. Wu, H., et al., *Metallothionein plays a prominent role in the prevention of diabetic nephropathy by sulforaphane via up-regulation of Nrf2.* Free Radic Biol Med, 2015. **89**: p. 431-442.
9. Wu, H., et al., *C66 ameliorates diabetic nephropathy in mice by both upregulating NRF2 function via increase in miR-200a and inhibiting miR-21.* Diabetologia, 2016. **59**(7): p. 1558-68.

10. Zhang, H., et al., *SP600125 suppresses Keap1 expression and results in NRF2-mediated prevention of diabetic nephropathy.* J Mol Endocrinol, 2018. **60**(2): p. 145-157.

11. Dong, W., et al., *Sodium butyrate activates NRF2 to ameliorate diabetic nephropathy possibly via inhibition of HDAC.* J Endocrinol, 2017. **232**(1): p. 71-83.

12. Sun, W., et al., *Epigallocatechin Gallate Upregulates NRF2 to Prevent Diabetic Nephropathy via Disabling KEAP1*. Free Radic Biol Med, 2017.

13. Bargonetti, J. and J.J. Manfredi, *Multiple roles of the tumor suppressor p53.* Curr Opin Oncol, 2002. **14**(1): p. 86-91.

14. Lane, D.P., *Cancer. p53, guardian of the genome.* Nature, 1992. **358**(6381): p. 15-6.

15. Levine, A.J., *p53, the cellular gatekeeper for growth and division.* Cell, 1997. **88**(3): p. 323-31.

16. Bhatt, K., et al., *MicroRNA-34a is induced via p53 during cisplatin nephrotoxicity and contributes to cell survival.* Mol Med, 2010. **16**(9-10): p. 409-16.

17. Zhou, L., et al., *Activation of p53 promotes renal injury in acute aristolochic acid nephropathy.* J Am Soc Nephrol, 2010. **21**(1): p. 31-41.

18. Yang, L., et al., *Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury.* Nat Med, 2010. **16**(5): p. 535-43, 1p following 143.

19. Molitoris, B.A., et al., *siRNA targeted to p53 attenuates ischemic and cisplatin-induced acute kidney injury.* J Am Soc Nephrol, 2009. **20**(8): p. 1754-64.

20. Niranjan, T., et al., *The Notch pathway in podocytes plays a role in the development of glomerular disease.* Nat Med, 2008. **14**(3): p. 290-8.

21. Saito, R., et al., *Systems biology analysis reveals role of MDM2 in diabetic nephropathy.* JCI Insight, 2016. **1**(17): p. e87877.

22. Tikoo, K., et al., *Change in histone H3 phosphorylation, MAP kinase p38, SIR 2 and p53 expression by resveratrol in preventing streptozotocin induced type I diabetic nephropathy.* Free Radic Res, 2008. **42**(4): p. 397-404.

23. Deshpande, S.D., et al., *Transforming growth factor-beta-induced cross talk between p53 and a microRNA in the pathogenesis of diabetic nephropathy.* Diabetes, 2013. **62**(9): p. 3151-62.

24. Tung, M.C., et al., *Mutant p53 confers chemoresistance in non-small cell lung cancer by upregulating Nrf2.* Oncotarget, 2015. **6**(39): p. 41692-705.

25. Faraonio, R., et al., *p53 suppresses the Nrf2-dependent transcription of antioxidant response genes.* J Biol Chem, 2006. **281**(52): p. 39776-84.

26. Wang, H.J., et al., *Dual pathways of p53 mediated glucolipotoxicity-induced apoptosis of rat cardiomyoblast cell: activation of p53 proapoptosis and inhibition of Nrf2-NQO1 antiapoptosis.* Metabolism, 2012. **61**(4): p. 496-503.

27. Sharma, K., et al., *Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease.* J Am Soc Nephrol, 2013. **24**(11): p. 1901-12.

28. Oliner, J.D., et al., *Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53.* Nature, 1993. **362**(6423): p. 857-60.

29. Brooks, C.L. and W. Gu, *p53 ubiquitination: Mdm2 and beyond.* Mol Cell, 2006. **21**(3): p. 307-15.

30. Komarov, P.G., et al., *A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy.* Science, 1999. **285**(5434): p. 1733-7.

31. Kato, M., et al., *An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy.* Nat Commun, 2016. **7**: p. 12864.

32. Allam, R., et al., *Mdm2 promotes systemic lupus erythematosus and lupus nephritis.* J Am Soc Nephrol, 2011. **22**(11): p. 2016-27.

33. Hoshino, A., et al., *Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic beta-cell function in diabetes.* Proc Natl Acad Sci U S A, 2014. **111**(8): p. 3116-21.

34. Thomasova, D., et al., *Murine Double Minute-2 Prevents p53-Overactivation-Related Cell Death (Podoptosis) of Podocytes.* J Am Soc Nephrol, 2015. **26**(7): p. 1513-23.

35. Gao, F., et al., *Notch pathway is involved in high glucose-induced apoptosis in podocytes via Bcl-2 and p53 pathways.* J Cell Biochem, 2013. **114**(5): p. 1029-38.

36. Hirano, T., et al., *A Novel Interaction between FLICE-Associated Huge Protein (FLASH) and E2A Regulates Cell Proliferation and Cellular Senescence via Tumor Necrosis Factor (TNF)-Alpha-p21WAF1/CIP1 Axis.* PLoS One, 2015. **10**(7): p. e0133205.

37. Wu, H., et al., *Metallothionein deletion exacerbates intermittent hypoxia-induced renal injury in mice.* Toxicol Lett, 2014. **232**(2): p. 340-348.

38. Wu, H., et al., *SRT2104 attenuates diabetes-induced aortic endothelial dysfunction via inhibition of P53.* J Endocrinol, 2018. **237**(1): p. 1-14.

39. Okano, Y., et al., *SNP (-617C>A) in ARE-like loci of the NRF2 gene: a new biomarker for prognosis of lung adenocarcinoma in Japanese non-smoking women.* PLoS One, 2013. **8**(9): p. e73794.

40. Fledderus, J.O. and R. Goldschmeding, *Nrf2 implicated as a novel therapeutic target for renal regeneration after acute kidney injury.* Nephrol Dial Transplant, 2013. **28**(8): p. 1969-71.

41. You, A., et al., *Transcription factor Nrf2 maintains the basal expression of Mdm2: An implication of the regulation of p53 signaling by Nrf2.* Arch Biochem Biophys, 2011. **507**(2): p. 356-64.

42. Hagemann, J.H., et al., *Nrf2 signalling promotes ex vivo tubular epithelial cell survival and regeneration via murine double minute (MDM)-2.* Nephrol Dial Transplant, 2013. **28**(8): p. 2028-37.

43. Winslow, R.L., et al., *Computational medicine: translating models to clinical care.* Sci Transl Med, 2012. **4**(158): p. 158rv11.

44. Mulay, S.R., et al., *Podocyte loss involves MDM2-driven mitotic catastrophe.* J Pathol, 2013. **230**(3): p. 322-35.

45. Patel, P., et al., *Transforming growth factor beta induces mesangial cell apoptosis through NO- and p53-dependent and -independent pathways.* J Investig Med, 2000. **48**(6): p. 403-10.

46. Shi, H., et al., *Effects of p53 on aldosterone-induced mesangial cell apoptosis in vivo and in vitro.* Mol Med Rep, 2016. **13**(6): p. 5102-8.

47. Jung, D.S., et al., *Apoptosis occurs differentially according to glomerular size in diabetic kidney disease.* Nephrol Dial Transplant, 2012. **27**(1): p. 259-66.

48. Menini, S., et al., *Increased glomerular cell (podocyte) apoptosis in rats with streptozotocin-induced diabetes mellitus: role in the development of diabetic glomerular disease.* Diabetologia, 2007. **50**(12): p. 2591-9.

49. Kato, M., et al., *A microRNA circuit mediates transforming growth factor-beta1 autoregulation in renal glomerular mesangial cells.* Kidney Int, 2011. **80**(4): p. 358-68.

50. Sun, W., et al., *Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1.* Free Radic Biol Med, 2017. **108**: p. 840-857.

51. Gao, P., et al., *Nrf2 ameliorates diabetic nephropathy progression by transcriptional repression of TGFbeta1 through interactions with c-Jun and SP1.* Biochim Biophys Acta, 2014. **1839**(11): p. 1110-20.

52. Secchiero, P., et al., *The MDM2 inhibitor Nutlin-3 attenuates streptozotocin-induced diabetes mellitus and increases serum level of IL-12p40.* Acta Diabetol, 2013. **50**(6): p. 899-906.

53. Zhao, H., et al., *Murine Double Minute-2 Inhibition Attenuates Cardiac Dysfunction and Fibrosis by Modulating NF-kappaB Pathway After Experimental Myocardial Infarction.* Inflammation, 2017. **40**(1): p. 232-239.

54. Gu, J., et al., *Inhibition of p53 prevents diabetic cardiomyopathy by preventing early-stage apoptosis and cell senescence, reduced glycolysis, and impaired angiogenesis.* Cell Death Dis, 2018. **9**(2): p. 82.

55. de Zeeuw, D., et al., *Rationale and trial design of Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes: the Occurrence of Renal Events (BEACON).* Am J Nephrol, 2013. **37**(3): p. 212-22.

56. Gold, R., et al., *Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis.* N Engl J Med, 2012. **367**(12): p. 1098-107.

57. Ellison, D.H., *Bardoxolone methyl in type 2 diabetes and advanced chronic kidney disease.* N Engl J Med, 2014. **370**(18): p. 1768.

58. Zhang, D.D., *Bardoxolone brings Nrf2-based therapies to light.* Antioxid Redox Signal, 2013. **19**(5): p. 517-8.

59. Cheng, Y., et al., *Up-regulation of Nrf2 is involved in FGF21-mediated fenofibrate protection against type 1 diabetic nephropathy.* Free Radic Biol Med, 2016. **93**: p. 94-109.

60. Gong, W., et al., *Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating CKIP-1 to resist HG-induced up-regulation of FN and ICAM-1 in GMCs and diabetic mice kidneys.* Free Radic Biol Med, 2017. **106**: p. 393-405.

61. Wei, J., et al., *Aldose reductase regulates miR-200a-3p/141-3p to coordinate Keap1-Nrf2, Tgfbeta1/2, and Zeb1/2 signaling in renal mesangial cells and the renal cortex of diabetic mice.* Free Radic Biol Med, 2014. **67**: p. 91-102.

62. Yang, Y., et al., *Therapeutic potential of digitoflavone on diabetic nephropathy: nuclear factor erythroid 2-related factor 2-dependent anti-oxidant and anti-inflammatory effect.* Sci Rep, 2015. **5**: p. 12377.

63. Pergola, P.E., et al., *Effect of bardoxolone methyl on kidney function in patients with T2D and Stage 3b-4 CKD.* Am J Nephrol, 2011. **33**(5): p. 469-76.

Figure Legends

Fig. 1. Inhibition of MDM2 by nutlin3a induced albuminuria and aggravated renal pathological features in the WT, but not the *Nrf2* KO, mice. (**A**) Schematic for STZ-induced diabetic model of C57BL/6 WT and *Nrf2* KO mice, in the presence or absence of nutlin3a. (**B**) Survival rate of the WT and *Nrf2* KO mice. (**C**) Blood glucose at 20, 21, 22, 23 and 24 weeks post DM onset. (**D**) UACR calculated at 20, 21, 22, 23 and 24 weeks post DM onset. (**E**) PAS staining and (**F**) Masson's trichrome staining were performed to evaluate renal pathology (Bar = 50 μm). (**G**) Glomerular area and (**H**) mesangial matrix expansion quantified from PAS staining. (**I**) Masson's positive area. For (**G-I**), the data is normalized to WT CTRL. All the data is presented as means ± SD (n = 8). *, $p < 0.05$ vs WT CTRL; †, $p < 0.05$ vs WT DM; ‡, $p < 0.05$ vs KO CTRL. Bars: solid red, WT CTRL; solid blue, WT CTRL/nutlin3a; solid magenta, WT DM; solid navy, WT DM/nutlin3a; hollow red, KO CTRL; hollow blue, KO CTRL/nutlin3a; hollow magenta, KO DM; hollow navy, KO DM/nutlin3a. Lines: red with solid up triangle, WT CTRL; blue with solid down triangle, WT CTRL/nutlin3a; magenta with solid left triangle, WT DM; navy with solid right triangle, WT DM/nutlin3a; red with hollow up triangle, KO CTRL; blue with hollow down triangle, KO CTRL/nutlin3a; magenta with hollow left triangle, KO DM; navy with hollow right triangle, KO DM/nutlin3a. Abbreviations: CTRL, control; DM, diabetes mellitus; KO, knockout; MDM2, mouse double minute 2; *Nrf2*, nuclear factor erythroid 2-related factor 2; PAS, periodic acid-Schiff; STZ, streptozotocin; UACR, urinary albumin to creatinine ratio; WT, wild-type.

Fig. 2. NRF2 was required for nutlin3a-enhanced renal oxidative stress and the expression of inflammatory and fibrotic factors. To assess renal oxidative stress and the expression of pro-inflammatory and pro-fibrotic genes, (**A**) iNOS protein level, (**B**) ROS and (**C**) MDA levels, and

protein levels of (**D**) ICAM-1, (**E**) VCAM-1, (**F**) TGF-β1 and (**G**) CTGF were determined. The data is normalized to WT CTRL and presented as means ± SD (n = 8). *, $p < 0.05$ vs WT CTRL; †, $p < 0.05$ vs WT DM; ‡, $p < 0.05$ vs KO CTRL. Bars are the same as in Fig. 1. Abbreviations: CTGF, connective tissue growth factor; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; ROS, reactive oxygen species; TGF-β1, transforming growth factor beta 1; VCAM-1, vascular cell adhesion molecule-1. Other abbreviations are the same as in Fig. 1.

Fig. 3. Nutlin3a decreased renal antioxidant activity via inhibition of *Nrf2* expression. Nutlin3a was tested for its effect on the expression of (**A**) MDM2 protein, (**B**) P53 protein, (**C, D**) *Nrf2* mRNA and protein, (**E, F**) *Nqo1* mRNA and protein, (**G, H**) *Ho1* mRNA and protein, as well as (**I**) *Mdm2* mRNA. The data is normalized to WT CTRL and presented as means ± SD (n = 8). *, $p < 0.05$ vs WT CTRL; †, $p < 0.05$ vs WT DM. Bars are the same as in Fig. 1. Abbreviations: *Ho1*, heme oxygenase-1, *Nqo1*; NAD(P)H dehydrogenase quinone 1. Other abbreviations are the same as in Fig. 1.

Fig. 4. Inhibition of P53 by PFT-α ameliorated DKD via NRF2. (**A**) Schematic for STZ-induced diabetic model of C57BL/6 WT and *Nrf2* KO mice, in the presence or absence of PFT-α. (**B**) Blood glucose levels and (**C**) UACR at 0, 4, 8, 12, 16, 20 and 24 weeks post DM onset. (**D**) PAS and (**E**) Masson's trichrome staining with (**F**) glomerular area, (**G**) mesangial matrix expansion and (**H**) Masson's positive area quantified. Levels of renal (**I**) MDA, (**J**) VCAM-1 protein and (**K**) TGF-β1 protein were determined. For (**F-K**), the data is normalized to WT CTRL and presented as means ± SD (n = 8). *, $p < 0.05$ vs WT CTRL; †, $p < 0.05$ vs WT DM; ‡, $p < 0.05$ vs KO CTRL.

Lines: red with solid up triangle, WT CTRL; blue with solid down triangle, WT CTRL/PFT-α; magenta with solid left triangle, WT DM; navy with solid right triangle, WT DM/PFT-α; red with hollow up triangle, KO CTRL; blue with hollow down triangle, KO CTRL/PFT-α; magenta with hollow left triangle, KO DM; navy with hollow right triangle, KO DM/PFT-α. Bars: solid red, WT CTRL; solid blue, WT CTRL/PFT-α; solid magenta, WT DM; solid navy, WT DM/PFT-α; hollow red, KO CTRL; hollow blue, KO CTRL/PFT-α; hollow magenta, KO DM; hollow navy, KO DM/PFT-α. Abbreviations: PFT-α, Pifithrin-α. Other abbreviations are the same as in Figs. 1, 2.

Fig. 5. NRF2 was indispensable for PFT-α-enhanced renal antioxidant activity. (**A**) Protein levels of P53, and mRNA and protein levels of (**B, C**) *Nrf2*, (**D, E**) *Ho1* and (**F, G**) *Mdm2* in the kidneys of the WT and *Nrf2* KO non-diabetic and diabetic mice, in the presence or absence of PFT-α. The data is normalized to WT CTRL and presented as means ± SD (n = 8). *, $p < 0.05$ vs WT CTRL; †, $p < 0.05$ vs WT DM; ‡, $p < 0.05$ vs KO CTRL; §, $p < 0.05$ vs KO DM. Bars are the same as in Fig. 4. Abbreviations are the same as in Figs. 1, 3, 4.

Fig. 6. NRF2 signaling was controlled by MDM2/P53 in HG-treated MMCs. The expression of (**A, B**) *Mdm2*, (**C**) P53, (**D, E**) *Nrf2* and (**F, G**) *Ho1*, as well as the levels of (**H**) ROS and (**I**) MDA were determined in nutlin3a-, si*Mdm2*- or PFT-α-treated MMCs, in the presence of HG. The Data is normalized to HG and presented as means ± SD (n = 3). *, $p < 0.05$ vs HG; †, $p < 0.05$ vs HG/NC. Bars: red, HG; blue, HG/DMSO; magenta, HG/nutlin3a; navy, HG/RFect^{PM}; green, HG/RFect^{PM}/NC; orange, HG/RFect^{PM}/si*Mdm2*, grey, HG/PFT-α. Abbreviations: HG, high glucose, MMCs, mouse mesangial cells; NC, negative control siRNA; RFect^{PM}, the transfection reagent; si*Mdm2*, *Mdm2* siRNA. Other abbreviations are the same as in Figs. 1-4.

Fig. 7. P53 mediated MDM2's effect on *Nrf2* expression and function in HG-treated MMCs. The expression of (**A, B**) *P53*, (**C, D**) *Nrf2*, (**E**) *Ho1*, (**F**) *Nqo1*, and the levels of (**F**) ROS and (**G**) MDA were determined in nutlin3a-, si*P53*- or nutlin3a/si*P53*-treated MMCs, in the presence of HG. The Data is normalized to HG and presented as means ± SD (n = 3). *, $p < 0.05$ vs HG; †, $p < 0.05$ vs HG/NC; ‡, $p < 0.05$ vs HG/nutlin3a. Bars: red, HG; blue, HG/DMSO; magenta, HG/nutlin3a; navy, HG/RFect^{PM}; green, HG/NC; orange, HG/si*P53*; grey, HG/nutlin3a/si*P53*. Abbreviation: si*P53*, *P53* siRNA. Other abbreviations are the same as in Figs. 1-3, 6.

Fig. 8. Possible role of MDM2/P53/NRF2 network in control of antioxidant activity in DKD. Oxidative stress leads to DM-induced renal inflammation, fibrosis, and pathological remodeling, all of which contribute to the pathogenesis of DKD. P53 is overexpressed under diabetic condition. In the nucleus, P53 inhibits the transcription of *Nrf2*, resulting in decreased expression of the NRF2 downstream antioxidant genes, such as *Nqo1* and *Ho1*, establishing an uncontrolled status of oxidative stress. By sequestering P53 from nuclear translocation and facilitating proteasomal degradation of P53, MDM2 enhances NRF2 antioxidant signaling which prevents the development of DKD. NRF2, in turn, activates Mdm2 gene transcription, forming a positive MDM2/P53/NRF2 feedback loop that controls renal antioxidant activity in DKD. This network can be modulated by pharmaceutical small molecules, such as the MDM2 inhibitor nutlin3a and the P53 inhibitor PFT-α. Symbols: ↓, activation; ⊥, inhibition.

Highlights

1. MDM2 controls diabetic and non-diabetic renal antioxidant capacity via NRF2.

2. NRF2 predominantly mediates MDM2's action on diabetic kidney disease.

3. P53 is a key factor through which MDM2 activates NRF2 in diabetic kidney disease.

4. P53 inhibition attenuates diabetic kidney desease.