Introduction

Aldose reductase (specifically AKR1B1, abbreviated “AR”) is a member of the aldo-keto reductase (AKR) superfamily representing the first and rate-limiting step of the polyol pathway, an alternate pathway of glucose metabolism. Besides reducing glucose to sorbitol, a wide range of aldehydes and their conjugates are also substrates of AR. Several studies have implicated AR in the pathogenesis of secondary diabetic complications, such as cataract genesis, retinopathy, neuropathy, and nephropathy (Yabe-Nishimura, 1998; Srivastava et al., 2011). However, more recent studies indicate that AR plays a more important role in mediating oxidative stress and detoxification (Srivastava et al., 2011). Previously, the pathogenesis of diabetic complications were thought to be tied specifically to osmotic stress generated by polyol flux (Narayanan, 2006), but recent studies demonstrate that oxidative stress caused by altered NADPH/NADP ratio also contributes to diabetic complications (Srivastava et al., 2005). As the key enzyme of polyol pathway, activation of AR induces the deposition of sorbitol causing increased levels of oxidative stress (Srivastava et al., 2011). Considering AR’s role in mediating oxidative stress, targeting AR with inhibitors to decrease the activity of polyol pathway is proving a viable way to ameliorate complications of diabetes (Brownlee, 2001).

As a multifunctional growth factor, transforming growth factor-β1 (TGFβ1) plays a crucial role in glomerulosclerosis and renal fibrosis. TGFβ1 induces extracellular matrix (ECM) deposition, and
breaks the balance between the production and degradation of ECM by down-regulating matrix metalloproteinase levels (Yamamoto et al., 1996). Previously we reported that TGFβ1 plays a role in the regulation of AR's expression, and the deposition of TGFβ1-induced ECMs could be ameliorated by AR inhibitors (Jiang et al., 2006). Further study showed mitogen-activated protein kinase (MAPK) signal pathways and transcription factor AP-1 are involved in regulating AR's expression stimulated by TGFβ1 (Jiang et al., 2008). Currently, the role of AR in mediating oxidative stress has attracted increasing attention. We speculate that oxidative stress also takes part in facilitating TGFβ1-induced AR expression. In diabetic nephropathy, the elevated level of reactive oxygen species (ROS) generated by hyperglycemia can activate a series of inflammatory factors, such as TNFα and TGFβ1, which stimulates cells to release more ROS in a positive feedback loop (Srivastava et al., 2011; Jiang et al., 2010). Furthermore, several reports have demonstrated that TGFβ1 itself is an inducer of ROS in many different cell types (Binker et al., 2011; Barnes and Gorin, 2011; Michaeloudes et al., 2011). As a consequence of increasing ROS, the cellular redox homeostasis is altered, which may lead to an up-regulation in expression of AR. Supporting this hypothesis, hydrogen peroxide (H₂O₂) was proven to activate expression of AR in vascular smooth muscle cells (VSMCs) (Spycher et al., 1997). Moreover, several redox-regulated transcription factor-binding sites were also found within the AR gene promoter, such as AP-1, NF-κB, and Nrf2, implicating AR is regulated by oxidative stress and could function via an anti-oxidant mechanism.

The transcription factor, Nrf2, has emerged as one of the most important cellular defense mechanisms to cope with oxidative stress. It regulates intracellular antioxidants, phase II detoxifying enzymes and several proteins that detoxify xenobiotics and neutralize ROS to promote cell survival and maintain redox homeostasis (Zhang, 2010). As a transcription factor, the consensus-binding site of Nrf2 within target gene promoters is known as the anti-oxidant response element (ARE) and exists in several anti-oxidative genes, such as NQO1, GST, and HO-1 (Hayes and McMahon, 2001; Nishinaka and Yabe-Nishimura, 2005). The presence of two AREs within the promoter of AR likely means it too is a target gene of Nrf2. This hypothesis was supported both by discovery that curcumin, a known Nrf2 activator, was able to induce AR expression (Kang et al., 2008) and indirect AR promoter activity experiments in HepG2 cells (Nishinaka and Yabe-Nishimura, 2005). Nevertheless, whether the increased AR expression and activity induced by TGFβ1 is mediated through the Nrf2 pathway remains unclear. Given the crucial role of TGFβ1 in glomerulosclerosis and the feedback on ROS release, we present our hypothesis that TGFβ1 triggers the production of ROS in mesangial cells of the kidney, which in turn activates the Nrf2 pathway leading to AR transcription and expression.

Recombinant DNA molecules

Luciferase reporter constructs containing the enhancer and promoter regions of the AR gene were kindly presented by Dr. Toru Nishinaka from Ohtani University, Osaka, Japan. Briefly, the enhancer region (~1044 to ~967) containing AREs was cloned into pGL3-luc basic construct. Accordingly, a single nucleotide mutation was performed by site-directed mutagenesis based upon the pGL3-AREs-luc construct (Nishinaka and Yabe-Nishimura, 2005). The Nrf2 expression plasmid and the NQO1-ARE reporter construct were described previously (Sun et al., 2009).

Cell culture, siRNA, transfection, and reporter gene assay

Human renal mesangial cells (HRMCs) were purchased from ScienCell Research Laboratories (Carlsbad, CA) and maintained in RPMI-1640 supplemented with 2% fetal bovine serum (FBS) and mesangial cell growth supplement (ScienCell Research Laboratories). For all experiments, HRMCs were used at passages 8–12. 293T cells were purchased from ATCC and maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FBS. Mouse embryonic fibroblasts (MEFs) (Leung et al., 2003; Chen et al., 2009) isolated from Nrf2 wild-type or knockout mice were maintained in DMEM supplemented with 10% FBS (Chen et al., 2009). Nrf2 siRNA, scrambled siRNA negative control and HiPerfect transfection reagent were purchased from Qiagen (Valencia, CA, USA) and transfection of Nrf2 siRNA was performed according to the manufacturer’s instruction. Briefly, the final concentration of Nrf2 siRNA was 25 nM and cells were exposed to siRNA for 48–72 h prior to analysis. For HRMCs, transfections were performed with Lipofectamine Reagent (Invitrogen, CA, USA) and Plus Reagent (Invitrogen) according to the manufacturer’s instructions. Reporter assays were performed using the Promega Dual-luciferase reporter gene assay system according to the manufacturer’s instructions. Reporter assays were performed using the Promega Dual-luciferase reporter gene assay system according to the manufacturer’s instructions. Briefly, firefly luciferase was driven by different DNA constructs to test the response and requirements of Nrf2 activation and renilla luciferase was used as a normalization control for transfection efficiency. All experiments were performed in triplicate unless otherwise noted. The mean ± standard deviation was calculated from three independent experiments.

ROS detection assay

After growth-arrest in 0.5% FBS for 24 h, cells were treated with TGFβ1 at specified doses and incubation times. At the conclusion of TGFβ1 exposure, cells were harvested and subjected to ROS detection by ESR spin trapping method (Zhang et al., 2007). Briefly, approximately 1 × 10⁶ cells were suspended in 500 μL PBS buffer containing 100 mM PBN; then ROS concentration was measured by EMX-E-8/2.7 ESR spectrometer (Bruker, MA, USA). All experiments were performed in triplicate unless otherwise noted.

Quantitative real-time PCR (qRT-PCR), immunoblot assay, and AR activity assay

HRMCs or MEFs were harvested after TGFβ1 treatment. The total RNA from HRMCs was extracted using Trizol solution (Invitrogen, Carlsbad, CA). Equal amounts of RNA (2 μg) were reverse-transcribed using the Transcriptor First Strand cDNA synthesis Kit (Roche, IN, USA). The primers for AR and GAPDH are shown in Table 1. The qPCR conditions were as follows: one cycle of initial denaturation (95 °C for 30 s), 40 cycles of amplification (denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s), followed by a cooling period (50 °C for 5 s). Relative mRNA expression was determined using the Lightcycler 480 software (Roche), which employs a modification

Materials and methods

Reagents

Polyclonal antibodies, including anti-AKR1B1, anti-Nrf2 were purchased from Santa Cruz (Santa Cruz, CA, USA). Human TGFβ1 was purchased from R&D Systems (Minneapolis, MN, USA). SYBR Green polymerase chain reaction (PCR) Master Mix was purchased from TaKaRa (Dalian, China). NADPH, Dl-Glyceraldehyde, N-tetraetyl-α-phenylintrone (PBN) and N-acetylcysteine (NAC) were purchased from Sigma Co. (St. Louis, MO, USA). Dual-Luciferase Reporter Assay System was purchased from Promega (Madison, WI, USA). All reagents were analytical grade.
of the delta–delta Ct method that adjusts for amplification efficiency between target and housekeeping genes (Pfaffl, 2001). The PCR data are expressed as relative fold change of the target gene between treated and control groups. The cycle point (Cp) values were analyzed by Student’s t-test to determine a P value. After harvest, cells were homogenized in lysis buffer (0.1 M Tris buffer (pH 7.4), 0.1 mM EDTA) in the presence of 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Roche, IN, USA). Protein was quantified using the standard BCA method and 30 µg of protein was loaded in each well of a 7.5% gel and subjected to SDS-PAGE. Gels were transferred to nitrocellulose membrane, which were then subjected to immunoblot analysis. For AR activity assay, the cells were collected and resuspended in TE buffer (pH 7.0), after several freeze/thaw cycles, the samples were centrifuged at 12,000 rpm for 10 min. The pellet was discarded, and the supernatant was mixed with phosphate buffer (67 mM, pH 7.0), ammonium sulfate (100 mM), NADPH (0.15 mM), β-Glyceraldehyde (0.04 mM). The mixture was incubated at 37°C for 5 min, then 60 µL HCl (0.5 M) was added and incubated at 60°C for 15 min, followed by cooling on ice. The lysate was mixed with 2 mL NaOH (6 M) containing imidazole (10 mM); then incubated at 37°C for 10 min prior to being measured for AR activity using SpectraMax M5 recording spectrophotometer (Molecular Devices Corp. USA). The Excitation and Emission wavelength is 360 nm and 460 nm, respectively. All experiments mentioned above were performed in triplicate unless otherwise noted.

Chromatin immunoprecipitation (ChIP) and qPCR

ChIP analysis was conducted according to protocols provided by Upstate (A Part of Millipore, MA, USA). Briefly, HRMCs (approximately 1 × 10⁷) were cross-linked with formaldehyde, collected in PBS, re-suspended in SDS lysis buffer and sonicated on ice. The lysates were then diluted with ChIP dilution buffer, pre-cleared with protein A agarose, and then incubated with indicated antibodies (4 mg/sample) overnight. The immune complexes were collected with protein A agarose, washed and eluted. DNA–protein cross-links were reversed and DNA was recovered. Relative amounts of DNA in the complex were quantified by real-time PCR using LightCycler 480 DNA SYBR green 1 kit (Roche). Primers shown in Table 1 were designed according to ARE sequences within the promoter region of AR gene. The confirmed NQO1 gene ARE sequence was used as positive control for Nrf2 interaction (Sun et al., 2009). The ChIP assay was repeated 3 times.

### Results

**TGFβ1 up-regulates the expression and activity of AR**

The expression of AR was analyzed in response to treatment with TGFβ1 in HRMCs. TGFβ1 (4 ng/mL) induced mRNA levels of AR as early as 6 h after treatment and followed a time-dependent increase, peaking at 24 h (Fig. 1A). However, the mRNA level returned to pre-TGFβ1 treatment levels at 36 and 48 h. Next, AR enzyme activity and protein expression were measured. Treatment of HRMCs with TGFβ1 also resulted in a significant time-dependent increase in AR activity and an observable increase in the protein level of AR (Fig. 1B and C). Similarly, the induction of AR mRNA, protein, and activity were observed in a dose-dependent manner when HRMCs were exposed to various concentrations of TGFβ1 for the maximum time-dependent response (Fig. 1D (24 h), E and F (36 h)). Protein expression by Western blot was quantified and graphed in Fig. 1G and H. Furthermore, we tested the correlation coefficient between AR protein level and activity using SPSS 19 where they were set as two variants. The correlation coefficients for the relationship of AR protein level and activity were r = 0.967 and r = 0.931 for Fig. 1B, G and H, respectively. We next analyzed the effect of actinomycin D or cycloheximide on TGFβ1-induced AR expression. As the qPCR data in Fig. 1I showed, both actinomycin D and cycloheximide blocked the mRNA expression of AR induced by TGFβ1, which means the increased mRNA level of AR is due to de novo transcription. The influence of cycloheximide reducing mRNA expression is likely due to inhibition of Nrf2 translation, thereby keeping AR mRNA levels low. These data demonstrate that exposure of HRMCs to TGFβ1 results in the up-regulation of AR mRNA and protein expression, which culminates in increased enzymatic activity of AR in a dose- and time-dependent manner.

**TGFβ1 increases AR expression through ROS**

We next tested our hypothesis that ROS is the mechanism of TGFβ1-induced expression of AR in HRMCs. First, ROS production in HRMCs was measured after treatment with TGFβ1. Treatment with 4 ng/mL of TGFβ1 rapidly induced production of ROS after only 30 min, which then decreased gradually but remained significantly elevated above the no TGFβ1 treatment for 4 h. Six hours following the exposure of TGFβ1, ROS levels were restored to basal levels (Fig. 2A). Next, the production of ROS was measured at 30 min in response to increasing doses of TGFβ1 (Fig. 2B). When cells were pre-incubated with ROS scavengers, NAC or super oxide dismutase (SOD), for 24 h prior to TGFβ1 treatment, the induction of ROS was inhibited significantly (Fig. 2C). Next, the TGFβ1 induction of AR expression was measured with NAC pretreatment. As expected, scavenging ROS with NAC prevented the increase of AR mRNA, protein, and activity that was formerly induced by TGFβ1 alone (Fig. 2D–F). Importantly, treatment with NAC alone did not alter any parameters of AR expression. Collectively, these data indicate that the induction of AR expression and activity by TGFβ1 is mediated through TGFβ1 stimulation of ROS.

**TGFβ1 production of ROS leads to Nrf2 pathway activation, resulting in increased AR expression**

When the level of cellular ROS is increased and the intracellular redox balance is altered, the Nrf2 pathway is activated by cysteine modifications to Nrf2’s negative regulator, Keap1. Keap1 is an E3 ligase targeting Nrf2 for degradation keeping basal levels low. The activation of Nrf2 by TGFβ1-induced ROS in HRMCs was analyzed. Treatment of HRMCs with two different doses of TGFβ1 (4 ng/mL and 10 ng/mL) increased Nrf2 protein levels as
early as 6 h (Fig. 3A and B), which then decreased gradually over 48 h. Additionally, a dose-dependent increase in Nrf2 was observed with increasing amounts of TGFβ1 (Fig. 3C). The activation of the Nrf2 pathway by ROS has been well established to be mediated by cysteine modification of Keap1, therefore, we examined whether TGFβ1 treatment altered total Keap1 protein levels. As expected, Keap1 levels showed no change, suggesting that TGFβ1-induced ROS likely also activates Nrf2 via cysteine modification of Keap1 (Fig. 3A–C). To verify the induction of Nrf2 in response to TGFβ1 occurs in response to increasing ROS levels, ROS was decreased by the use of scavengers, NAC, and SOD. As a result of pretreatment with either NAC or SOD, TGFβ1-induction of Nrf2 was blocked (Fig. 3D). Next the relationship between Nrf2 and AR expression was interrogated through modulating Nrf2 levels by siRNA in the presence or absence of TGFβ1. Again, TGFβ1 up-regulated the expression of AR mRNA, protein, and activity of AR (Fig. 3E–G). However, when Nrf2 was knocked down by siRNA, the induction of AR expression by TGFβ1 was partially blocked (Fig. 3E–G). Taken together, these data clearly demonstrate that TGFβ1-induced Nrf2 is mediated through ROS and that Nrf2 serves, at least in part, as an intermediary between TGFβ1 and increasing AR expression.

Nrf2 regulates AR transcription activity

Our data indicate that Nrf2 plays a role in TGFβ1 induced AR expression. However, whether the regulation of AR by Nrf2 is direct or indirect remains unknown. Next, we analyzed the ability of Nrf2 to specifically regulate AR expression using dual luciferase assays. Several regions or mutations of the AR promoter were evaluated for the ability of Nrf2 to regulate firefly-luciferase activity (using the pGL3 basic vector as backbone). Transfection of HEK 293T with Nrf2 was able to activate both the full 1.06 kb fragment of the AR promoter, as well as a truncated fragment containing 2 AREs (putative Nrf2 binding sites) driving firefly-luciferase. However, when each ARE sequence was mutated (Nishinaka and Yabe-Nishimura, 2005), the transcriptional activity in the presence of Nrf2 significantly decreased (mGST-ARE served as a positive control; Fig. 4A, lower panel). Immunoblot data is provided to demonstrate the amount of transfected Nrf2 was similar in all luciferase construct conditions (Fig. 4A, upper panel). These experiments were repeated in HRMCs and yielded similar results to those in 293T (Fig. S1).

To confirm the direct binding of Nrf2 with the ARE fragment within the AR promoter, Chromatin immunoprecipitation (ChIP) was performed and results were analyzed by both endpoint and qPCR. As one of the Nrf2 downstream genes, NQO1’s promoter ARE sequence has been previously published, therefore the NQO1-ARE was analyzed as a positive control, and β-actin promoter and the AR-intron were utilized as negative controls in these experiments. To enhance Nrf2’s binding to target genes, cells were treated with tBHQ, a well-known and strong Nrf2 inducer. With the treatment of tBHQ, the binding of Nrf2 with NQO1-ARE increased dramatically (Fig. 4B). Interestingly, TGFβ1 also increased the binding of Nrf2 with NQO1-ARE (Fig. 4B), further supporting the activation of Nrf2 by TGFβ1 (Fig. 3A–C). The binding of Nrf2 to the AR-ARE was also increased when treated with either tBHQ or TGFβ1, which indicate
the TGFβ1 induced AR expression is likely through the binding of Nrf2 with the ARE sequence in the AR promoter (Fig. 4C). Importantly, neither tBHQ nor TGFβ1 was capable of facilitating Nrf2 binding to the negative control (β-actin) promoter (Fig. 4D) or AR intron region (Fig. 4E). In support of the results obtained by qPCR, isolated DNA from the ChIP experiments were also subjected to conventional PCR and visualized by ethidium bromide agarose gel (Fig. 4F). Given that our luciferase and ChIP data showed AR to be an authentic Nrf2 downstream gene, we next measured the expression of AR in Nrf2 deficient mouse embryonic fibroblast (MEF) cells. Notably, there was no significant difference in basal AR expression in Nrf2+/+ compared to Nrf2−/− MEF cells. However, treatment with TGFβ1 induced AR protein (Fig. 4G) only in Nrf2+/+, but not Nrf2−/− MEF cells. Taken together, these results show that Nrf2 mediates AR gene activity via two AREs in the promoter region and that Nrf2 is capable of binding the endogenous AR promoter directly. Additionally, our results demonstrate a requirement for Nrf2 in TGFβ1’s ability to modulate AR expression, likely due to the TGFβ1 induction of ROS (Figs. 2A and 3D), which also activates Nrf2.

Discussion

As the key enzyme of the polyol pathway, AR executes an important role in regulating glucose and aldehyde metabolism, making it intimately coupled with complications associated with diabetes mellitus. Though the pathological consequences of increased flux to the polyol pathway arise principally from high glucose levels, there are reports demonstrating that osmotic or oxidative stress may also play a role. Therefore, AR plays a role not only in diabetes mellitus, but also in diseases without hyperglycemia such as asthma, uveitis, and colon cancer; diseases
where inflammation plays a key part (Srivastava et al., 2011). We have reported that the expression of AR also correlates with the severity of non-diabetic nephritis; including foot process disease, lupus nephritis, focal and segmental glomerulosclerosis, and others (Jiang et al., 2004, 2006). Combined, the role of AR in mediating both hyperglycemic and normo-glycemic pathologies is well documented, making the identification of AR’s regulatory networks vitally important to understanding AR’s role in disease onset and progression. Previously, we reported that TGFβ1 is capable of inducing AR expression in mesangial cells, however, the detailed mechanism of TGFβ1’s regulation of AR has remained unknown.

In the present study, the regulatory mechanism of TGFβ1 on AR was dissected and clearly demonstrated using multiple approaches. First, we confirmed that TGFβ1 is able to induce AR mRNA, protein, and enzyme activity in both a dose- and time-dependent manner. Notably, previous studies by our group have not included assays of AR activity, which is important to ensure the increase in AR’s mRNA and protein levels is translating to increased AR function. Second, we demonstrated that the positive regulation of AR by TGFβ1 is due, at least in part, to TGFβ1’s ability to stimulate ROS. Third, we established Nr2f2 as the mediator between TGFβ1 induced ROS and subsequent increases in AR mRNA, protein, and enzymatic activity. Lastly, we provide the first evidence that Nr2f2 binds the promoter of AR directly (ChIP assay), and that Nr2f2 is required for TGFβ1’s induction of AR mRNA and protein levels.

Our group has found that TGFβ1 induces AR expression in mesangial cells, however, this was previously reported to occur via the MAPK signal pathway and AP-1 (Jiang et al., 2008). Importantly, in that previous study no experiments were designed to interrogate the role of TGFβ1 induced ROS and whether the regulation on AR may also occur in an indirect manner (e.g., ROS-induced expression of Nr2f2). Although the well-known downstream signaling pathway of TGFβ1 is through Smads, MAPKs are a second downstream pathway which can cross-talk with Smads (Javelaud and Mauviel, 2005). Therefore the regulation of AR by TGFβ1 may be “directly” controlled through MAPK and AP-1, as we reported earlier, whereas stimulation of ROS, which activates the Nr2f2 pathway, may be an alternative, “indirect” manner by which TGFβ1 can regulate AR expression. Interestingly, a recent report by Shoeb et al. demonstrated a correlation between AR and Nr2f2 signal pathways. They showed inhibition of AR prevented LPS-induced inflammation, whereas the Nr2f2 signal pathway activated accordingly with LPS (Shoeb et al., 2011). These data support ours showing that AR has a role in anti-oxidant stress. Inhibition or genetic ablation of AR exacerbates Nr2f2 responses to LPS, suggesting that if you remove AR from Nr2f2’s detoxifying arsenal, the system works harder to remove cellular stress (like LPS). In light of the findings by Shoeb et al. TGFβ1 activation of AR may in fact be a novel arm of the Nr2f2 anti-oxidant defense pathway, which is supported by our data presented here.

TGFβ1 plays a critical role in renal diseases, in particular, glomerulosclerosis. In addition to TGFβ1 activity, ROS also have
important roles in the progression of glomerulosclerosis (Barnes and Gorin, 2011; Touyz and Briones, 2011; Kuo et al., 1999; Kerns et al., 2010). For example, NAD(P)H oxidase homolog, Nox4, accounts for ROS-induced mesangial cell activation, where it plays an essential role in TGFβ1 signaling of fibroblast activation and differentiation into myofibroblasts and promotes ECM production (Barnes and Gorin, 2011). Importantly, TGFβ1 can also involve AR in the deposition of extracellular matrix (ECM), including fibronectin and type IV collagen, which can lead to glomerulosclerosis (Jiang et al., 2006). Information on the role of ROS in mesangial cell signaling is incomplete, however, and further research is needed on the role of ROS during fibrosis. Besides its function on ECM production, TGFβ1 also has a documented anti-inflammatory and anti-oxidative stress role (Churchman et al., 2009). HO-1, a well-known downstream gene of Nrf2, is also regulated by TGFβ1 via the Nrf2 signaling pathway in aortic smooth muscle cells (Churchman et al., 2009). Notably, our current data indicate that TGFβ1 has a strong, yet transient induction of ROS, which subsequently activates the Nrf2 pathway, illustrating a dual role for TGFβ1 in the onset and progression of glomerulosclerosis.

Though a handful of reports have been made detailing AR’s regulation by Nrf2 (Nishinaka and Yabe-Nishimura, 2005; Kang et al., 2007, 2008), none have yet described that the TGFβ1-dependent expression of AR happens via the Nrf2 pathway in human mesangial cells upon oxidative stress. The current study explored another role of TGFβ1-induced ROS, focusing on the downstream signal pathways of ROS and subsequent alterations in gene expression, like AR. As a major ROS-induced signal pathway, Nrf2 has an important role in attenuating oxidative stress by up-regulating the expression of downstream genes, which include intracellular redox-balancing proteins, phase II detoxifying enzymes and transporters. The involvement of Nrf2 in regulating AR expression brings
up an interesting paradox. Several investigations into the therapeutic role of AR inhibitors suggest too much AR activity contributes to the pathogenesis of retinopathy, neuropathy, and nephropathy, mainly via accumulation of sorbitol inducing osmotic stress (Schemmel et al., 2010; Gaynor et al., 1997). However, alternate hypotheses on ARs function imply (1) decreasing cellular availability of NADPH could compromise antioxidant defenses leading to disease progression (Srivastava et al., 2005, 2011); or (2) alternate substrates such as lipid peroxidation-derived aldehydes (LDAs) contribute to ARs role in oxidative stress signaling (Ramana, 2011). Additionally, recent evidence of the therapeutic benefits to Nrf2 activation in diabetic nephropathy has been well documented (Pergola et al., 2011; Palsamy and Subramanian, 2011; Song et al., 2009; Li et al., 2011). These data lead to a possible contradiction in the role of AR in diabetic complications. Importantly, more work must be done to decipher the interplay of oxidative, osmotic, and inflammatory stress signals in such complex disease processes as diabetes. For now, our data confirm the anti-oxidative stress role of TGFβ1 through up-regulation of Nrf2’s downstream genes, like AR. The results presented here clearly demonstrate the role of ROS and the Nrf2 signal pathway in TGFβ1-dependent AR expression in human mesangial cells, which is helpful to understand the mechanism of TGFβ1 and AR expression in oxidative stress or fibrosis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejcb.2012.07.004.

References


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