INTRODUCTION

Complications of diabetes mellitus (DM) occur in the eyes (retinopathy) (Heng et al., 2013; Gorin et al., 2015; Khan et al., 2017), nerves (neuropathy) (Said, 2007; Vinik et al., 2013) and kidneys (nephropathy) (Thomas et al., 2006; Caramori et al., 2013; Forbes and Cooper, 2013; Thomas et al., 2015). Among these complications, diabetic nephropathy (DN) is the leading cause of renal failure in patients with diabetes mellitus which ultimately results in end-stage renal disease (ESRD) (Molitch et al., 2004; Thomas et al., 2015; Papadopoulou-Marketou et al., 2017). In the initial stage of diabetic nephropathy (DN), commonly observed signs include albuminuria mainly resulting from reduction of the glomerular filtration rate (GFR) (Jha et al., 2016). Increasing albuminuria, reduction of GFR or mesangial expansion, glomerulosclerosis, podocyte apoptosis, and tubulointerstitial fibrosis are well-established features seen in DN progression (Jha et al., 2016). Generally, DN can be divided into multiple stages; (1) increased GFR (hyperfiltration) phase with increased volume of the kidney; (2) normal filtration phase with thickening of the glomerular basement membrane (GBM); (3) microalbuminuria (>30 mg/day); (4) macro-albuminuria (>300 mg/day); (5) uremia with ESRD (Rocco and Berns, 2009; Palatini, 2012; Reidy et al., 2014; Lv et al., 2015). In addition to functional changes, structural changes are also observed; glomerular basement membrane (GBM) thickening, mesangial expansion and overexpression of ECM proteins, tubulointerstitial fibrosis and glomerulosclerosis (Mason and Wahab, 2003; Tervaert et al., 2010; Caramori et al., 2013). Several lines of evidence strongly suggest that oxidative stress serves as an etiological factor in the initiation and progression of DN (Jha et al., 2016). In particular, renal oxidative stress is known to be closely associated with the activation or the overexpression of various NADPH oxidase (Nox) isozymes (Jha et al., 2016). This review will discuss about the function of Nox isozymes in pathology of DN and recent on-going development of Nox inhibitors for treatment of DN.

STRUCTURE AND REGULATION OF NOX ISOZYMES

The gp91phox (renamed as Nox2) was the first identified isozyme, and it was in phagocytic cells. Subsequently, six additional homologues have been isolated in non-phagocytic cells (Nox1, Nox3, Nox4, Nox5, Duox1 and Duox2) (Bae et al., 2002; Bae et al., 2003; Bae et al., 2004; Bae et al., 2005; Bae et al., 2006; Bae et al., 2007; Bae et al., 2008; Bae et al., 2009; Bae et al., 2010; Bae et al., 2011; Bae et al., 2012; Bae et al., 2013; Bae et al., 2014; Bae et al., 2015; Bae et al., 2016; Bae et al., 2017; Bae et al., 2018; Bae et al., 2019; Bae et al., 2020). The gp91phox is composed of two catalytic subunits, Nox2a and Nox2b, and two regulatory subunits, p47phox and p67phox. The Catalytic subunits, composed of three domains: an N-terminal domain (NTD), a transmembrane domain (TMD), and a C-terminal domain (CTD), are responsible for catalysis. The regulatory subunits, composed of an N-terminal domain (NTD), a transmembrane domain (TMD), and a C-terminal domain (CTD), are responsible for regulation. The NTD contains a conserved oxido-reductase domain, which is responsible for catalysis. The TMD contains a transmembrane domain, which is responsible for membrane localization. The CTD contains a regulatory domain, which is responsible for regulation. The p47phox and p67phox are responsible for the activation of the gp91phox. The p47phox is responsible for the activation of the gp91phox, and the p67phox is responsible for the activation of the gp91phox. The gp91phox is activated by the p47phox and p67phox, and the activation of the gp91phox is responsible for the generation of reactive oxygen species (ROS).

Several recent studies have reported that reactive oxygen species (ROS), superoxide anion and hydrogen peroxide (H$_2$O$_2$), play important roles in various cellular signaling networks. NADPH oxidase (Nox) isozymes have been shown to mediate receptor-mediated ROS generation for physiological signaling processes involved in cell growth, differentiation, apoptosis, and fibrosis. Detectable intracellular levels of ROS can be induced by the electron leakage from mitochondrial respiratory chain as well as by activation of cytochrome p450, glucose oxidase and xanthine oxidase, leading to oxidative stress. The up-regulation and the hyper-activation of NADPH oxidases (Nox) also likely contribute to oxidative stress in pathophysiologic stages. Elevation of the renal ROS level through hyperglycemia-mediated Nox activation results in the oxidative stress which induces a damage to kidney tissues, causing to diabetic nephropathy (DN). Nox inhibitors are currently being developed as the therapeutics of DN. In this review, we summarize Nox-mediated ROS generation and development of Nox inhibitors for therapeutics of DN treatment.

Key Words: NADPH oxidase, Oxidative stress, Signal transduction, Kidney, Diabetic nephropathy, Nox inhibitor
All Nox isozymes are single polypeptide enzymes and share common features. Nox isozymes are structurally divided into two functional domains. The long carboxyl terminal region of Nox contains NADPH- and FAD-binding sites homologous to that of ferredoxin-NADP⁺ reductase (FNR) (Segal et al., 1992; Sumimoto et al., 1992). The six transmembrane α-helical domains also found in the carboxyl terminal region bearing tandem heme groups homologous to that in ferric reductase (F₄₅₀) (Fig. 1A) (Finegold et al., 1996). The sequential electron transfer from NADPH to FAD and heme groups stimulates the reduction of oxygen molecules leading to generation of reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide. Among the seven Nox isozymes in human, Nox1, Nox3, and Nox4 have similar structures with Nox2 requiring p22phox integral protein for the activation and formation of Nox complex. Nox5 and Duox1/2 isozymes do not require p22phox integral protein, but contain the EF hand domain responsible for binding of intracellular calcium (Fig. 1B) (Bae et al., 2011).

Nox2 isozyme requires one integral protein, p22phox, multiple cytosolic proteins (p47phox, p40phox, and p67phox) and a small GTPase Rac (Bokoch and Zhao, 2006; Bedard and Krause, 2011).

**Fig. 1.** NADPH Oxidase (Nox) structure and Nox isozymes. (A) General structure of Nox isozyme. Nox contains six transmembrane domains with two heme binding sites and long COOH-terminal region for NADPH binding site and FAD-binding site as prosthetic group. (B) Regulation of Nox isozymes. Nox2-p22⁺⁺⁺⁺ dimer associates p47phox through interaction of SH3 of p47phox with PRR of p22phox. The PRR domain of p47phox serves as the binding site for p67phox. Subsequently, the p67phox protein recruits p40phox and Rac protein. Nox1 also binds to p22phox and requires Nox organizer 1 (NoxO1), Nox activator 1 (NoxA1), and Rac protein. Nox4 requires only p22phox for the activity. Nox5 and Duox 1/2 isozymes contain Ca²⁺-binding EF hand domain at N-terminus region, indicating that these isozymes are regulated by intracellular calcium mobilization.

https://doi.org/10.4062/biomolther.2019.188
Lee et al. (2007; Bae et al., 2011; Jha et al., 2016) for its activity. The formation of Nox2 complex is mediated by sophisticated protein-protein interactions (Fig. 1B). Nox2 tightly associates with the integral protein p22phox whose proline-rich region (PRR) functions as the binding site for the Src homology domain 3 (SH3) of p47phox. This interaction is key to subsequent formation of active Nox2 complex. The p47phox also contains phox (PX) domain in the NH-terminal region, two SH3 domains in the central region, and auto-inhibitory region (AIR) and PRR in the COOH-terminal region. In the resting stage, AIR interacts with tandem SH3 domains to keep intramolecular folding consistent with inactive state. Various serine/threonine kinases including PKC, PKA, MAPK, and Akt phosphorylate the COOH-terminal region of p47phox to interrupt intramolecular SH3-AIR interaction and then to induce the opening of SH3 domain for interaction with PRR of p22phox (Sumimoto et al., 1996; Groemping et al., 2003; Bedard and Krause, 2007). Subsequently, the PRR domain of p47phox serves as the binding site for p67phox (Bae et al., 2011). The p67phox protein contains two SH3 domains in its COOH-terminal region that interacts with the PRR domain of p47phox and four tetratricopeptide repeats (TPR) in the NH-terminal region that functions as the binding site of small G protein Rac. The p67phox protein also contains the PB1 domain between the two SH3 domains which mediates molecular interaction with the PB1 domain of p40phox. Receptor-mediated Nox2 complex formation induces electron transfers among NADPH, FAD and heme group, leading to generation of superoxide anions.

Nox1 was identified as a homologue of Nox2 from non-phagocytic colon epithelium (Suh et al., 1999; Bae et al., 2011). Nox1 also binds to p22phox and requires Nox organizer 1 (NoxO1) and Nox activator 1 (NoxA1) as the homologues and functional counterparts of p47phox and p67phox of Nox2 complex respectively as well as the small G protein Rac (Fig. 1B) (Geiszt et al., 2003; Kawahara et al., 2005; Cheng et al., 2006). NoxO1 contains two SH3 domains in the central region for interaction with the PRR domain of p22phox and one PRR domain in the COOH-terminal region for binding to the SH3 domain of NoxA1. It has generally been assumed that the Nox1 complex is constitutively active because a domain homologous to the AIR domain of p47phox is missing in NoxO1 (Sumimoto, 2008; Dutta and Rittinger, 2010). However, it has been reported that Nox1 activity is transiently increased in response to various agonists including EGF (Lassegue et al., 2001; Park et al., 2006; Schroder et al., 2007; Choi et al., 2008; Lee et al., 2009, 2013), suggesting that an additional mechanism is in operation for the regulation of Nox1 activity. Joo et al. (2016) reported a molecular regulatory process in which Casitas B–lineage lymphoma (Cbl) E3 ligase is recruited to Nox1 complex through the interaction between NoxO1 and growth receptor–bound protein 2 (Grb2) leading to the disassembly of Nox1 complex and a subsequent reduction in

Fig. 2. Distribution of Nox isozymes and its components in the kidney.
ROS generation upon epidermal growth factor (EGF) stimulation.

The primary structure of Nox3 shares 56% homology with that of Nox2 (Kikuchi et al., 2000; Jha et al., 2016). Nox3 also requires p22phox and NoxO1 for the activation (Fig. 1B). However, the molecular mechanism for Nox3 complex formation remains to be elucidated (Banfi et al., 2004a; Cheng et al., 2004; Ueno et al., 2005; Ueyama et al., 2006; Bedard and Krause, 2007). Nox4 was initially identified from kidney and requires only p22phox for the activity (Fig. 1B). However, no cytosolic protein regulating Nox4 activity has been reported. Many reports suggested that Nox4 seems to be constitutively active and Nox4 activity is dependent largely on its expression level in cells. However, Nox4 isozyme activity is regulated by receptor-mediated cell signaling networks, suggesting that unknown protein or proteins regulate Nox4 complex formation and stimulates its activity (Bedard and Krause, 2007; Leto et al., 2009; Block and Gorin, 2012; Lassegue et al., 2012; Lambeth and Neish, 2014). Poldip2 (polymerase delta-interacting proteins2), a p22phox interacting protein, has been identified as a positive regulator for Nox4 activity in SMC migration (Lyle et al., 2009). However, molecular mechanisms by which poldip2 associates and interacts with Nox4-p22phox complex remain to be determined.

Unlike Nox1-4 isozymes, Nox5 does not require p22phox or cytosolic accessory proteins for activation. Given that Nox5 isoyme contains long intracellular NH3-terminus region containing Ca2+-binding EF hand domain, Nox5 isozyme is likely regulated by intracellular calcium mobilization (Fig. 1B) (Banfi et al., 2001, 2004b). As in Nox5, the EF-hand domain is localized in the NH3-terminus region of Duox 1/2 isozymes and functions as the binding site of intracellular calcium (Fig. 1B) (Ameziane-El-Hassani et al., 2005; Bae et al., 2011). Recently, we reported that testosterone binds to GPRC6A located in plasma membrane and subsequently induces the activation of Gq protein, IP3 generation, and [Ca2+]i mobilization ultimately leading to Duox1 activation (Ko et al., 2014).

**EXPRESSION OF NOX ISOZYMES IN KIDNEY CELLS**

It is now clear that multiple Nox isozymes including Nox1, Nox2, Nox4 and Nox5 (in case of human genomes) and their accessory proteins are expressed in kidney tissues (Fig. 2). Together, these isozymes represent one of the major sources of ROS in normal and pathological states of kidney (Jha et al., 2014; Holterman et al., 2015; Cha et al., 2017). The pathological states of kidney in particular includes diabetic nephropathy and fibrosis in which the major function of kidney, elimination of metabolic wastes in blood through glomerular filtration, is severely compromised (Jha et al., 2016). The renal vessels are composed of vascular smooth muscle cells (VSMCs) and

![Diagram](https://doi.org/10.4062/biomolther.2019.188)
endothelial cells (ECs) expressing Nox1 and Nox4 which are responsible for ROS generation in response to angiotensin II (Fig. 2) (Wingler et al., 2001). Glomerulus plays an important role in maintaining kidney function and is mainly composed of mesangial cells and podocytes. Many reports have shown that upregulation of Nox1, Nox2, Nox4, and Nox5 and the regulatory subunits p22phox, p47phox and p67phox contributes to mesangial cell hypertrophy, tissue expansion, ECM protein accumulation and podocyte apoptosis in glomerulonephritis (Jha et al., 2016). Proximal tubule cells express Nox1, Nox2, Nox4 and Nox5 isoforms (Yang et al., 2012; Yu et al., 2014; Holterman et al., 2015), and macula densa expresses Nox2 and Nox4 as well as p47phox, p67phox and p22phox (Fig. 2) (Holterman et al., 2015).

**ACTIVATION OF NOX IN DIABETIC NEPHROPATHY**

High glucose (HG)-induced kidney damages are associated with several metabolic risk factors (Fig. 3). Activation of renin-angiotensin-aldosterone pathway, activation of PKC, production of advanced glycation end products (AGEs) and TGFβ-induced fibrosis have been reported as high risk factors for the initiation and progression of DN. A high HG level induces increased generation of advanced glycation end products (AGEs) which serve as ligands for cellular receptor RAGE, transducing signals for inflammation (Schmidt et al., 1995; Hofmann et al., 1999). AGEs from ECM fragments including protein and lipid species thereby lead to structural changes in kidney tissues (Silbiger et al., 1993; Forbes et al., 2003). Activation of signaling networks by AGE stimulates nuclear factor-kB (NF-kB) activity which induces the generation of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Neumann et al., 1999). AGEs are also considered as potent stimulators for VEGF expression and PKC activation, leading to upregulation of TGFβ1 expression, which in turn is causally linked to expression of ECM protein for mesangial expansion (Osicka et al., 2000; Lal et al., 2002; Singh et al., 2014). Activation of TGFβ1 also stimulates Nox4 expression (Fig. 3) (Jiang et al., 2014). Importantly, knockdown of Nox4 expression suppressed TGFβ1-dependent fibrotic markers including fibronectin, collagen I, and α-smooth muscle actin (Jiang et al., 2014). Moreover, Nox inhibitor treatment inhibited TGFβ-induced Smad2/3 activation and then suppressed expression of marker proteins for fibrosis, indicating that Nox-dependent ROS generation may have critical roles in TGFβ1-mediated fibrosis in kidney tissues (Jiang et al., 2014).

Activation of protein kinase C (PKC) is involved in hyperglycemia-dependent nephropathy. HG-RAGE-phospholipase Cβ (PLC) pathway and angiotensin-angiotensin receptor II-PLCβ1 pathway can stimulate DAG generation and intracellular calcium mobilization through hydrolysis of phosphatidylinositol 4,5, bisphosphate (PIP2). Elevated levels of intracellular calcium and DAG activate renal PKC affecting vascular functions through inducing changes in endothelial permeability, vasoconstriction, extracellular matrix (ECM) maintenance, cytokine activation and leukocyte adhesion (Fig. 3). Of interest, PKC inhibition ameliorates structural and functional abnormalities in the kidney in db/db mice (Koya et al., 2000). Importantly, HG-induced translocation of PKCα to renal membranes stimulates Nox2 activation. In type I diabetic mice, treatment of PKCα inhibitor (Ro-32-0432) attenuates Nox-dependent superoxide production. Renal extracellular matrix accumulation of fibronectin and collagen IV is decreased by Nox inhibitor apocynin (Thallas-Bonke et al., 2008). These reports together suggest that Nox2 activation by PKCα is the key downstream event of the AGE-receptor signaling in diabetic nephropathy.

Recently, oxidative stress has come to be recognized as a key player in the pathogenesis of DN. Most ROS produced intracellularly originate from the mitochondrial respiratory chain, and they certainly can bring damages in renal tissues when mitochondria become dysfunctional. Relatively small amounts (1-2%) of total O2 consumed during mitochondrial respiration are normally converted to ROS (Chance et al., 1979; Kudin et al., 2004). However, the formation of ROS is increased from dysfunctional mitochondria in hyperglycemia stage. ROS accumulation can lead to oxidative stresses in the cell, leading to damages of cellular components including protein and DNA. Ultimately, the oxidative stress leads to renal fibrosis and decline in renal function (Badal and Danesh, 2014). ROS mediate activation of a number of pathways including PKC, NF-kB, hexosamine synthesis and AGE (Nishikawa et al., 2000).

As has been described above, ROS can also be produced by activities of Nox isozymes. Many reports have indicated that HG, angiotensin II or TGFβ stimulate Nox4-derived ROS generation, resulting in fibrotic response in mesangial cells, podocytes and tubular cells (Jha et al., 2016). The pathogenic process leading to fibrosis has been studied with Nox4-deficient mice (Jha et al., 2014; Gorin et al., 2015). Interestingly, treatment of Nox inhibitors (GKT137831 or APX-115) resulted

---

**Table 1. NADPH oxidase inhibitors**

| Compound     | Mechanism of action       | Chemical name                                                                 | Chemical structure | Target                              | References                                      |
|--------------|---------------------------|-------------------------------------------------------------------------------|--------------------|-------------------------------------|------------------------------------------------|
| GKT137981    | Dual Nox1/4 inhibitor     | 2-(2-Chlorophenyl)-4-(3-dimethylamino)phenyl)-5-methyl-1H-pyrazolo[4,3-c]     |                    | Liver fibrosis, kidney fibrosis, lung fibrosis | Laleu et al., 2010; Gaggini et al., 2011; Aoyama et al., 2012; Green et al., 2012; Jha et al., 2014; Gorin et al., 2015; Jha et al., 2016 |
| APX-115      | Pan-Nox inhibitor         | 3-Phenyl-4-propyl-1-(pyridin-2-yl)-1H-pyrazol-5-oxyhydrochloride              |                    | Diabetic Nephropathy                | Cha et al., 2017; Kwon et al., 2017; Dorotea et al., 2018 |
in resistance to DN progression as evidenced by reduction in ECM accumulation and albuminuria. In addition, inflammation events in Nox4 deficient renal cells were accompanied by reduced levels of NF-κB activity and subsequently of chemokines and MCP-1 and in renal tubular cell or podocyte in response to HG stimulation (Xu et al., 2010; Jha et al., 2014).

**DEVELOPMENT OF NOX INHIBITOR FOR THE TREATMENT OF DN**

Oxidative stress plays an important role in DN, and clinical trials for therapeutic intervention for reduction of such oxidative stress are ongoing. That Nox isozymes are sources of oxidative stress for kidney is now well-established. Therefore, it is tempting to speculate that Nox isozymes will be viable therapeutic targets for DN treatment. Nox isozymes are expressed in various renal cells; Nox4 in EC, Nox1 in SMC, Nox1, Nox2, Nox4, and Nox5 in glomeruli. Nox4 and Nox1 in tubule cells. Of importance, studies with diabetic nephropathy animal models with Nox gene mutation suggest that pan-Nox inhibitors are more effective rather than isozyme-specific inhibitors.

**GKT137831 (Setanaxib)**

Pyrazolopyridine compound, a dual inhibitor of Nox1 and Nox4 was developed by Genkyoext. GKT136901 was the lead compound identified from a high-throughput screening (Table 1) (Laleu et al., 2010). GKT137831 compound as a Nox1/4 inhibitor was subsequently obtained from a structure-activity relationship study of GKT136901. GKT137831 has been applied to various disease models. These preclinical studies showed that GKT137831 have therapeutic potential in fibrotic and inflammatory diseases of the liver (Aoyama et al., 2012), lung (Green et al., 2012) and other organs as well as kidney. According to the preclinical data, GKT137831 attenuated various parameters of glomerular injury, such as ECM accumulation, glomerular structural changes and albuminuria in streptozotocin-induced diabetic ApoE−/− mice (Jha et al., 2014). In another study, application of GKT137831 significantly reduced mesangial matrix expansion, urinary albumin excretion, and podocyte loss in OVE mice as a type I diabetes model (Gorin et al., 2015). GKT137831 is not only a dual inhibitor for the Nox1 and Nox4 isozymes but also a partial inhibitor of Nox5 (Gaggini et al., 2011; Jha et al., 2016). GKT137831 is now enrolled in a phase 2 clinical trial in primary biliary cholangitis (PBC, a fibrotic orphan disease) and type I diabetes and kidney disease (DKD).

**APX-115**

APX-115 is first-in-class pan Nox inhibitor developed by Aptabio (Table 1). The Apx-115 compound protects against diabetic nephropathy in type II diabetic model db/db mice. Specifically, treatment of APX-115 significantly improved insulin resistance in diabetic mice. Oxidative stress as measured by plasma 8-isoprostane level was decreased by the APX-115 treatment. APX-115 also suppressed urinary albumin excretion and preserved creatinine clearance in db/db mice. In diabetic kidneys, APX-115 significantly suppressed mesangial expansion. Comparative study showed that APX-115 is more effective than dual Nox1/4 inhibitor GKT137831 (Cha et al., 2017). Another study compared APX-115 and Losartan (renin-angiotensin system inhibitor) in Streptozotocin (STZ)-induced type I diabetic mouse. APX-115 inhibited renal oxidative stress and prevented albuminuria, glomerular hypertrophy, tubular injury, podocyte injury, fibrosis and inflammation in diabetic kidneys as effectively as losartan alone which is currently used as the standard treatment against kidney injuries in diabetic patients (Kwon et al., 2017). Moreover, treatment with APX-115 in STZ-induced diabetic rat was also as effective as losartan (Dorolea et al., 2018). APX-115 is more effective than GKT137831 based on inflammation or fibrosis. This suggests that pan-Nox inhibitors are more effective therapeutics for DN treatment than Nox isozyme-specific inhibitors. This is not surprising given that multiple Nox isozymes are expressed in overlapping manners in multiple cell types of kidney, which all appear to be affected by ROS one way or another. Of note, APX-115 treatment decreased expression of all Nox isozyme expression at the protein level in the kidney. Pan-Nox inhibition might therefore be a promising therapy for diabetic nephropathy.

**CONCLUSION**

Currently, no effective therapy exists for treatment of DN. Based on the damage ROS brings to kidney during pathogenesis of DN, Nox isozymes have emerged as promising targets. It should be noted that chronic hyperglycemia in diabetes stimulates the expression and activation of Nox isozymes in renal tissues leading to a sustained oxidative stress. There are various pathways that may contribute to stimulation of Nox-dependent ROS generation. Therefore, Nox isozymes in renal tissues should be effective therapeutic targets for DN treatment. Now, two Nox inhibitors (GKT137831 and APX-115) are enrolled in clinical phase II for DN patients. These Nox inhibitors may represent best hope to date to many DN patients with no other recourse.

**ACKNOWLEDGMENTS**

This work was supported by Aging project (2017M3A9D 8062955 to YSB) and Bio-SPC (2018M3A9G1075771 to YSB) funded by the National Research Foundation of Korea (NRF) and Ministry of Science and ICT.

**REFERENCES**

Ameziane-El-Hassani, R., Morand, S., Boucher, J. L., Frapart, Y. M., Apostolou, D., Agnandji, D., Gnidehou, S., Chayon, R., Noel-Hudson, M. S., Francon, J., Lalaoui, K., Vinion, A. and Dupuy, C. (2005) Dual oxidase-2 has an intrinsic Ca2+-dependent H2O2-generating activity. *J. Biol. Chem.* 280, 30046-30054.

Aoyama, T., Paik, Y. H., Watanabe, S., Laleu, B., Gaggini, F., Fioraso-Cartier, L., Molango, S., Heitz, F., Merlot, C., Szynadlewiez, C., Page, P. and Brenner, D. A. (2012) Nicotinamide adenine dinucleotide phosphate oxidase in experimental liver fibrosis: GKT137831 as a novel potential therapeutic agent. *Hepatology* 56, 2316-2327.

Badal, S. S. and Danesh, F. R. (2014) New insights into molecular mechanisms of diabetic kidney disease. *Am. J. Kidney Dis.* 63, S63-S83.

Bae, Y. S., Oh, H., Rhee, S. G. and Yoo, Y. D. (2011) Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells* 32, 491-509.

Banfi, B., Malgrange, B., Knisz, J., Steger, K., Dubois-Dauphin, M. and...
Lee, J. H., Joo, J. H., Kim, J., Lim, H. J., Kim, S., Curtiss, L., Seong, J. K., Cui, W., Yabe-Nishimura, C. and Bae, Y. S. (2013) Interaction of NADPH oxidase 1 with Toll-like receptor 2 induces migration of smooth muscle cells. Cardiovasc. Res. 99, 483-493.

Lee, M. Y., San Martin, A. and Griendling, K. K. (2009) Mechanisms of vascular smooth muscle NADPH oxidase 1 (Nox1) contribution to injury-induced neointimal formation. Arterioscler. Thromb. Vasc. Biol. 29, 480-487.

Lelo, T. L., Morand, S., Hurt, D. and Ueyama, T. (2009) Targeting and regulation of reactive oxygen species generation by Nox family NADPH oxidases. Antioxid. Redox Signal. 11, 2607-2619.

Lv, M., Chen, Z., Hu, G. and Li, Q. (2015) Therapeutic strategies of diabetes. Therapeutic targets of diabetes. 781-790.

Lyle, A. N., Deshpande, N. N., Taniyama, Y., Seidel-Rogol, B., Pounkos, L. D., Poushoney, L. D., Papaharakambis, C., Lassegue, B. and Griendling, K. K. (2009) Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. Circ. Res. 105, 249-259.

Mason, R. M. and Wahab, N. A. (2003) Extracellular matrix metabolism in diabetic nephropathy. J. Am. Soc. Nephrol. 14, 1358-1373.

Molitch, M. E., DeFronzo, R. A., Franz, M. J., Keane, W. F., Mogensen, C. E., Parving, H. H., Stoffers, M. W. and American Diabetes, A. (2004) Nephropathy in diabetes. Diabetes Care 27, S79-S83.

Neumann, A., Schinzel, R., Palm, D., Riederer, P. and Munch, G. (1999) High molecular weight hyaluronic acid inhibits advanced glycation endproduct-induced NF-kappaB activation and cytokine expression. FEBS Lett. 453, 283-287.

Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kaneeda, Y., Yone, M. A., Beek, D., Oates, P. J., Hammes, H. P., Giardino, I. and Brownlee, M. (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic nature. Diabetes 404, 787-790.

Osicka, T. M., Yu, Y., Panagiotopoulos, S., Clavent, S. P., Kriazis, Z., Pike, R. N., Pratt, M. L., Russo, L. M., Kemp, B. E., Comper, W. D. and Jerums, G. (2000) Prevention of albuminuria by am. J. Kidney Dis. 34, 985-990.

Reidy, K., Chang, H. M., Hostetter, T. and Susztak, K. (2014) Molecular mechanisms of diabetic kidney disease. J. Clin. Invest. 124, 2333-2340.

Rocco, M. V. and Bems, J. S. (2009) KDQO1 in the era of global guidelines. Am. J. Kidney Dis. 54, 781-787.

Said, G. (2007) Diabetic neuropathy—a review. Nat. Clin. Pract. Neurol. 3, 331-340.

Schmidt, A. M., Horii, O., Chen, J. X., Li, J. F., Crandall, J., Zhang, J., Cao, R., Yan, S. D., Brett, J. and Stern, D. (1995) Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. J. Clin. Invest. 96, 1395-1403.

Schorer, K., Helmcke, I., Palfi, K., Krause, K. H., Busse, R. and Brandes, R. P. (2007) Nox1 mediates basic fibroblast growth factor-induced migration of vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 27, 1736-1743.

Segal, A. W., West, I., Wijnenjes, F., Nugent, J. H., Chavan, A. J., Haley, B., Garcia, R. C., Rosen, H. and Scrase, G. (1992) Cytochrome b-245 is a flavocytochrome containing FAD and the NADPH-binding site of the microsomal oxidase of phagocytes. Biochem. J. 284, 781-788.

Silbiger, S., Crowley, S., Shan, Z., Brownlee, M., Satiriano, J. and Schlundorf, D. (1993) Nonenzymatic glycation of mesangial matrix and prolonged exposure of mesangial matrix to elevated glucose reduces collagen synthesis and proteoglycan charge. Kidney Int. 43, 853-864.

Singh, V. P., Bari, A., Singh, N. and Jaggi, A. S. (2014) Advanced glycation end products and diabetic complications. Korean J. Physiol. Pharmacol. 18, 1-14.

Suh, Y. A., Arnold, R. S., Lassegue, B., Shi, J., Xu, X., Sorescu, D., Chung, A. B., Griendling, K. K. and Lambeth, J. D. (1999) Cell transformation by the superoxide-generating oxidase Nox1. Nature 401, 79-82.

Sumimoto, H. (2008) Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J. 275, 3984.

Sumimoto, H., Hata, K., Mizuki, K., Ito, T., Kage, Y., Sakaki, Y., Fukumaki, Y., Nakamura, M. and Takeshige, K. (1996) Assembly and activation of the phagocyte NADPH oxidase. Specific interaction of the N-terminal Src homology 3 domain of p47phox with p22phox is required for activation of the NADPH oxidase. J. Biol. Chem. 271, 22152-22158.

Sumimoto, H., Sakamoto, N., Nozaki, M., Sakaki, Y., Takeshige, K. and Minakami, S. (1992) Cytochrome b558, a component of the phagocyte NADPH oxidase, is a flavoprotein. Biochem. Biophys. Res. Commun. 186, 1368-1375.

Tervaert, T. W., Mooyaart, A. L., Amann, K., Cohen, A. H., Cook, H. T., Drachenberg, C. B., Ferraro, F., Fogo, A. B., Haas, M., de Heer, E., Joh, K., Noel, L. H., Radhakrishnan, J., Sehgal, S. V., Bajema, I. M., Buijin, J. A. and Renal Pathology, S. (2010) Pathologic classification of diabetic nephropathy. J. Am. Soc. Nephrol. 21, 556-563.

Thallas-Bonke, V., Thorge, S. R., Coughlan, M., Fukumi, Y., Yapi, F. Y., Sourcis, K. C., Penfold, S. A., Bach, L. A., Cooper, M. E. and Forbes, J. M. (2008) Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alpha-dependent pathway. Diabetes 57, 460-469.

Thomas, M. C., Brownlee, M., Susztak, K., Sharma, K., Jandelet-Dahm, K. A., Zoungas, S., Rossing, P., Groop, P. H. and Cooper, M. E. (2015) Diabetic kidney disease. Nat. Rev. Dis. Primers 1, 15018.

Thomas, M. C., Weekes, A. J., Broadley, O. J., Cooper, M. E. and Mathew, T. H. (2006) The burden of chronic kidney disease in Aus- tralian patients with type 2 diabetes (the NEFRON study). Med. J. Aust. 185, 140-144.

Ueno, N., Takeya, R., Miyano, K., Kikuchi, H. and Sumimoto, H. (2005) The NADPH oxidase Nox3 constitutively produces superoxide in a p22phox-dependent manner: its regulation by oxidase organizers and activators. J. Biol. Chem. 280, 23328-23339.

Ueyama, T., Geiszl, M. and Leto, T. L. (2006) Involvement of Rac1 in activation of multicomponent Nox1- and Nox3-based NADPH oxidi- sases. Mol. Cell. Biol. 26, 2160-2174.

Vinik, A. I., Nevoret, M. L., Casellini, C. and Parson, H. (2013) Diabetic nephropathy. Endocrinol. Metab. Clin. North Am. 42, 747-787.

Wingler, K., Wunsch, S., Kreutz, R., Rothermund, L., Paul, M. and Schmidt, H. H. (2001) Upregulation of the vascular NADPH-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. Free Radic. Biol. Med. 31, 1456-1464.

Xu, Y., Ruan, S., Xie, H. and Lin, J. (2010) Role of LOX-1 in Ang II-induced oxidative functional damage in renal tubular epithelial cells. Int. J. Mol. Med. 26, 679-690.

Yamaoka, H., Zhang, Y., Cuzzi, M., Villar, V. A., Escano, C., L. D. A., Yu, P., Grandy, D. K., Felder, R. A., Armando, I. and Jose, P. A. (2012) Paroxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-
induced inhibition of NADPH oxidase. *Free Radic. Biol. Med.* **53**, 437-446.
Yu, P., Han, W., Villar, V. A., Yang, Y., Lu, Q., Lee, H., Li, F., Quinn, M., Gildea, J. J., Felder, R. A. and Jose, P. A. (2014) Unique role of NADPH oxidase 5 in oxidative stress in human renal proximal tubule cells. *Redox Biol.* **2**, 570-579.