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EDITORIAL

Diabetic nephropathy and transcription factors

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Diabetic nephropathy (DN) is a major microvascular complication of diabetes mellitus (DM) and is an important cause of increased morbidity and mortality in patients with DM. Multiple factors, including an interaction between hyperglycemia-induced metabolic and hemodynamic changes [1] and genetic predisposition [2], have been shown to contribute to the development of DN. Currently, the mainstay of therapy for DN is improving glucose control and lowering systemic blood pressure with renin–angiotensin system inhibitors. However, despite those therapeutic regimens, the prevalence of DN has been increasing, and novel strategies are urgently needed.

DN is primarily glomerular injury, which is histologically defined as glomerular basement membrane thickening and excess accumulation of mesangial extracellular matrix proteins, although tubular injury or tubulointerstitial fibrosis is also a prominent component of the disease [3, 4]. Hyperglycemia present in the diabetic milieu induces a number of pathways, such as the activation of protein kinase C [5], accumulation of inflammatory mediators or growth factors [6] and advanced glycation end products (AGEs) [7], and the generation of reactive oxygen species (ROS) [8], in a variety of cell types in the kidney. Nevertheless, knowledge of the mechanism of gene regulation that elicits the biological response to glucose-mediated biochemical derangements is lacking. Recently, investigations in mesangial cells and in glomerular cells in vitro and in vivo have highlighted transcription factors that participate in the regulation of gene expression in diabetic circumstances.

For example, the Smad family of proteins, including Smad1 and Smad3, are activated in response to stimuli such as transforming growth factor beta (TGF-β) and AGEs to upregulate the production of extracellular matrix proteins by mesangial cells at the transcriptional level [9–11]. The transcription factor NF-κB is activated by cellular stimulation with ROS or cytokines induced by high glucose, and contributes to microinflammation of glomeruli [12]. STAT1 and STAT3, which are activated by high glucose or by increased angiotensin II in glomerular mesangial cells, are implicated in the production of extracellular matrix proteins and control of cellular growth [13].

Diverse cellular signaling pathways may converge on a transcription factor, and transcriptional dysregulation in the diabetic kidney can occur at multiple levels, including alterations in upstream signals or the transcription factor itself. In this respect, transcription factors are the final effector proteins at the site of gene regulation to mediate the cellular responses to pathogenic factors in diabetes. Therefore, transcription factors may represent attractive therapeutic targets that could circumvent many of the problems with signal redundancy and crosstalk that are often experienced in therapies targeting upstream of the cellular signals.

Despite the apparent progress in this field of research, difficulties in developing strategies for "drugging" transcription factors have been encountered. Some transcription factors, such as estrogen receptor in endocrine therapy for cancers [14] and glucocorticoid receptor in anti-inflammation or immunosuppression therapy [15], are directly regulated by, e.g., synthetic steroid ligands. For the other transcription factors, however, indirect targeting designs are available: decoy oligonucleotides to disrupt

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STAT3– or NF-κB–DNA interactions [16, 17], quadruplex stabilizer in c-Myc regulation [18], and small interfering RNA or antisense oligonucleotide technology for many other factors [19]. Since the issue of the delivery of the drug or the molecule is important in a small nucleic acid based strategy, more realistic options for targeting transcription factors are required.

Recently, it was reported that high glucose induces *O*-GlcNAcylation of carbohydrate response element binding proteins (ChREBP) in rat mesangial cells [20]. ChREBP is a basic helix–loop–helix/leucine zipper transcription factor that is expressed in several metabolically relevant tissues, including liver, adipocytes, pancreatic β-cells, and certain cancer cells, and is a mediator of glucose-responsive gene activation [21–23]. Upon activation by glucose, ChREBP translocates from the cytosol into the nucleus, thereby forming a heterodimer with Max-like protein X (Mlx) that binds to the carbohydrate response element (ChRE) for transcriptional regulation of genes associated with glucose, lipid, fatty acid, and steroid metabolism [24]. We previously reported that, in response to high glucose, ChREBP upregulates hypoxia-inducible transcription factor (HIF)-1α mRNA expression via direct binding to the HIF-1α gene promoter in diabetic mesangial cells [25]. Upregulated HIF-1α in turn induces its target genes, such as connective tissue growth factor and plasminogen activator inhibitor-1, promoting extracellular matrix accumulation in diabetic glomeruli. The discovery of the ChREBP–HIF-1α axis demonstrated a previously unknown role of HIF-1α in diabetic glomerulopathy and provided a mechanism for the diversity of glucose signal outputs that leads to diabetic kidney injury [25]. In addition, we found that ChREBP transcriptionally upregulates platelet-derived growth factor C for the production of type IV and VI collagens in mesangial cells (unpublished observation). *O*-GlcNAcylation of ChREBP in mesangial cells, induced either by high glucose or the drug *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNAc), augments protein stability, transcriptional activity, and nuclear translocation of ChREBP, resulting in the upregulation of ChREBP targets including HIF-1α and genes downstream [20]. Importantly, 6-diazo-5-oxonorleucine (DON), a drug that decreases *O*-GlcNAcylation, mitigated HIF-1α induction and fibrotic response via *O*-GlcNAcylated ChREBP in mesangial cells in high ambient glucose [20]. Although there are concerns over cell-specific drug delivery, it is worth noting that pharmacological modification of ChREBP has emerged in anticipation of it becoming a therapeutic strategy for DN.

Another example of the evolving therapeutic tactics employed for DN is the augmentation of antioxidant defenses by upregulation of the Nrf2/Keap1 pathway [26]. Nrf2 is a transcription factor that controls the expression of cytoprotective genes harboring the antioxidant response element (ARE) in their promoters. Nrf2 is latently sequestered in the cytoplasm by the inhibitory protein Keap1. Upon cellular exposure to oxidative or electrophilic stress, Nrf2 dissociates from Keap1 and migrates into the nucleus, where it heterodimerizes with other transcription factors such as small Maf proteins and then binds to ARE in the gene promoter. Nrf2 activates the transcription of genes encoding antioxidant enzymes and phase II detoxifying enzymes, thereby eliciting protection against cellular threats such as oxidative stress and inflammation that are common in diabetic milieu [26]. Indeed, Nrf2-knockout mice were observed to suffer severe streptozotocin-induced diabetic kidney complications [27]. Moreover, pharmacological or dietary activation of Nrf2 resulted in the amelioration of kidney damage in a diabetic mouse model through the reduction of oxidative damage or ROS in those animals [28]. A good illustration of the impact of an Nrf2 activator in the treatment of DN was provided by a recent phase 2 clinical trial in which bardoxolone methyl, a potent synthetic triterpenoid that activates Nrf2, was administered to patients with type 2 diabetes and chronic kidney disease [29]. Estimated GFR, blood pressure, and urinary albumin-to-creatinine ratio increased significantly while body weight decreased significantly in the bardoxolone methyl group as compared with the placebo group, indicating a promising positive effect of Nrf2-targeted therapy for DN [29]. Unexpectedly, however, a phase 3 clinical trial of bardoxolone methyl was terminated prematurely due to unexplained adverse cardiovascular events. That said, the potentially valuable properties of bardoxolone methyl in the treatment of diabetes and chronic kidney disease has motivated the organization of a new domestic phase 2 clinical trial of this Nrf2 activator in Japan.

In conclusion, progress is being made in research aiming at the modulation of transcription factors with potential clinical benefits, although the issue of druggability must still be resolved. Precisely targeting transcription factors may minimize the redundancy of mechanisms downstream of complex signaling pathways, thus potentially improving therapeutic approaches. The development of new concepts and technology for targeting transcription factors is needed to create novel therapies for DN.

Compliance with ethical standards

Conflict of interest statement M. Haneda received lecture fees from Boehringer Ingelheim GmbH, Mitsubishi Tanabe Pharma Corporation, Novo Nordisk Pharma Ltd., Taisho Pharmaceutical Co., Ltd., Sanofi K.K., Astellas Pharma Inc., Kowa Pharmaceutical Co., Ltd., Novartis Pharma K.K., AstraZeneca K.K., Ono Pharmaceutical Co., Ltd., and Taisho Toyama Pharmaceutical Co., Ltd.
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Y. Makino declares that he has no conflict of interest.

Ethics policy This article does not report any studies with human or animal subjects that were performed by any of the authors.

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