

Interactions of EGFR and the Hippo Pathway in Diabetic Nephropathy

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Abstract

Diabetic nephropathy (DN) is one of the most important complications of diabetes mellitus (DM), and it continues to rank as the leading cause of end-stage renal disease (ESRD) in the U.S. In our recently published manuscript, both in vitro and in vivo data showed that in response to high glucose, YAP expression and activation in renal proximal tubule epithelial cells were upregulated through activation of an EGFR- PI3K-Akt-CREB dependent pathway and subsequent activation of CTGF and AREG gene expression. The existence of crosstalk between EGFR and Hippo signaling pathway in diabetic kidney indicates the Hippo pathway may serve as an alternative novel therapeutic target of DN treatment.

Keywords: YAP; Hippo signaling; EGFR signaling; Diabetic Nephropathy

Abbreviations: EGFR: Epidermal Growth Factor Receptor, YAP: Yes-associated Protein, TAZ: Transcriptional co-activator with PDZ-binding Motif, CTGF: Connective Tissue Growth Factor, AREG: Amphiregulin, AMPK: Adenosine Monophosphate-activated Protein Kinase, PAI-1: Plasminogen Activator Inhibitor-1, LATS: Large Tumor Suppressor Kinase, TGF- β : Transforming Growth Factor-beta

Introduction

Diabetes mellitus (DM) is a growing worldwide epidemic health problem. More than 350 million people worldwide are affected by DM, and one in three U.S. adults could have DM in 2050, if the current trends continue [1]. Diabetic nephropathy (DN) is one of the most important DM complications, and it continues to rank as the leading cause of end-stage renal disease (ESRD) in the U.S. Patients must undergo either dialysis or a kidney transplantation once DN progress to ESRD, which produces a huge economic burden for society [2,3]. There is a pressing need to develop novel therapeutics for preventing or delaying the progression of DN. Progressive glomerulosclerosis and renal interstitial fibrosis are two characteristic pathological changes in the kidney in DN. Glomerular sclerosis has been at the center of attention for nephrologists [4]; however, increasing evidence suggests the renal tubule is a primary site of injury during DN progression, and a significant positive correlation between development of interstitial fibrosis and subsequent loss of renal function is seen in DN patients [5,6]. Activation of the EGFR (Epidermal Growth Factor Receptor) has been implicated in diabetic kidney injury [7-10]; studies by us and others have found that chronic EGFR activation in diabetic kidney is detrimental, and inhibition of EGFR activation by pharmacologic or genetic strategies markedly preserved renal function and slowed DN progression [11-14].

The Hippo signaling pathway, a kinase cascade that is conserved from *Drosophila* to mammals, controls the balance of cell proliferation, cell differentiation and apoptosis to define organ size or initiate tumorigenesis via tuning the phosphorylation and activation of YAP (Yes-associated protein)/TAZ (transcriptional co-activator with PDZ-binding motif), which serve as transcriptional co-activators for numerous target genes in nucleus. Upon activation of the Hippo pathway in response to different extracellular cues, YAP/TAZ are phosphorylated at specific serine/threonine residues, which results in inactivation of YAP/TAZ by cytoplasmic sequestration and/or proteasome-mediated degradation, thereby inactivating expression of

their numerous downstream target genes [15,16]. Increasing evidences suggest that YAP is an adaptor protein that modulates multiple signal transduction pathways in many cell types [17]. Activation of TGF- β signaling is well-known to be implicated in DN development and progression, and Smad3 is a crucial mediator of TGF- β signaling in fibroblasts [18,19]. Interestingly, recent studies revealed association of YAP with Smad2/3 to activate CTGF gene expression, and CTGF has been strongly associated with the development and progression of diabetic kidney injury via interaction with multiple ECM proteins [20-25]. In addition, some studies have implicated YAP and TAZ in mechanical signaling and tissue remodeling independent of the canonical mammalian Hippo pathway under various stress conditions [26-29]. Moreover, a recent study suggested that activation of YAP/TAZ in fibroblasts and subsequent activation of a renal profibrotic factor PAI-1 gene, SERPINE1, expression is involved in lung fibrogenesis [30]. Interestingly, YAP was also found to be inactivated in response to energy stress via direct activation of AMPK or through AMPK dependent LATS activation, whereas release of energy stress by administration of glucose activated YAP, which increased t downstream target gene expression [31,32].

Conclusion

Our recently published manuscript in Journal of American Society of Nephrology showed that YAP expression and activation in both type I and type II diabetic renal proximal tubule epithelial cells were upregulated through activation of an EGFR-PI3K-Akt-CREB dependent pathway; in contrast, TAZ expression in proximal tubule epithelial cells was reduced. Moreover, we also found that two downstream target gene of YAP, CTGF and AREG, expression were markedly upregulated both in vitro and in vivo in high glucose milieu [33]. Under normal physiologic conditions, we found YAP expression

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in proximal tubule epithelial cells is mainly located in cytoplasm, whereas it is highly expressed in podocyte nuclei similarly to what was found in previous studies [34]. However in the diabetic condition, we observed much higher expression of YAP in both cytosol and nucleus of renal proximal tubule epithelial cells compared with non-diabetic kidneys. Previous studies by us and others have indicated that EGFR activation in renal proximal tubule epithelial cells in response to various insults is closely related to the development of renal interstitial fibrosis through direct activation of myofibroblasts and/or interaction with TGF- β [35-41]. Therefore, EGFR dependent upregulation and activation of YAP and subsequent target gene expression in diabetic renal proximal tubule epithelial cells may be involved in initiation and progression of interstitial fibrosis in DN. More investigation on the potential mechanisms are still needed, but we postulate the following five possible mechanisms by which YAP activation in renal proximal tubule epithelial cells may mediate renal interstitial fibrosis: 1) CTGF is expressed in glomerular epithelial cells, proximal tubule epithelial cells and interstitial fibroblast cells [42]. Upregulation of CTGF per se and/or interaction with TGF- β can initiate or promote development of kidney interstitial fibrosis [20-25]; 2) Activated YAP directly activates TGF- β gene expression in the proximal tubule epithelial cells to promote interstitial fibrosis development similar to what has been reported in skin [43]; 3) The upregulated amphiregulin (and EGFR ligand) in diabetic renal proximal tubule epithelial cells activates EGFR to induce proximal tubule epithelial cells dedifferentiation through an autocrine mechanism, or activates EGFR expressed on fibroblasts to induce interstitial fibroblast proliferation and/or differentiation to myofibroblast through a paracrine mechanism; 4) Activation of CTGF and TGF- β signaling in diabetic renal proximal tubule epithelial cells induces excess ECM production thereby increasing kidney tissue stiffness, which further enhance YAP transcriptional activity via a positive feedback mechanism; 5) High glucose directly elevates YAP transcriptional activity by inhibition of AMPK and subsequent fibrogenesis in diabetic kidney.

In conclusion, this study for the first time has documented Hippo pathway activation in diabetic kidney, which suggests a novel potential therapeutic target for DN treatment.

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