NRF2: A potential target for the treatment of diabetic nephropathy

Received 23 September 2020

Accepted 3 February 2021

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Abstract

One of the major complications of diabetes mellitus is diabetic nephropathy (DN), the pathogenesis of which is primarily driven by oxidative stress. As a major regulator of antioxidant responses, the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) has recently attracted much interest. NRF2 is a primary defense mechanism against the cytotoxic effects of oxidative stress, involving heterogeneous detoxification, the production of antioxidants and anti-inflammatory molecules, DNA repair, nuclear chaperones, and proteasome systems. A myriad of studies in pre-clinical models of DN have consistently demonstrated a beneficial effect of NRF2 activation, suggesting that NRF2 is likely a promising target for treating DN. This has been further supported by findings from clinical trials of bardoxolone methyl, an activator of NRF2, despite the unexpected adverse cardiovascular effects. This review summarizes the support for therapeutic targeting of NRF2 in DN and emphasizes the need for the optimization of NRF2-based treatment with the minimization of potential adverse effects.

Keywords

diabetic nephropathy • nuclear factor erythroid 2-related factor 2 • oxidative stress

1. Introduction

Diabetes mellitus type 1 and type 2 will inevitably result in diabetic nephropathy (DN) at some point, which features a progressive leak of circulating albumin into the urine and thus can be empirically staged according to the severity of the leakage, *i.e.*, normal albuminuria, moderately increased albuminuria (previously known as microalbuminuria), and severely increased albuminuria (previously known as macroalbuminuria) [1–3]. Although long-term epidemiologic studies have shown that not all diabetic patients will progress to DN, it has been estimated that approximately 25–40% of diabetic patients eventually develop kidney disease [4]. While poor glycemic control is a well-known risk factor for albuminuria and DN [5–7], a large number of patients still develop DN despite adequate glycemic control. Although the complete risk factors for this progression are not fully understood, researchers likely include hereditary, environmental, other pre-existing kidney diseases, and the severity of diabetes [8–10]. Oxidative stress, in particular, is considered to be a key factor for the development of various diabetic complications, including DN [11, 12]. Therefore, as a major regulator of the antioxidant response, nuclear factor erythroid 2-related factor 2 (NRF2) is a potential therapeutic target for the prevention or treatment of DN.

2. Oxidative Stress: A Key Player in the Pathogenesis of DN

A consequence of physiological cellular oxygen metabolism is the inevitable production of potentially harmful reactive oxygen species (ROS) [13]. This oxidant formation must be

balanced by oxidant removal. Oxidative stress is the result of an imbalance between pro-oxidants and antioxidants which may lead to chronic tissue and organ injury such as DN [11]. Several macromolecules are involved in increased ROS production in the kidney in diabetic conditions [14]. These include NAD(P)H oxidase, advanced glycation end products (AGE), defective polyol pathway, uncoupling of nitric oxide synthase (NOS), and oxidative phosphorylation of mitochondrial respiratory chains [15–18]. Increased abundance of ROS triggers activation of protein kinase C, mitogen-activated protein kinases, and various cytokines and transcription factors. These general inflammatory markers are also common in the pathophysiology of DN and are often found alongside histological lesions including glomerular and tubular basal changes, increased production of extracellular matrix (ECM) which further results in glomerular and tubular basement membrane thickening, dilatation of interstitial matrix of the glomerulus, diffuse glomerulosclerosis, nodular glomerulosclerosis, and hyalinization of afferent arterioles [19–22].

3. NRF2: The Highly Conserved Master Regulator of Antioxidative Self-defense

As a result of oxidative stress caused by ROS [23, 24], mammalian cells trigger an effective cytoprotective mechanism in the form of the Cap 'n' Collar (CNC) basic-leucine zipper (bZip) nuclear transcription factor, NRF2. This defense mechanism includes pathways for xenobiotic detoxification, antioxidant, anti-inflammatory responses, DNA repair, molecular chaperone, and proteasome systems [25, 26]. NRF2, also known as nuclear factor erythroid-derived 2-like 2, is a bZip transcription factor with a CNC structure [27].

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HUMAN_(HOMO_SAPIENS)	MGLDILWRQDILGVSRVDFDSQRKYELEKQKKLEKERQQLQKEQKFAFQQLQDEET	64
RAT_(RATTUS_NORVEGICUS)	MMDL EL PPPGL QSQQDMDL DILWRQDILGVSRVDFDSQRKYELEKQKKLEKERQQLQKEQKFAFQQLQDEET	80
MOUSE_(MUS_MUSCULUS)	MMDL EL PPPGL QSQQDMDL DILWRQDILGVSRVDFDSQRKYELEKQKKLEKERQQLQKEQKFAFQQLQDEET	80
CHICKEN_(GALLUS_GALLUS)	MNL DILWRQDILGARREVDFDSQRKYELEKQKKLEKERQQLQKEQKFAFQQLQDEET	64
HUMAN_(HOMO_SAPIENS)	GEFLPIQPACHI CSETSGSANYSQVAHI PKSD.....ALYFEDCMQLLIAQTFFPVDNEVSSATFCSLVPI PG	133
RAT_(RATTUS_NORVEGICUS)	GEFLPIQPACHI CTDTSGSVSYSQVAHI PKQD.....ALYFEDCMQLLIAETFFPVDNE.....SLALDPS	142
MOUSE_(MUS_MUSCULUS)	GEFLPIQPACHI CTDTSGSASYSQVAHI PKQD.....ALYFEDCMQLLIAETFFPVDNE.....SLALDPS	142
CHICKEN_(GALLUS_GALLUS)	GEFVPAQPAQRVGSSEN.....AEPII SFSQSTDTSKPEEALS FDCMQLLIAEAPFI DDNEASPAAFCSLVPI QD	135
HUMAN_(HOMO_SAPIENS)	HI ESPVFI ATNCAQSPET SVAQVAPVDLDGMCDI EQVVEELLSI PELQCLNI ENDKLVETT MVPSPKALITEVDNYHFY	213
RAT_(RATTUS_NORVEGICUS)	HVESSVFTTPDCAQSLDSSLETAMTDLSSI QDMEQVWQELFSI PELQCLNTENKQQAETTTVPSPKALITEVDNSYHF	221
MOUSE_(MUS_MUSCULUS)	HAESSVFTAPHCAQSLN SSLEAAMTDLSSI QDMEQVWQELFSI PELQCLNTENKQLADTTAVRSPKALITEVDNSYHF	221
CHICKEN_(GALLUS_GALLUS)	SD...PVFI SANCTCPP...SSPGI VPLTDAENMNT EQVVEELLSI PELQCLNI ENDNLAEVSTI TSPETKPAEMHNSDY	211
HUMAN_(HOMO_SAPIENS)	SSI PSM EKEVGNCSPHFLNAFEDSFSSI LSTEDPNQLTVNSLNSDAT VNTDFGDEFYSAFI AEPSI SNSMPSPATLS S	292
RAT_(RATTUS_NORVEGICUS)	YSSI PSLEKEVDSCSPHFLHGFEDSFSSI LSTDDASQL...NSLDSNPTLNTDFGDEFYSAFI AEPSGGGSMPSAAI SCS	299
MOUSE_(MUS_MUSCULUS)	YSSI PSLEKEVGNCSPHFLHGFEDSFSSI LSTDDASQL...TSLDSNPTLNTDFGDEFYSAFI AEPSDGGGSMPSAAI SCS	299
CHICKEN_(GALLUS_GALLUS)	YNSLP...MRKDVNCGPDELNI EGFSSI LQPDSSQLNVNSLNSLT LSSDFCEDFYTNFI CAKGDGDTGTN TI SCS	289
HUMAN_(HOMO_SAPIENS)	LSEL LNGL DVSDLSLCKAFNQNPESTAEFNDSDSGI SLNTSPSRASPEHVSSESSYGDTL LGLSDSEMEELDSAPGSV	372
RAT_(RATTUS_NORVEGICUS)	LSEL LGGPI EGDDL SLCKAFNCKT ECTVEFNDSDSGI SLNTSPSRASPEHVSSESSYGDPPPGSDSEMEELDSAPGSV	379
MOUSE_(MUS_MUSCULUS)	LSEL LGDTI EGDDL SLCKAFNPKAECTMEFNDSDSGI SLNTSPSRASPEHVSSESSYGDPPPGSDSEMEELDSAPGSV	379
CHICKEN_(GALLUS_GALLUS)	LADI LSEPI DLSDFPLVRAFNDDSGTVP EONDSDSGI SLNANSI ASPEHVSSESTCGDKTF CQSDSEMEEDMSSPGSV	369
HUMAN_(HOMO_SAPIENS)	KQNGPKT PVHSSGDMVCLSPSQGQSTHVHACCENTPEKELPVSPGHKPTPFTKDKSSRLEAHLTRDELRAKALH P	451
RAT_(RATTUS_NORVEGICUS)	KQNGPKAQPTHSSGDTVCLSPAQGHSAAVHSSCCENTTTKKEVPVSPGHCKVPFTKDKSSRLEAHLTRDELRAKALH P	459
MOUSE_(MUS_MUSCULUS)	KQNGPKAQPAHSPGDTVCLSPAQGHSAVHSSCCENTTTKKEVPVSPGHCKAPFTKDKSSRLEAHLTRDELRAKALH P	459
CHICKEN_(GALLUS_GALLUS)	PCGNAS...VYSSRFPCVCLSPVEPTGTQPSLRMNTPKDPPAGCPKAPFTKDKPSGRLEAHLTRDEORAKALQ P	445
HUMAN_(HOMO_SAPIENS)	FPVEKI NLPVDFNEMWSKEQFNEAQLALI RDI RRRGKNKVAACNCRKRKLENI VELEQDL DHLKDEKEKLLKEKGEND	531
RAT_(RATTUS_NORVEGICUS)	FPVEKI NLPVDFNEMWSKEQFNEAQLALI RDI RRRGKNKVAACNCRKRKLENI VELEQDL GHKDEKEKLLREKGEND	539
MOUSE_(MUS_MUSCULUS)	FPVEKI NLPVDFNEMWSKEQFNEAQLALI RDI RRRGKNKVAACNCRKRKLENI VELEQDL GHKDEKEKLLREKGEND	539
CHICKEN_(GALLUS_GALLUS)	FPVEKI NLPVDFNEMWSKEQFNEAQLALI RDI RRRGKNKVAACNCRKRKLENI VELEQDL SHLKDEKEKLLKEKGEND	525
HUMAN_(HOMO_SAPIENS)	KS LHLK KKLSTLY LEVFSMLRDEDGKPYSPSEYSLQQT RDGNVFLVPKSKKPDVKK	588
RAT_(RATTUS_NORVEGICUS)	RNLHLK KKLSTLY LEVFSMLRDEDGKPYSPSEYSLQQT RDGNVFLVPKSKKPDVKK	596
MOUSE_(MUS_MUSCULUS)	RNLHLK KKLSTLY LEVFSMLRDEDGKPYSPSEYSLQQT RDGNVFLVPKSKKPDVKK	596
CHICKEN_(GALLUS_GALLUS)	KS LRLK KKLSTLY LEVFSMLRDEDGKPYSPSEYSLQQT RDGNVFLVPKSKKPDVKK	582

Figure 1. NRF2, a basic leucine zipper transcription factor. The black box represents completely preserved residues, pink indicates residues with similar properties, blue indicates those with weakly similar properties, and white boxes represent those not identical to human NRF2. NRF2, nuclear factor erythroid 2-related factor 2.

Homologs to human NRF2 exist in various organisms such as rats, mice, and chickens (Figure 1 and Table 1). NRF2 has seven highly conserved domains called NRF2-ECH homology (Neh) domains (Neh1~7 domains). The Neh1 domain, a CNC-bZip domain, is necessary for the formation of a heterodimer with small musculoaponeurotic fibrosarcoma (sMaf) and DNA binding [28]. The Neh2 domain binds with its cytosolic repressor, kelch-like ECH-associated protein 1 (KEAP1), via DLG and ETGE motifs within Neh2, leading to the degradation of NRF2 [29]. The Neh3 domain is necessary for transcriptional activation [30], while Neh4/5 domains are independent transactivation domains [31]. Besides, the recently uncharacterized Neh6 domain is involved in the process of NRF2 degradation [32]. Finally, the Neh7 domain helps to suppress NRF2/antioxidant response element (ARE) via serving as a binding site for RXR α [33].

4. Signaling Pathways Regulating the NRF2 Antioxidant Response

Under basal conditions, NRF2 is restricted to the cytoplasm at very low levels by the binding of the DLG and ETGE motifs of the Neh2 domain to KEAP1, leading to degradation by the proteasome [31]. However, when the

cells are exposed to oxidative stress, the binding of the DLG motif of NRF2 to KEAP1 is inhibited, suppressing the rate of degradation [34]. Freed from KEAP1, NRF2 translocates to the nucleus where it forms a heterodimer with sMaf proteins, its obligatory partner, and binds to the ARE sequence initiating the transcription of a multitude of NRF2 downstream genes (Figure 2). These NRF2-regulated genes are classified into intracellular redox-balancing proteins and phase II detoxifying enzymes and transporters, such as glutamate-cysteine ligase (GCL), glutathione s-transferase (GST), and multidrug resistance-associated protein (MRP) [35–37]. As the primary regulator of these cytoprotective genes, the ubiquitously expressed NRF2 is largely responsible for maintaining cellular homeostasis under oxidative stress [38]. Along with the NRF2-mediated protective pathways for toxic or oxidant exposure, NRF2 is also involved in the mechanisms of cellular differentiation, proliferation, inflammation, and lipid synthesis [38, 39].

The activation of NRF2 is under tight regulation, and various diseases including neurodegenerative diseases, malignancy, and cardiovascular diseases are associated with abnormal expression and function of NRF2 [40]. Along with the KEAP1-dependent mechanism of activation described

Species	Accession	Max score	Total score	Query cover (%)	E value%	Identity
<i>Homo sapiens</i>	NP_001138884.1	1206	1206	100%	0.0	100.00%
<i>Rattus norvegicus</i>	NP_113977.1	964	964	100%	0.0	81.22%
<i>Mus musculus</i>	NP_035032.1	952	952	100%	0.0	80.54%
<i>Gallus gallus</i>	NP_990448.1	726	726	99%	0.0	65.82%

Table 1. Protein sequence and homology analysis by NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for NRF2 in various organisms

Figure 2. The mechanism diagram illustrates the KEAP1-dependent and GSK3β-mediated KEAP1-independent regulation pathway of the NRF2 antioxidant response. Under basal conditions, the DLG and EGTE motifs of NRF2 bind KEAP1 in the cytoplasm. This binding localizes NRF2 to the cytoplasm and causes its ubiquitination and degradation via the proteasome. However, when kidney cells are exposed to diabetic oxidative stress, this degradation is inhibited and NRF2 is separated from KEAP1 at the DLG motif. This structural change causes NRF2 to escape the control of KEAP1 entirely allowing NRF2 nuclear translocation, where NRF2 forms a heterodimer with its essential partner, the sMaf proteins, and binds to the ARE sequence to initiate transcription of several NRF2 downstream genes which eliminate stress or oxidative damage. In response to diabetic oxidative stress, GSK3β is overactivated to promote the excretion of NRF2 from the nucleus and then degradation. ARE, antioxidant response element; GSK3β, glycogen synthase kinase 3β; HO-1, heme oxygenase 1; KEAP1, kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; sMaf, small musculoaponeurotic fibrosarcoma.

above, a glycogen synthase kinase 3β (GSK3β)-mediated KEAP1-independent mechanism is responsible for NRF2 degradation in the setting of oxidative stress-induced NRF2 [41, 42]. By the promotion of its nuclear elimination and degradation, GSK3β is a negative regulator of NRF2 (Figure 2). It is important to turn on GSK3β in response to remove NRF2 from the nucleus where it is liberated and migrates into the nucleus, which does not affect the underlying activity of NRF2 but primarily regulates its activity in the later stages of the NRF2 antioxidant response [41]. Several signaling cascades

can modulate NRF2 activity through KEAP1-dependent and KEAP1-independent mechanisms, it is important that NRF2 must be expressed correctly, neither too much nor too little, to protect the cell. Therefore, the modulation of targets such as GSK3β may represent additional therapeutic strategies for the KEAP1-independent regulation of NRF2 activity.

5. Therapeutic Targeting of NRF2 to Treat DN: Evidence from Pre-Clinical Studies

Nearly all relevant pre-clinical studies have indicated a beneficial effect of NRF2 in DN. Specifically, these studies have shown that the renoprotective activities of several drugs in pre-clinical models of type 1 or 2 DN are mediated through the NRF2–ARE signaling axis. In this regard, the beneficial effects of resveratrol [43], rosuvastatin [44], curcumin [45], salvianolic acid A [46], telmisartan [47], mycophenolate mofetil [48], fenofibrate [49], and hepatocyte growth factor [50] by *in vivo* or *in vitro* models of DN have been demonstrated to be conveyed at least in part by activating the NRF2 antioxidant response. Further, these findings were later corroborated by selective silencing of KEAP1, the endogenous NRF2 inhibitor, in kidney cells. More specifically, Zheng et al. [51] demonstrated a reduced expression of transforming growth factor- β (TGF β) and matrix proteins in human renal mesangial cells under normal and hyperglycemic conditions by RNA interference of KEAP1. Later, a myriad of studies focused on the development of highly selective small-molecule activators of NRF2, typically triggering indirect activation of NRF2 *via* the suppression of KEAP1. Tert-butylhydroquinone and sulforaphane, the two most popularly used NRF2 activators, have been shown to alleviate DN in mouse and rat models [52]. With these data, it would be tempting to speculate that KEAP1 knockout animals may be resistant to the development of DN. However, mice with KEAP1 genetic ablation do not survive >3 weeks after birth [53], suggesting that targeting KEAP1 chronically to activate NRF2 may yield unintended side effects. In fact, RTA 405, a synthetic triterpenoid analog of bardoxolone methyl, another KEAP1 inhibitor, aggravated proteinuria, glomerulosclerosis, and tubular damage in Zucker diabetic fatty rats with type 2 diabetes (T2D) [54]. These findings suggest that NRF2 antioxidant response is indeed a great therapeutic target for DN, but KEAP1 blockade-based NRF2 activation may not be the optimal approach. Further studies are merited to explore other therapeutic strategies to harness the beneficial aspects of NRF2 activation for the treatment or prevention of DN.

6. Therapeutic Targeting of NRF2 to Treat DN: Evidence from Clinical Trials

To date, the most extensively studied NRF2 activator in patients with DN is bardoxolone methyl, also referred to as “RTA 402,” “CDDO-methyl ester,” and “CDDO-Me.” This semisynthetic triterpenoid is based on the scaffold of oleanolic acid and is orally bioavailable [53]. Bardoxolone methyl

binds to KEAP1 and inhibits its ubiquitination, which allows for NRF2 nuclear translocation [55, 56]. The earliest sign of the potential renoprotective activity of bardoxolone was revealed in a retrospective analysis of bardoxolone methyl in advanced solid tumors and lymphomas during a phase I first-in-human trial [57]. In this study, there was an overall 26% increase in estimated glomerular filtration rate (eGFR) for all patients. Later, in a multinational, randomized, double-blinded, placebo-controlled phase 2 outcome-trial which enrolled 227 adults with T2D and stage IV chronic kidney disease (CKD) (52-week bardoxolone methyl treatment: renal function in CKD/T2D [BEAM] study), demonstrated rapid improvements in the eGFR (20–45 mL/min/1.73 m²). The changes observed within 4 weeks were largely reversible when the drug was stopped due to the direct hemodynamic effect of this strategy [57]. In recent years, typical interventions seem to delay the decline in renal function to <1 mL/min/1.73 m²/year. However, bardoxolone methyl treatment demonstrated improvements of between 5 and 10 mL/min/1.73 m²/year. Due to the significantly increased hospitalization and increased mortality resulting from heart failure (96 [8.8%] with bardoxolone methyl vs. 55 [5%] to placebo), the BEACON trial was given up [58]. Nevertheless, promising data from bardoxolone trials in other kidney diseases such as Alport Syndrome has rekindled the interest in therapeutic targeting of NRF2 in DN *via* the use of other NRF2 activators or *via* optimizing a combined regimen of bardoxolone with cardiovascular drugs [58].

7. Conclusion

The pathogenesis of DN is likely to be heavily driven by oxidative stress. As a master regulator of antioxidative self-defense in mammalian cells, NRF2 has attracted much interest in the quest for DN therapy. Given the consistent findings in support of a beneficial effect of activating NRF2 *in vitro* and in pre-clinical models of DN, it is conceivable that NRF2 is a promising target for treating or preventing DN. Furthermore, clinical trials of bardoxolone, an NRF2 activator, yielded promising results despite unexpected adverse cardiovascular events. Currently, therapeutic targeting of NRF2 in DN is largely limited to KEAP1 inhibitors. Future studies are warranted to explore KEAP1-independent NRF2 regulatory signaling that may be utilized to harness NRF2 for the treatment and possible prevention of DN while minimizing the potential adverse effects.

Author Contributions

JZ wrote the original draft; JZ and FA contributed to the discussion; JZ, FA, JDB, JL, and JS contributed to the review and editing; and all authors approved the final version of the paper. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

None declared.

Funding Sources

None.

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