The physiological and pathophysiologica roles of carbohydrate response element binding protein in the kidney

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Abstract. Glucose is not only the energy fuel for most cells, but also the signaling molecule which affects gene expression via carbohydrate response element binding protein (ChREBP), a Mondo family transcription factor. In response to high glucose conditions, ChREBP regulates glycolytic and lipogenic genes by binding to carbohydrate response elements (ChoRE) in the regulatory region of its target genes, thus elucidating the role of ChREBP for converting excessively ingested carbohydrates to fatty acids as an energy storage in lipogenic tissues such as the liver and adipose tissue. While the pathophysiological roles of ChREBP for fatty liver and obesity in these tissues are well known, much of the physiological and pathophysiological roles of ChREBP in other tissues such as the kidney remains unclear despite its high levels of expression in them. This review will thus highlight the roles of ChREBP in the kidney and briefly introduce the latest research results that have been reported so far.

Key words: Carbohydrate response element binding protein (ChREBP), Thioredoxin-interacting protein (TXNIP), Diabetic nephropathy (DN), Diabetes mellitus (DM)

Introduction

Glucose is one of the most fundamental nutrients for most cells [1]. Once the ingested glucose is absorbed from the small intestine into the bloodstream, it is delivered to the various organs of the body. After glucose is taken up by the cells, it undergoes a series of enzymatic reactions as it is metabolized via glycolysis and is either used for ATP synthesis or stored as triglyceride/glycogen in the cells. Along with its role as a basic nutrient, glucose also has the role of regulating gene expression itself [1-3]. This means that glucose is not only the most important energy source for the cells, but also functions as a kind of signaling molecule. The transcription factor which mediates the regulation of gene expression by glucose is carbohydrate response element binding protein (ChREBP), a Mondo family transcription factor.

The ChREBP transcription factor was initially isolated from rat liver nuclear extracts as a protein that binds to the carbohydrate response element (ChoRE) of L-type pyruvate kinase gene (PKLR, coding L-PK protein) in 2001 by Yamashita et al. [4]. ChREBP is known to be highly expressed in metabolic tissues such as the liver, adipose tissue, pancreas, and kidney, and plays an important role in homeostasis by regulating the expression of genes related to glucose and lipid metabolism in response to glucose [5]. The major target genes include the glycolytic enzyme PKLR and the lipid synthetic enzymes like fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) [4-7]. It is thus believed that the main physiological role of ChREBP is to convert excessively ingested carbohydrates to fatty acids as an energy storage. When the cells take up glucose, ChREBP, which remains in the cytoplasm, recognizes glucose or their metabolites and is activated. Activated ChREBP is then translocated to the nucleus and binds to ChoRE together with its heterodimeric partner Mlx to induce the transcription of its cognate target genes [8-11] (Fig. 1). There are several controversial models for the mechanism of recognition of glucose or its metabolites by ChREBP. In one theory, it is proposed that the dephosphorylation of ChREBP by xylulose 5-phosphate-activated protein...
phosphatase 2A (PP2A) is involved in ChREBP nuclear translocation [12]. In another theory, it is proposed that glucose metabolites, such as glucose-6-phosphate and fructose 2,6 bisphosphate, directly bind to ChREBP, thereby altering the three-dimensional structure of ChREBP and activating it [13-15]. In addition, the transcriptional activity regulation by posttranslational modification, such as O-linked N-acetylglucosamine (GlcNAc) transferase (OGT)-mediated O-GlcNAcylation and p300-mediated acetylation, have also been reported [16-21].

In addition to the above mentioned mechanisms, the discovery of a ChREBP-β isoform in adipocytes in 2012 provided an opportunity to further understand the complexity of the regulatory mechanism of ChREBP activation [22]. This isoform lacking the N-terminal inhibitory region had constitutive transcriptional activation ability and showed robust transcriptional activity as compared with the α isoform. As ChREBP-β is induced by the ChREBP-α isoform (a canonical ChREBP isoform) through ChoRE in the alternative ChREBP-β promoter, it forms a positive feedback loop and is believed to be a surrogate marker for ChREBP activity. Recently, however, Recazens et al. reported that ChREBP-β deficiency showed limited impact on gene expression, glucose homeostasis, and energy balance in mice [23]. Thus, the physiological role of ChREBP-α might be predominant over that of ChREBP-β in vivo.

Due to the role of ChREBP as a “thrifty gene” that stores energy as fat [24], many studies have been conducted on it from the viewpoint of metabolic regulation in main lipogenic tissues, such as liver and adipose tissue. On the other hand, much of the physiological roles of ChREBP besides those in such tissues remains unclear despite its high levels of expression in other tissues such as the kidney. Furthermore, activation mechanisms of ChREBP have been studied mainly by using mouse liver tissue or liver-derived cells, and it is still unclear whether it is also applicable in the kidney or other tissues. Therefore, in this review, we will examine the role of ChREBP in the kidney, and briefly summarize the latest research results that have been reported so far. See other reviews published elsewhere for a comprehensive picture of ChREBP research [25-27].

The Physiological Roles of ChREBP in the Kidney

Regardless of the fact that ChREBP is known to be
significantly expressed in the kidney tissue since its gene discovery [4, 5], there have been few reports concerning the physiological roles of ChREBP in this tissue. As the kidney is not a main lipogenic tissue for energy storage, this indicates there must be other optimal physiological role for ChREBP in this organ. As far as we know, ChREBP does not seem to be indispensable for kidney development or its blood filtration function since the levels of uric protein or serum creatinine in ChREBP-deficient mice fed with normal chow are the same as those of wild-type mice (male, ICR background, 8–10 weeks old, Suzuki et al., unpublished data). Thus, the physiological function of ChREBP in kidney tissue still remains elusive.

**The Pathophysiological Roles of ChREBP in the Kidney**

On the other hand, ChREBP is implicated in the pathophysiology of some kidney diseases such as diabetic nephropathy (DN) and chronic renal failure. The role of ChREBP in DN was first reported by Proctor et al. in 2006 [28]. In that paper, they reported that there was significant increase in triglyceride content in the kidney of Akita mice, a genetic type 1 diabetes model developing DN, in which there was also a concomitant increase of lipogenic gene expression including Chrebp. Kim et al. reported that 5/6 nephrectomized rats, which is a chronic renal failure model, fed with regular diet showed lipid accumulation in the remnant kidney and also showed upregulation of lipogenic genes like Chrebp [29]. Furthermore, there are other papers showing Chrebp upregulation and lipid accumulation in rodent kidney under diabetic conditions [30–32], supporting the involvement of ChREBP in the pathology of DN.

More than 1/3 of diabetic patients will eventually develop DN, which is clinically defined by the presence of elevated urine albumin excretion and/or decreased glomerular filtration rate, resulting in end-stage renal disease [33, 34]. Indeed, the excess mortality rate associated with type 1 and type 2 diabetes is largely confined to those with DN [33, 35, 36]. Although the intensive management of diabetic patients includes controlling blood glucose levels and blood pressure as well as the blockade of the renin-angiotensin-aldosterone system in order to slow the progression of established DN [33, 37–39], and SGLT2 inhibitors have recently been shown to significantly lower the risk of worsening kidney function [40, 41], there still remains unmet clinical needs for therapeutic strategies to halt or reverse its progression. On this point, the precise molecular mechanisms for the involvement of ChREBP in the pathogenesis of DN has been increasingly attracting attention from both the basic and clinical sides of the medical research field.

**The Molecular Mechanisms of ChREBP Involvement for DN Development**

The first mechanistic insight of the involvement of ChREBP in DN was reported by Isoe et al. in 2010 [42]. From in vitro study using mouse and human mesangial cells, they showed that ChREBP directly regulates hypoxia-inducible factor-1α gene (HIF1A, coding HIF-1α protein) via ChoRE and thereby induced downstream fibrosis-related genes, resulting in the development of high blood glucose-mediated diabetic glomerulopathy. In addition, in 2014, Park et al. proposed that high glucose-mediated ChREBP O-GlcNAcylation is involved in the induction of Hif1a in cultured rat mesangial cells and streptozotocin-treated rat glomeruli [43]. These reports provided molecular insights for the pathogenesis of ChREBP-driven DN. However, at that time, there was no precise expression analysis of ChREBP in the kidney, and it was unclear whether glomerular mesangial cells were the main cell type which showed ChREBP expression in kidney tissue.

Each kidney is composed of millions of nephrons which consist of glomeruli, renal tubules, and collecting ducts [44, 45]. Each segment possesses its respective original function and is known to interact functionally with each other. In this context, we recently performed a detailed analysis of ChREBP expression by laser microdissection-coupled expression analysis using renal sections from wild-type (ICR) and type 1 diabetes mellitus (T1DM) model mice (iNOS-TG) [46, 47]. This study showed that ChREBP was predominantly expressed in the proximal tubules, and to a lesser extent in the glomeruli [47] (Fig. 2). A comparison of the expression levels of Chrebp and its target gene mRNA in the proximal tubules between wild-type and diabetic iNOS-TG mice show that ChREBP target genes such as thioredoxin-interacting protein (Txnip) were significantly upregulated in iNOS-TG mice, although total Chrebp mRNA were at similar levels [47]. TXNIP is a ubiquitously expressed thioredoxin (TXN)-binding protein and is the most prominent glucose-responsive gene among ChREBP target genes [19, 48]. TXNIP can impair the protein reducing activity of TXN and thus generates excess reactive oxygen species (ROS), leading to cell apoptosis [49]. Furthermore, in vitro analysis using human proximal tubule-derived cell line suggested that the ChREBP-mediated upregulation of TXNIP was involved in ROS production [47], implying the existence of a mechanism for inducing renal tubular injury in DN (Fig. 3).

Although the glomeruli, particularly their mesangial
cells, have been the focus of intense investigation in DN research, the alterations of the proximal tubule condition are reported to be far more intimately related to the loss of renal function and are said to more accurately predict the progression of DN than glomerular changes [33, 50-52], although the detailed link between proximal tubular damage and DN progression is yet unproven. It is also proposed that, in addition to ROS production, high glucose conditions make for hypoxic conditions due to increased consumption of oxygen and induction of HIF1A [53, 54], further supporting the involvement of a putative ChREBP-HIF-1α axis in the development of DN. Therefore, more precise analysis of the role of ChREBP in TXNIP-mediated ROS generation and HIF1A induction in the proximal tubules, especially analysis using in vivo models, is strongly needed.

In addition, increased expressions of sterol regulatory element-binding protein (SREBP) isoforms and angiotensin II are common features of models of progressive renal injury induced by diabetes. Overexpression of SREBP-1a also induced tubulointerstitial injury and cellular lipid loading in mice tubules [55, 56]. Autophagy has further been reported to play an essential role in regulating intracellular lipid metabolism, with autophagy impairment contributing to lipid accumulation in tubular epithelial cells during kidney fibrosis [57]. Moreover, ChREBP signaling downregulates SIRT1 signaling and results in autophagy impairment in the liver [58]. Thus, it is possible that a link exists between ChREBP and autophagy in renal tubular cells that might play a role in the development of DN.

We should note here that, as mentioned above, we could detect Chrebp mRNA from glomerular fraction of mice kidney, though to a lesser extent than that from

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**Fig. 2** The expression of Chrebp in wild-type mouse kidney
Immunohistochemical staining of Chrebp protein in renal cortex of wild-type mouse (male, ICR background, 14 weeks old, Scale bar: 50 μm). Immunohistochemical analyses of Chrebp protein revealed its predominant expression in the renal tubules as reported in our previous report [47].

**Fig. 3** Involvement of ChREBP in development of diabetic nephropathy
A close-up image of a nephron structure is shown in the left panel. Each kidney is composed of millions of nephrons which consist of glomeruli, proximal/distal renal tubules, and collecting ducts. Arrowheads indicate the direction of either bloodstream or pre-urine flow. The right panel shows the schematic roles of ChREBP in the glomerulus and proximal tubules in the development of diabetic nephropathy. In the glomerulus (where ChREBP is lowly expressed), O-GlcNAcylated ChREBP upregulates HIF1A expression, thereby inducing downstream fibrosis-related genes, leading to glomerular fibrosis. On the other hand, in the proximal tubules (where ChREBP is highly expressed), ChREBP upregulates TXNIP, thereby inducing ROS production and cell apoptosis, resulting in tubular injury. These lead to the development of diabetic nephropathy.
proximal tubules [47]. However, the main cell types which express *Chrebp* in glomerulus are elusive, and it is a matter of great importance to identify the cells in the glomerulus which are responsible for the expression of *Chrebp*.

**The Promise of ChREBP Inhibition as a Novel Therapeutic Strategy for DN**

Recently, two groups reported that congenital ChREBP deficiency alleviated streptozotocin-induced albuminuria, inflammation, and glomerular hypertrophy, which are the hallmarks of DN [32, 59, 60]. This improvement of renal condition was presumably mediated by a decreased ROS-mediated apoptosis induced by TXNIP [59]. As these model mice are systemically ChREBP-deleted, there still exists the possibility that the improvement of metabolic state in other tissues, such as the liver, indirectly ameliorated the condition of the renal phenotypes. It is also unclear whether the inhibition of ChREBP affects its progression or not. Thus, future analysis using conditional knockout mice in which ChREBP is deleted specifically in kidney or after tamoxifen administration might provide valuable insights. However, given the fact that TXNIP deficiency also ameliorates mice DN [61], these results clearly suggest the promise of ChREBP inhibition as a novel therapeutic strategy for DN.

In addition to the above findings in which rodent models were used, there are some studies which reported high levels of expression of ChREBP and its target genes in diabetes mellitus (DM) patients. Chen Y. *et al.* reported that the serum levels of pre-inflammatory cytokines, such as TNFα, IL-1β, and IL-6, and ChREBP in type 2 diabetes mellitus (T2DM) patients, were significantly upregulated with positive correlation, although it was unclear what tissues the serum ChREBP was derived from [30]. In a study by another group, Chen N. *et al.* reported a significant increase of ChREBP protein expression in both glomeruli and tubules of DN patients [59]. Additionally, the elevated level of expression of TXNIP in diabetic kidneys was positively correlated with that of ChREBP [59], further supporting the notion that the abnormal expressions of ChREBP and TXNIP were associated with the development of DN.

**Concluding Remarks**

From the latest findings introduced in this review, it is clear that ChREBP functions more than simply as a “thrifty gene” to store excessive energy as fat. Rather, ChREBP is a pleiotropic transcription factor which has specific roles for adapting to the fluctuations in environmental glucose levels in each tissue where ChREBP is expressed. Thus, the detailed investigation of ChREBP especially in non-main lipogenic tissues such as the kidney is an urgent requisite for the comprehensive understanding of the role of this protein.

As for therapeutic strategy, targeting ChREBP seems to be promising for DN. However, ChREBP inhibition is beneficial for DN, whereas ChREBP activation seems to be beneficial in other tissues such as pancreas for the induction of beta-cell proliferation [27, 62]. Furthermore, over-activation of ChREBP in beta cells leads to cell apoptosis due to excess ROS production induced by TXNIP [7, 63]. Thus, the optimal activation/inhibition of ChREBP and drug delivery strategy must be established for its therapeutic application to individual diseases.

For tissue-specific regulation of ChREBP activity, further analysis of the molecular mechanisms of ChREBP regulation in each tissue is required. Given that transcription factors generally form transcriptional coregulator complexes in a tissue-specific manner [64-67], it would be of interest to search for such protein complexes which ChREBP forms using a proteomic approach [67-71], and to determine whether these complexes could be a potential drug target for tissue-specific ChREBP activation/inhibition.

In addition, there are still presently unknown roles of ChREBP in other tissues and such roles have been continuously investigated and reported on. The involvement of ChREBP in fructose absorption in small intestine is one example [72-75]. Therefore, understanding the physiological and pathophysiological roles of ChREBP in each tissue would be beneficial for future drug development and also for understanding the systemic regulation of coordinated response to glucose.

**Disclosure Summary**

The authors have nothing to disclose. The authors declare no conflicts of interest.

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