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Lipid accumulation is ahead of epithelial-to-mesenchymal transition and therapeutic intervention by acetyl-CoA carboxylase 2 silence in diabetic nephropathy ☆

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ABSTRACT

Objective. The study investigated the relationship between epithelial-to-mesenchymal transition (EMT) and lipotoxicity in diabetic nephropathy as well as the protective effect of acetyl-CoA carboxylase 2 (ACC2) silence.

Methods. High glucose (30 mmol/L) cultured human proximal tubular epithelial cells (HK-2 cells) were used. Triglyceride content, fatty acid β-oxidation rate, malonyl CoA content, and marker proteins of EMT, including E-cadherin (E-cad), α-smooth muscle actin (α-SMA) and transforming grow factor-β (TGF-β), were assessed. Silence of ACC2 was achieved by ACC2-shRNA lentivirus transfection.

Results. In cultured human proximal tubular cells, high glucose induced fatty acid deposit before phenotypical and morphological changes of EMT. At 48 h, more triglyceride content, more malonyl CoA content and lower fatty acid β-oxidation rate were detected. However, increased expression of TGF-β, accompanied by loss of E-cad and acquisition of α-SMA, was observed at 98 h but not at 48 h. The silence of ACC2 in HK-2 cells led to restored cell morphology with less lipid deposition and less malonyl-CoA content, which resulted from faster β-oxidation rate.

Conclusion. The progress of lipotoxicity participates in the development of diabetic nephropathy in early stage before EMT. The manipulation of lipid metabolism might act as a promising therapeutic intervention for diabetic nephropathy.

Abbreviations: ACC2, acetyl-CoA carboxylase 2; p-ACC2, phosphorylated acetyl-CoA carboxylase 2; DM, diabetes mellitus; DN, diabetic nephropathy; ESRD, End Stage Renal Disease; FFAs, free fatty acids; SREBP-1, sterol regulatory element binding protein-1; PPARs, peroxisome proliferator-activated receptors; CPT-I, carnitine palmitoyl transferase I; ORO, Oil-red O; E-cad, E-cadherin; α-SMA, α-smooth muscle actin; TGF-β, transforming growth factor-β; EMT, epithelial-to-mesenchymal transition; AMPK, AMP activated protein kinase; ER, endothelial reticulum; ROS, reactive oxygen species.

☆ Disclosure: None.

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1. Introduction

As of 2011, approximately 366 million individuals were affected with diabetes mellitus (DM) worldwide, and this number is expected to exceed 550 million by 2030 [1]. The alarming prevalence of DM is a serious worldwide concern in terms of poor prognosis with complications and medical costs. Of all the complications, diabetic nephropathy (DN), which contributes to a large proportion of the reduced life expectancy in individuals with diabetes, is of particular importance. DN has been the leading cause of End Stage Renal Disease (ESRD) in the US, accounting for 50% of all ESRD patients [2]. Therefore, new research in disease pathophysiology, detection and novel treatments is hoped to alleviate the burden of diabetic nephropathy in the future.

Although the mechanisms underlying DN are not fully understood yet, emerging evidences have suggested a role of lipotoxicity in kidney dysfunction. Lipotoxicity is termed as accumulation of excess lipids in non-adipose tissues, which are shunted into non-oxidative pathways that disrupt normal cellular signaling leading to cell dysfunction and apoptosis [3]. Over the past decades, progress has been made in the investigation of lipotoxicity in DM, which includes multiple organs such as the endocrine pancreas, heart, and so on. Although not fully elucidated, the mechanism that contributes to cell dysfunction in DM possibly stems from the influence of glucose on intracellular fatty acid metabolism. The excessive levels of fatty acids lead to decreased insulin secretion, impaired insulin gene expression and beta-cell death by apoptosis [4]. Increased myocardial lipid content was also shown to be involved in the process of altered cardiac structure and function [5]. As far as the kidney is concerned, intraglomerular lipid deposits in the diabetic human kidneys were first described as early as in 1936 [6]. Many animal models of diabetes display upregulated lipogenic genes and lipid deposits in glomerular and tubular cells [7,8]. Therapeutic strategies targeting lipogenic proteins, such as sterol regulatory element binding protein-1 (SREBP-1) [9] ameliorates diabetic kidney injury *in vivo* and protects cell dysfunction *in vitro*. With detailed signal pathway to be elucidated, these investigations done *in vitro* and *in vivo* all support the hypothesis that lipotoxicity plays a role in genesis and propagation of DN. A recent genome-wide association study in Japanese patients reveals the association between a single nucleotide polymorphism in the acetyl-CoA carboxylase 2 (ACC2) with the susceptibility to type 2 diabetes mellitus associated nephropathy [10]. The following analyses done in other ethnic groups suggest the association still holds [11,12]. As ACC2 plays a critical role in lipid metabolism, these unbiased human genome-wide association studies warrant further explorations of lipotoxicity in DN in human.

Together with renal hypertrophy and matrix protein accumulation, tubulointerstitial fibrosis has been established as major pathological feature of diabetic nephropathy [13], and epithelial-to-mesenchymal transition (EMT) is considered to be central to the process of tubulointerstitial fibrosis [14]. As mentioned previously, although the role of lipotoxicity has gained more and more attention in the development of diabetic nephropathy, the question is still open whether lipotoxicity, which is necessarily accompanied by disturbance of lipid metabolism causing lipid accumulation, is the result or the cause of renal injury. To solve the puzzle, the relationship between lipotoxicity and a well established process, such as EMT, is worth being investigated.

As a crucial enzyme for fatty acid metabolism, mammalian acetyl-CoA carboxylase 2 (ACC2) has gained enormous exploration in the area of metabolic syndrome, atherosclerosis, cardiomyopathy, and DM. In particular, the inhibition of ACC2 has been predicted to reduce intracellular lipids by enhancing the use of fat for energy production. ACCs catalyze the formation of malonyl-CoA from acetyl-CoA. There are two isoforms of ACC. ACC1 is localized in cytosol, and its function is to synthesize Malonyl-CoA, which is used for de novo synthesis of fatty acids. ACC2, localized at the mitochondrial surface, regulates fatty acid oxidation by catalyzing the carboxylation of acetyl-CoA to malonyl-CoA. Malonyl-CoA inhibits carnitine palmitoyl transferase I (CPT-I), which is the rate limiting step in fatty acid uptake and oxidation by mitochondria in non-lipogenic tissues. Genetic deletion as well as pharmacological inhibition of ACC2 has been reported to result in reduced malonyl-CoA levels and enhanced fatty acid oxidation. Given the gene susceptibility of DN associated with ACC2 polymorphism, the role of ACC2 in the pathophysiology of DN remains unknown. Identification of lipid metabolism abnormalities associated with ACC2 would improve our understanding of pathogenesis of DN, and shed new insights on therapeutics benefits.

Because of a high energy demand and relatively little glycolytic capacity, mitochondrial β-oxidation of FFA is particularly the major source of ATP production in proximal tubular cells [15]. Proximal tubule is also where the lipid accumulation was detected in Type 1 diabetes kidneys [9]. Therefore, in the current study, we used cultured human tubular epithelial cells to investigate the relationship between two processes, EMT and lipotoxicity. We also tested our hypothesis that silence of ACC2, which would lead to faster β-oxidation, has protective effect to the lipotoxicity with restored cell morphology and function. We observed lipid accumulation occurred before early EMT, and these processes can be restored by ACC2 silence. These data support that lipotoxicity participates in the progression of chronic kidney disease in diabetes in very early stage, and interventions targeting lipid metabolism, such as ACC2, represent a novel treatment for DN.

2. Experimental procedures

2.1. Cell culture and treatment

HK-2 cells, a proximal tubule cell line immortalized by transduction with human papillomavirus type 16 E6/E7 (American Type Cell Collection, Rockville, MD), were grown in Dulbecco's MEM (Gibco, USA) that was supplemented with 10% FBS as described previously [16]. Three groups of culture conditions were investigated, which were normal glucose medium (5.5 mmol/L glucose), normal glucose medium plus mannitol (5.5 mmol/L glucose plus 24.5 mmol/L mannitol) for high osmotic pressure control, and high glucose medium (30 mmol/L). HK-2 cells were seeded at a density of 0.5×10^6 cells on 60-mm Petri dishes in 5.5 mmol/L glucose. All cell lines were grown at 37 °C in a humidified atmosphere of 5% CO_2. Pictures were obtained using Leica 090-135.001 microscope (Leica, Wetzlar, Germany) and graphed using