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High glucose up-regulates angiotensin II subtype 2 receptors via interferon regulatory factor-1 in proximal tubule epithelial cells

Quaisar Ali, Rifat Sabuhi, and Tahir Hussain

Department of Pharmacological and Pharmaceutical Science, Heart and Kidney Institute, College of Pharmacy, University of Houston, 521 Science and Research Building 2, Houston, TX 77204-5037, USA

Tahir Hussain: thussain2@uh.edu

Abstract

Earlier studies have reported an increase in the proximal tubule AT₂ receptor (AT₂R) expression in diabetes, with a beneficial role in kidney function and blood pressure regulation. Here, we demonstrate that the increase in AT₂R protein expression is associated with an increased expression of transcription factor IRF-1 in hyperglycemic rat and in high glucose-treated HK2 cells. Knock-down of IRF-1 by siRNA in HK2 cells prevented high glucose-induced AT₂R up-regulation. The data suggest that IRF-1 is a transcriptional regulator of AT₂R expression in hyperglycemia, and warrant further studies to understand the physiological role of IRF-1 along with AT₂R in diabetic kidney.

Keywords

Angiotensin receptors; Transcription factor; Hyperglycemia; Kidney

Introduction

Angiotensin II (Ang II) is major effector of renin angiotensin system and mediates its cellular and physiological actions by acting on AT₁ and AT₂ receptor (AT₂R) subtypes [1]. Most of the cellular and physiological actions of Ang II such as cellular growth, proliferation, vasoconstriction, antinatriuresis, and increase in blood pressure are mediated via AT₁ receptor [1]. The functions associated with the AT₂R are less studied, in part, due to its lower expression in adult tissues [1]. However, AT₂R has been suggested as a functional antagonist of AT₁ receptors and thereby opposes the actions of Ang II mediated via AT₁ receptor [2, 3]. Thus, the activation of AT₂R has been shown to cause vasodilatation, natriuresis, and decrease in blood pressure [3]. The signaling pathways that mediate AT₂R functions, include NO/cGMP generation, activation of phosphatases and p38 MAP kinase pathways [3–5].

The AT₂R expression is abundant in fetus but regresses after birth [1, 6]. The expression of AT₂R is regulated via transcription factors of IRF-1 and CEB/P β in response to glucocorticoids and growth factors [6–8]. Recently, increase in the AT₂R expression in adult animal tissues, especially in the kidney has been reported in certain pathophysiological conditions such as diabetes and renal injury [9–12]. The enhanced expression of AT₂R in the proximal tubules of obese Zucker rats [4] and streptozotocin-treated diabetic rats [5] has

a direct role in natriuresis and diuresis. This function of AT₂R on renal sodium excretion may be protective against blood pressure increase in hyperglycemic obese Zucker rats [13]. Hyperglycemia or high glucose concentration has been reported to stimulate various proinflammatory genes, including various transcription factors contributing to tissue damage [14–16]. While we have reported an increase in AT₂R expression in the proximal tubules of obese and other diabetic rat models [4, 5], cause-effect relationship between high glucose and AT₂R expression and the molecular mechanisms responsible for this association is not known. Therefore, we utilized HK2 cells, a proximal tubule epithelial cell line derived from human kidney, which express AT₂R (able to inhibit Na⁺, K⁺-ATPase, unpublished data) for testing our hypothesis that high glucose regulates AT₂R expression via interferon regulatory factor-1 (IRF-1). We found that high glucose increases AT₂R expression, and that IRF-1 knockdown by siRNA abolished the effect of high glucose on the AT₂R expression.

Materials and methods

Chemicals

Human kidney proximal tubule epithelial (HK2) cells were purchased from ATCC (Chicago, IL). Keratinocyte-serum free media (K-SFM) and fetal bovine serum (FBS) were purchased from Invitrogen Corporation, NY. siRNA IRF-1 (h), control siRNA, siRNA transfection reagent containing lipofectamine, polyclonal antibodies for IRF-1, IRF-2, and monoclonal antibody for β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal AT₂R antibody was purchased from either Alpha Diagnostics Intl (San Antonio, TX) or Santa Cruz Biotechnology (Santa Cruz, CA). HRP-coupled anti-IgG and enhanced chemiluminescence substrates were obtained from Alpha Diagnostics Intl (San Antonio, TX). Other chemicals used in the study were purchased from Sigma- Aldrich (St. Louis, MO).

Cell culture

HK2 cells were cultured using K-SFM supplemented with 5% FBS, epidermal growth factor (EGF) and bovine pituitary extract (BPE). The cells used in the experiment were between passages 4–12. Cells were seeded at 1×10^6 in 100×20 mm culture dish.

Treatment with glucose

At 50–60% confluency, the HK2 cells were treated with normal (5 mM) and high glucose (25 mM) for 24 h. In another set of experiments, the cells also were treated with equimolar glucose + sorbitol (20 mM sorbitol + 5 mM glucose) to examine the role of hyperosmolarity per se on AT₂R expression. After treatment with glucose or sorbitol, the cell were lysed and processed for western blotting as described in “Western blotting” section.

siRNA transfection of HK2 cells

The transfection of HK2 cells with siRNA IRF-1 (CUCCA CCUCUGAAGCUACATT), which was a pool of three different siRNA duplexes with mRNA location on position 851, 854, and 1652 downstream of the IRF-1 gene. There are several IRF-1 binding motifs present in the upstream of the promoter in positions between –453 and –225 of the AT₂R gene. Briefly, the cells at 50–60% confluency were incubated with serum free medium for 3–4 h before transfection. Thereafter, the transfection was performed using lipofectamine as transfecting reagent. The cells were incubated with the siRNA IRF-1 at different concentrations (0.01–0.1 μ M). After 6 h of incubation, the existing media was replaced with a fresh media and the cells were incubated for additional 24 and 48 h. The significant knockdown of IRF-1 was achieved at 0.1 μ M siRNA at 48 h and this regimen was used in subsequent experiments for testing the effect of glucose on AT₂R expression. After siRNA

transfection (0.1 μ M, 48 h), the cells were incubated with normal and high glucose for 24 h. Cells treated with transfecting reagent alone served as control, which were also incubated with normal and high glucose. Also, we used control siRNA (Santa Cruz, CA Cat # 37007) with scrambled sequence, which does not interfere with any known cellular mRNA.

Proximal tubules from lean and obese Zucker rats

Male obese and lean Zucker rats (12 weeks of age) were purchased from Harlan, Indianapolis, IN. The rats were housed in the University of Houston animal care facility. Food and water were supplied ad libitum during this period.

The rats were anaesthetized with inactin (100 mg/kg body wt). After midline incision, kidneys were selectively infused with Krebs buffer containing collagenase and hyaluronidase. Kidney cortices were dissected and used to prepare proximal tubule suspension by the percoll density-gradient centrifugation method [4, 5]. Proteins in the proximal tubules were determined by BCA method using a kit (Pierce, Rockford, IL).

Western blotting

The HK2 cells were washed with cold phosphate buffered saline and lysed in 0.3 ml of lysis buffer containing 0.5 M Tris base (pH 6.8), 1% SDS, 1 mM EDTA, 1 mM PMSF, and protease inhibitor (aprotinin, calpain inhibitors, leupeptin, pepstatin, and trypsin inhibitor). The cell lysates were used for protein estimation by BCA method using a kit. Equal amounts (60 μ g for AT₂, IRF-1 and IRF-2) of proteins from HK2 cells were subjected to SDS-PAGE and transferred onto immobilon P (blot). Similarly, proteins in proximal tubules from lean and obese Zucker rats were determined and 60 μ g of the protein samples were subjected to western blotting for AT₂R, IRF-1 and IRF-2 using respective protein specific polyclonal antibodies. Polyclonal IgG-linked with horseradish peroxidase and the ECL system were used to detect the signal, which were analyzed by FluorChem 8800 (Alpha Innotech Imaging System, San Leandro, CA). β -actin was used as a loading control.

In a separate set of experiments, we have determined the specificity of the AT₂R antibody in Sprague-Dawley rat kidneys selectively treated with AT₂ siRNA for 5, 7, and 14 days. The AT₂ antibody was able to detect changes at various levels of AT₂R expression at different days of siRNA treatment. The changes in AT₂R protein expression were in parallel to the changes in AT₂ mRNA expression (unpublished data). This test supports the validity of the AT₂R measurement by either of the methods, i.e., western blotting or qRT-PCR.

qRT-PCR

Quantitative PCR (qRT-PCR) was performed both in HK2 cells and in rat proximal tubules by using the SYBR Green detection method. After treating HK2 cells with normal and high glucose, RNA was isolated by RNeasy mini kit (Qiagen, Valencia, CA). The RNA (50 ng) isolated from HK2 cells was used for making cDNA by using Advantage RT-PCR kit (Clontech Mountain View, CA). The resulting cDNA was used for measuring AT₂R by RT-PCR machine (Applied Biosystem 7300). The primer used for amplification of AT₂R gene in HK2 cells was: forward 5'CCCGTGACCAAG TCCTGAA3'; reverse 5'GCAAATGATGAAGGCCAG AA3' and for GAPDH (as internal control) was forward 5'CAAGGCTGTGGGCAAGGT3'; reverse 5'GGAAGGC CATGCCAGTGA3'. The results obtained were compared between normal and high glucose-treated cells. The protocol used for qRT-PCR experiments in kidney cortex of lean and obese Zucker rats was similar to that of HK2 cells but the primer used for amplifying AT₂ in rat kidney cortex was: forward 5'GCTGTTGTGTTGGCATTCA3'; reverse 5'ATCCAAGAAGGTCAGAACATGGA3' and GAPDH primer was: forward 5'GTAGTCGCCGTGCCTACCAT3'; reverse 5'TCCGGAATCGAACCCTGAT3'. Two different primers were designed based on human

and rat genes to ensure the primers' specificity in respective source of samples, i.e., human derived HK2 cells and rat proximal tubules.

Statistical analysis

Results are expressed as the mean $\pm$ SEM. The data were subjected to statistical analysis (Student's *t* test) and one-way ANOVA followed by Newman–Keuls test using GraphPad Prism 4, San Diego, CA. Values at $P < 0.05$ were considered to be statistical significant.

Results

AT₂R and IRF-1 expression in HK2 cells and obese Zucker rat proximal tubules

Western blotting demonstrates the presence of IRF-1 as approximately 52 kDa band and AT₂R as 45 kDa band in HK2 cell lysates as well as in the proximal tubules. Densitometric analysis of the bands revealed that 24 h incubation of HK2 cells with high glucose (25 mM) caused increase in the expression of both AT₂R and IRF-1 as compared to the control HK2 cells incubated with normal (5 mM) glucose (Figs. 1aA, 4). Sorbitol (20 + 5 mM glucose) treatment of HK2 cells for 24 h had no significant effect on AT₂R expression (Fig. 1aC) suggesting that AT₂ protein expression change during high glucose incubation was not due to hyper-osmolality of the medium. Similarly, densitometric analysis revealed that the expression of IRF-1 as well as of AT₂R in the proximal tubules was significantly elevated in obese compared with lean Zucker rats (Fig. 1bA, bC).

The qRT-PCR analysis also revealed a significant increase in AT₂ mRNA levels in both the high glucosetreated cells as compared to control HK2 cells (Fig. 1aB) and the proximal tubules of obese Zucker rats as compared to their lean controls (Fig. 1bB).

Effect of IRF-1 knockdown on glucose-induced up-regulation of AT₂R

To study the role of IRF-1 in high glucose-induced AT₂R up-regulation, we optimized the conditions to knock-down IRF-1 using IRF-1 siRNA (10 and 100 nM) for 24 and 48 h (Fig. 2). Treatment with siRNA (100 nM) for 48 h down regulated IRF-1 protein expression by 50% in HK2 cells. IRF-1 siRNA did not affect the expression of IRF-2, suggesting the specificity of the siRNA (Fig. 3). The reduction in IRF-1 expression was associated with the reduction in AT₂ expression in HK2 cells. However, incubation of 100 nM siRNA-treated cells with high glucose restored the expression of both the IRF-1 and the AT₂R (Fig. 3). In another set of experiments, we used higher siRNA concentration (500 nM) in order to prevent the effect of high glucose on IRF-1 expression. Higher siRNA concentration was able to maintain lower IRF-1 expression even in the presence of high glucose. This lowering of IRF-1 expression abolished glucose-induced AT₂R up-regulation (Fig. 4). The data clearly suggest that glucose induces an IRF-1 dependent upregulation of AT₂R.

Discussion

This study directly demonstrates that high glucose induces AT₂R expression in the proximal tubule epithelial cells and this process is mediated via an increase in the transcription factor IRF-1 expression.

Earlier, we have reported AT₂R expression increase in the proximal tubules of obese Zucker rats and streptozotocin-induced diabetic rats [4, 5]. The AT₂R upregulation potentially promotes renal sodium excretion and protects against blood pressure increase in these animals [4]. The incubation of HK2 cells with high glucose concentration suggests that glucose has a positive regulatory role in AT₂R expression. Hyperglycemia is known to activate various stress kinases leading to hypertrophy and tissue damage, including kidney

damage [17]. The increase in AT₂R expression in response to high glucose could be a compensatory mechanism to improve kidney function and to prevent blood pressure increase in diabetic animals. In pursuit of understanding the mechanism of glucose-induced AT₂R expression, we found that the increase in AT₂R mRNA was associated with the increase in AT₂R protein, and increase in IRF-1 expression in the proximal tubules of obese Zucker rats. These findings suggest a potential association between the increases in AT₂ and IRF-1, the notion supported by the IRF-1 knock-down studies. However, high glucose reverted the knock-down of IRF-1 expression by siRNA (100 nM). The reversal in IRF-1 expression was associated with simultaneous reversal in the reduction of AT₂R expression. This suggested that high glucose is a potent stimulus, which overcame effectiveness of 100 nM siRNA on IRF-1 expression and hence on AT₂R expression. In order to maintain the knock-down of IRF-1 in the presence of high glucose, we used higher concentration of siRNA (500 nM). With this strategy, we were able to keep the low expression of IRF-1, and high glucose was unable to increase AT₂R expression, suggesting a role of IRF-1.

How does glucose regulate IRF-1 expression is not clear. However, p38 pathway may be a plausible mechanism responsible for high glucose-induced IRF-1 up-regulation/activation leading to AT₂R transcription. Activation of p38 has been implicated in the cellular response to hyperglycemia. High levels of glucose stimulate p38 in proximal tubular cells and other kidney cells such as mesangial cells [18]. Also, enhanced p38 activation is reported in the proximal tubules of obese Zucker rats [19]. Moreover, the activated p38 pathway causes activation of a number of transcription factors, including IRF-1 [20–25]. Since IRF-1 regulates AT₂R transcription, it might be possible that glucose-induced AT₂R up-regulation involves IRF-1 via p38 pathway. Glucose also is known to stimulate various inflammatory signals leading to tissue injury, including kidney [17]. For example, high glucose concentrations activate tumor necrosis factor- α (TNF- α) and migration inhibitory factor (MIF) which leads to phosphorylation of ERK1/2 pathway [18, 26]. The increase in AT₂R expression in response to high glucose appears to be beneficial, in terms of renal function and protection against blood pressure increase [13]. However, it is not clear whether increase in the IRF-1 expression in response to hyperglycemia has other cellular and physiological consequences. IRF-1 is known to act as a transcriptional activator and is regulated by different cytokines such as IFN- α , IFN- β and mediates cell growth, transformation, and apoptosis [25, 27]. IRF-1 is dramatically upregulated in certain pathophysiological conditions [27]. If increase in the IRF-1 expression and hence in AT₂R expression and function are beneficial to the diabetic kidney and blood pressure control, these molecules provide novel targets to treat kidney disease and hypertension. Studies for understanding the role of selective activation of AT₂R in long-term blood pressure control are underway.

In summary, we found that the expression of AT₂R and IRF-1 is increased in proximal tubules of obese Zucker rats and in HK2 cells treated with high concentration of glucose. The siRNA knock-down studies suggest that glucose is a potent stimulator of IRF-1 expression, which mediates increase in AT₂R expression in response to high glucose. While there is reasonable physiological relevance of AT₂R, the role of IRF-1 in renal physiology and blood pressure control needs to be investigated.

Acknowledgments

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References

1. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger TH. International union of pharmacology XXIII: the angiotensin receptors. *Pharmacol Rev.* 2000; 52:415–472. [PubMed: 10977869]
2. Masaki H, Kurihara T, Yamaki A, Inomata N, Nozawa Y, Mori Y, Murasawa S, Kizima K, Maruyama K, Horiuchi M, Dzau VJ, Takahashi H, Iwasaka T, Inada M, Matsubara H. Cardiac-specific overexpression of angiotensin II AT₂ receptor causes attenuated response to AT₁ receptor-mediated pressor and chronotropic effects. *J Clin Invest.* 1998;527–535.10.1172/JCI1885 [PubMed: 9449684]
3. Akishita M, Ito M, Lehtonen JY, Daviet L, Dzau VJ, Horiuchi M. Expression of the AT₂ receptor developmentally programs extracellular signal-regulated kinase activity and influences fetal vascular growth. *J Clin Invest.* 1999; 103(1):63–71.10.1172/JCI5182 [PubMed: 9884335]
4. Hakam AC, Hussain T. Renal angiotensin II type-2 receptors are upregulated and mediate the candesartan-induced natriuresis/diuresis in obese Zucker rats. *Hypertension.* 2005; 45:270–275.10.1161/01.HYP.0000151622.47814.6f [PubMed: 15596573]
5. Hakam AC, Siddiqui AH, Hussain T. Renal angiotensin II AT₂ receptors promote natriuresis in streptozotocin-induced diabetic rats. *Am J Physiol Renal Physiol.* 2006; 290:F503–F508.10.1152/ajprenal.00092.2005 [PubMed: 16204414]
6. Matsubara H, Shibasaki Y, Okigaki M, Mori Y, Masaki H, Kosaki A, Tsutsumi Y, Uchiyama Y, Fujiyama S, Nose A, Iba O, Tateishi E, Hasegawa T, Horiuchi M, Nahmias C, Iwasaka T. Effect of angiotensin II type 2 receptor on tyrosine kinase Pyk2 and c-Jun NH₂-terminal kinase via SHP-1 tyrosine phosphatase activity: evidence from vascular-targeted transgenic mice of AT₂ receptor. *Biochem Biophys Res Commun.* 2001; 282(5):1085–1091.10.1016/j.ejphar.2004.11.040 [PubMed: 11302725]
7. Gendron L, Laflamme L, Rivard N, Asselin C, Payet MD, Gallo-Payet N. Signals from the AT₂ (angiotensin type 2) receptor of angiotensin II inhibit p21ras and activate MAPK (mitogen-activated protein kinase) to induce morphological neuronal differentiation in NG108-15 cells. *Mol Endocrinol.* 1999; 13(9):1615–1626.10.1210/me.13.9.1615 [PubMed: 10478850]
8. Kijima K, Matsubara H, Murasawa S, Maruyama K, Mori Y, Inada M. Gene transcription of angiotensin II type 2 receptor is repressed by growth factors and glucocorticoids in PC12 cells. *Biochem Biophys Res Commun.* 1995; 216(1):359–366.10.1006/bbrc.1995.2632 [PubMed: 7488113]
9. Kumar AM, Gupta RK, Spitzer A. Intracellular sodium in proximal tubules of diabetic rats. Role of glucose. *Kidney Int.* 1998; 33:792–797.10.1038/ki.1988.69 [PubMed: 3386134]
10. Ichiki T, Inagami T. Transcriptional regulation of the mouse angiotensin II type 2 receptor gene. *Hypertension.* 1995; 25(4 Pt 2):720–725. [PubMed: 7721422]
11. Horiuchi M, Yamada T, Hayashida V, Dzau VJ. Interferon regulatory factor-1 up-regulates angiotensin II type 2 receptor and induces apoptosis. *J Biol Chem.* 1997; 272(18):11952–11958.10.1074/jbc.272.18.11952 [PubMed: 9115258]
12. Lee JH, Xia S, Ragolia L. Up-regulation of AT₂ Receptor and iNOS impairs angiotensin II-induced contraction without endothelium influence in young normotensive diabetic rats. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R144–R154.10.1152/ajpregu.00191.2008 [PubMed: 18463192]
13. Siddiqui AH, Ali Q, Hussain T. Protective role of angiotensin II subtype 2 receptor in blood pressure increase in obese Zucker rats. *Hypertension.* 2009; 53:256–261. doi: 10.1161/hypertension.126086. [PubMed: 19114640]
14. Benndorf RA, Krebs C, Hirsch-Hoffmann B, Schwedhelm E, Cieslar G, Schmidt-Haupt R, Steinmetz OM, Meyer-Schwesinger C, Thaiss F, Hadda M, Fehr S, Heilmann A, Helmchen U, Hein L, Ehmke H, Stahl RA, Böger RH, Wenzel UO. Angiotensin II type 2 receptor deficiency aggravates renal injury and reduces survival in chronic kidney disease in mice. *Kidney Int.* 2009; 75(10):1039–1049.10.1038/ki.2009.2 [PubMed: 19212419]
15. Mezzano S, Droguett A, Burgos ME, Ardiles LG, Flores CA, Aros CA, Caorsi I, Vío CP, Ruiz-Ortega M, Egido J. Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy. *Kidney Int Suppl.* 2003; 86:S64–S70.10.1046/j.1523-1755.64.s86.12.x [PubMed: 12969130]

16. Samikkannu T, Thomas JT, Bhat J, Wittman V, Thekkumkara TJ. Acute effect of high glucose on long-term cell growth: a role for transient glucose increase in proximal tubule cell injury. *Am J Physiol Renal Physiol*. 2006; 291(1):F162–F175.10.1152/ajprenal.00189.2005 [PubMed: 16467130]
17. Satriano J. Kidney growth, hypertrophy and the unifying mechanism of diabetic complications. *Amino Acids*. 2007; (2):331–339.10.1007/s00726-007-0529-9 [PubMed: 17443269]
18. Zhang SL, Tang SS, Chen X, Filep JG, Ingelfinger JR, Chan JS. High levels of glucose stimulate angiotensinogen gene expression via the P38 mitogen-activated protein kinase pathway in rat kidney proximal tubular cells. *Endocrinology*. 2000; 141:4637–4646.10.1210/en.141.12.4637 [PubMed: 11108278]
19. Banday AA, Fazili FR, Marwaha A, Lokhandwala MF. Mitogen-activated protein kinase upregulation reduces renal D1 receptor affinity and G-protein coupling in obese rats. *Kidney Int*. 2007; 71:397–406.10.1038/sj.ki.5002055 [PubMed: 17191082]
20. Cheong J, Coligan JE, Shuman JD. Activating transcription factor-2 regulates phosphoenolpyruvate carboxykinase transcription through a stress-inducible mitogen-activated protein kinase pathway. *J Biol Chem*. 1998; 273:22714–22718.10.1074/jbc.273.35.22714 [PubMed: 9712902]
21. Faure V, Hecquet C, Courtois Y, Goureau O. Role of interferon regulatory factor-1 and mitogen-activated protein kinase pathways in the induction of nitric oxide synthase-2 in retinal pigmented epithelial cells. *J Biol Chem*. 1999; 274:4794–4800.10.1074/jbc.274.8.4794 [PubMed: 9988718]
22. Seymour KJ, Roberts LE, Fini MA, Parmley LA, Oustitch TL, Wright RM. Stress activation of mammary epithelial cell xanthine oxidoreductase is mediated by p38 MAPK and CCAAT/enhancer-binding protein-beta. *J Biol Chem*. 2006; 281:8545–8558.10.1074/jbc.M507349200 [PubMed: 16452486]
23. Horiuchi M, Koike G, Yamada T, Mukoyama M, Nakajima M, Dzau V. The growth-dependent expression of angiotensin II type 2 receptor is regulated by transcription factors interferon regulatory factor-1 and -2. *J Biol Chem*. 1995; 270:20225–20230.10.1074/jbc.270.34.20225 [PubMed: 7650042]
24. Sanchez-Niño MD, Sanz AB, Ihalmo P, Lassila M, Holthofer H, Mezzano S, Aros C, Groop PH, Saleem MA, Mathieson PW, Langham R, Kretzler M, Nair V, Lemley KV, Nelson RG, Mervaala E, Mattinzoli D, Rastaldi MP, Ruiz-Ortega M, Martin-Ventura JL, Egido J, Ortiz AJ. The MIF receptor CD74 in diabetic podocyte injury. *J Am Soc Nephrol*. 2009; 20(2):353–362.10.1681/ASN.2008020194 [PubMed: 18842989]
25. Giovanna R, Gianna F, Maria V, Chiantore MV, Percario ZA, Vannucchi S, Elisabetta A. IRF-1 as a negative regulator of cell proliferation. *J Interferon Cytokine Res*. 2002; 22(1):39–44.10.1089/107999002753426473 [PubMed: 11846974]
26. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005; 54:1615–1625. doi: 10.2337. [PubMed: 15919781]
27. Tadatsug T, Kouetsu O, Akinori T, Nobuyuki T. IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol*. 2001; 19:623–655. doi: 10.1146/annurev.immunol.19.1.623. [PubMed: 11244049]

Fig. 1.

a HK2 cells (A) AT₂ receptor expression in HK2 cells treated for 24 h with normal (5 mM) and high glucose (25 mM). *Upper panel*: representative western blots. *Lower panel*: bar graph of band density of AT₂ receptor normalized with β-actin. (B) AT₂ mRNA expression measured by qRT-PCR in HK2 cells treated for 24 h with normal (5 mM) and high (25 mM) glucose. (C) AT₂ receptor expression in HK2 cells treated for 24 h with normal glucose (5 mM) and sorbitol (sorbitol 20 mM + glucose 5 mM). *Upper panel*: representative western blot. *Lower panel*: bar graph of band density of AT₂ receptor normalized with β-actin. **b** Proximal tubules (A) AT₂ receptor expression in the proximal tubules of lean and obese Zucker rats. *Upper panel*: representative western blot. *Lower panel*: bar graph of band density of AT₂ receptor expressed as percent of lean. (B) AT₂ receptor mRNA expression measured by qRT-PCR in proximal tubules of lean and obese Zucker rats. (C) Expression of IRF-1 in proximal tubules of lean and obese Zucker rats. *Upper panel*: representative western blot. *Lower panel*: bar graph of band density of IRF-1 normalized with β-actin. Values are represented as mean ± SEM, * significantly different from lean rats (Student's *t* test, *P* < 0.05, *n* = 4 in each group)

Fig. 2. Concentration and time course study of siRNA transfection: IRF-1 expression in HK2 cells transfected with **a** different concentrations of siRNA IRF-1 (10, 100 and 500 nM) for 48 h and **b** 100 nM siRNA IRF-1 for 24 and 48 h

Fig. 3.

Effect of glucose (25 mM) on the expression of AT_2 receptor and IRF-1 in HK2 cells transfected with 100 nM IRF-1 siRNA. *Upper Panels:* western blots of IRF-1, IRF-2, AT_2 receptor and β -actin. *Lower panels:* bar graphs of band density of IRF-1, IRF-2, AT_2 normalized with β -actin. Values are represented as mean $\pm$ SEM, * significantly different from control HK2 cells (one-way ANOVA followed by Neuman-Keuls test, $P < 0.05$, $n = 3$).

Fig. 4.

Effect of glucose (25 mM) on the expression of AT_2 receptor and IRF-1 in HK2 cells transfected with 500 nM IRF-1 siRNA. *Upper Panels:* western blots of IRF-1, IRF-2, AT_2 receptor and β -actin. *Lower panels:* bar graphs of band density of IRF-1, IRF-2, AT_2 normalized with β -actin. Values are represented as mean $\pm$ SEM, * significantly different from control HK2 cells (one-way ANOVA followed by Neuman–Keuls test, $P < 0.05$, $n = 3$). *CT* control, *HG* high glucose, *SS* scrambled sequence