Transforming growth factor in diabetes and renal disease

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Abstract: Renal lipid metabolism may play important roles in renal inflammation, glomerulosclerosis and tubulointerstitial injury in diabetic nephropathy. These alterations in lipids are associated with (1) decreased expression of PPAR-γ mRNA and protein, (2) increased abundance of the sterol regulatory element binding protein-1 (SREBP-1), key regulator of fatty acid synthesis, (3) decreased abundance of farnesoid X receptor (FXR), a negative regulator of fatty acid synthesis and promoter of fatty acid oxidation, (4) downregulation of peroxisome proliferator-activated receptor delta (PPAR-γ), key regulator of fatty acid oxidation, (5) increased abundance of the sterol regulatory element binding protein-2 (SREBP-2), key regulator of cholesterol synthesis, and (6) downregulation of ATP binding cassette A1 (ABCA1), key regulator of cholesterol efflux. These lipid alterations are also associated with marked downregulation of the podocyte markers podocin and zonula occludens-1 (ZO-1) and proteinuria. Treatment of ZDF rats with the PPAR-γ agonist rosiglitazone results in normalization of the renal lipid metabolism pathways and prevention of lipid and adipophilin accumulation, restoration of podocin and ZO-1 expression, and prevention of proteinuria. Thus, our results indicate that renal lipid accumulation significantly contributes to renal cell injury and treatment with PPAR agonist significantly ameliorates podocyte injury, glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. PPAR-FXR-SREBP pathway may play a critical role in regulation of lipid homeostasis and fibrosis in the kidney. [Nature and Science. 2009;7(1):91-95]. (ISSN: 1545-0740).

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1. Introduction

Diabetes mellitus is the leading cause of cardiovascular and renal disease. The pathogenesis of diabetic nephropathy is multi-factorial. Hypertension, hyperglycemia, profibrotic growth factors, including angiotensin II, transforming growth factor (TGF) and vascular endothelial growth factor (VEGF), proinflammatory cytokines, oxidative stress, and advanced glycation end products (AGEs) have been determined to play important roles in the pathogenesis of diabetic nephropathy (Wendt et al. 2003; Bohlender et al. 2005; Brownlee 2005; Chen et al. 2005; Cohen et al. 2005; Nicholas et al. 2005; Wellen and Hotamisligil 2005; Yamagishi et al. 2007; Goh and Cooper 2008). In addition, abnormal lipid metabolism and renal accumulation of lipids have also been proposed to play a similar important role in the pathogenesis of diabetic nephropathy (Moorhead et al. 1982; Sun et al. 2002; Jiang et al. 2005a; Jiang et al. 2005b).

Several studies in human subjects and in experimental animals with diabetes have shown a correlation between serum lipids, renal lipids, and proteinuria and progressive decline in renal function (Bonnet and Cooper 2000; Spencer et al. 2004). Renal lipid accumulation mediated by increased renal lipid synthesis is involved in the nephropathy seen in animal models of type I diabetes, diet induced obesity and insulin resistance, and aging (Sun et al. 2002; Jiang et al. 2005a; Jiang et al. 2005b).

Diabetic renal disease is associated with lipid deposits in the kidney and TNF-α, TGF-β1, TGF-β2, plasminogen activator inhibitor-1 (PAI-1), nephrin, podocin, ABCA1, α-actin, PPAR, VEGF, COX-2, and HIF expressions, etc. (Sun et al. 2002; Lim et al. 2005).

Transforming growth factor-βs (TGF-βs) in diabetes and renal disease

Transforming growth factor alpha (TGF-α) is upregulated in some human cancers. It is produced in macrophages, brain cells, and keratinocytes, and induces epithelial development. It is closely related to EGF, and can also bind to the EGF receptor with similar effects . TGFα stimulates neural cell proliferation in the adult injured brain (Fallon et al. 2000). TGFα was cited in the 2001 NIH Stem Cell report to the U.S. Congress as promising evidence for the ability of adult stem cells to restore function in neurodegenerative disorders.

TGF-β acts synergistically with TGF-α in inducing cellular transformation. Specific receptors for TGF-β activation trigger apoptosis when activated. Many cells synthesize TGF-β and almost all of them have specific receptors for this peptide. TGF-β1, TGF-β2, and TGF-β3 all function through the same receptor signaling systems.

The peptide structures of the three members of the TGF-β family are highly similar. They are all encoded as large protein precursors; TGF-β1 contains 390 amino acids and TGF-β2 and TGF-β3 each contain 412 amino acids. They each have an N-terminal signal peptide of 20-30 amino acids that they require for secretion from a cell, a pro-region (called latency associated peptide, and a 112-114 amino acid C-terminal region that becomes the mature TGF-β molecule following its release from the pro-region by proteolytic cleavage. The mature TGF-β protein dimerizes to produce a 25 KDa active molecule with many conserved structural motifs. TGF-β has nine cysteine residues that are conserved among its family; eight form disulfide bonds within the molecule to create a cysteine knot structure characteristic of the TGF-β superfamily while the ninth cysteine forms a bond with the ninth cysteine of another TGF-β molecule to produce the dimer. Many other conserved residues in TGF-β are thought to form secondary structure through hydrophobic interactions. The region between the fifth and sixth conserved cysteines houses the most divergent area of TGF-β molecules that is exposed at the surface of the molecule and is implicated in receptor binding and specificity of TGF-β.

TGF-β induces apoptosis in numerous cell types. TGF-β can induce apoptosis in two ways: The SMAD pathway or the DAXX pathway. The SMAD pathway is the classical signaling pathway that TGF-β family members signal through. In this pathway, TGF-β dimers binds to a type II receptor which recruits and phosphorylates a type I receptor. The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD). SMAD3, an R-SMAD, has been implicated in inducing apoptosis. The R-SMAD then binds to the common SMAD (coSMAD) SMAD4 and forms a heterodimeric complex. This complex then enters the cell nucleus where it acts as a transcription factor for various genes, including those to activate the mitogen-activated protein kinase 8 pathway, which triggers apoptosis.

TGF-β may also trigger apoptosis via the death associated protein 6 (DAXX adapter protein). DAXX has been shown to associate with and bind to the type II TGF-β receptor kinase. TGF-β plays a crucial role in the regulation of the cell cycle.

A study at the Saint Louis University School of Medicine of USA has found that cholesterol suppresses the responsiveness of cardiovascular cells to TGF-β and its protective qualities, thus allowing atherosclerosis to develop. It was also found that statins, drugs that lower cholesterol levels, enhance the responsiveness of cardiovascular cells to the protective actions of TGF-ß, thus helping prevent the development of atherosclerosis and heart disease. TGF increases with the renal disease.

Discussion

Since the description by Kimmelstiel and Wilson of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney, several investigators have shown presence of lipid deposition in the kidneys of diabetic humans and experimental animals. The results from our longitudinal studies in ZDF rats indicate that at the initial stage of diabetic nephropathy, there are multiple disturbances in the lipid metabolic pathways and significantly increased lipid deposition in kidney, including cholesterol, triglyceride, ceramide and glucosylceramide. Analysis of transcriptional factors and their target enzymes that play an important role in regulation of lipid metabolism demonstrated significant a) augmentation of *de novo* fatty acid synthesis and b) concomitant decreased fatty acid oxidation in the initial stage of diabetic kidney development. Moreover, there was c) increased cholesterol synthesis and uptake, and d) decreased cholesterol efflux in the young ZDF rat kidney. Thus, the combined effects of these disturbances in renal

lipid metabolism result in the net increased accumulation of lipids in the kidney. One of the novel and interesting findings in our present study is the demonstration of decreased FXR expression in ZDF rat kidney. FXR has been shown to inhibit SREBP-1c expression in the liver. In the liver FXR has also been shown to induce fatty acid oxidation via stimulation of PPAR and to have anti-fibrotic effect via decreasing TGF-M expression. Thus, the decrease in FXR activity in the kidney could mediate the upregulation of fatty acid synthesis, downregulation of fatty acid oxidation, and increased expression of TGF-M in the ZDF rat kidney.

In view of the toxic effects elicited by lipids on various target tissues and cells, we speculate that the ectopic accumulation of excess lipids in the kidney ultimately result in lipid-mediated cell injury or renal lipotoxicity. This could contribute to the pathogenesis of diabetic nephropathy. The lipotoxicity encompass various pathophysiological events including lipid-mediated cell injury. Lipotoxicity has been well documented in several non-adipose tissues including pancreatic cells, heart, liver and skeletal muscle, and has a profound impact in the pathogenesis and target organ damage in the metabolic syndrome.

We have observed that the increases in renal lipid content was already evident in 6 week old ZDF rats, prior to onset of hyperglycemia, glomerulosclerosis, and proteinuria indicating that these lipid alterations may play an important role in the progression of the diabetic renal injury. In support of this hypothesis, studies in renal mesangial and tubular cells grown in culture have shown that incubation of these cells with low density lipoprotein (LDL) or very low density lipoprotein (VLDL) cause upregulation of growth factors, including TGF-b, PDGF, and plasminogen activator inhibitor-1 (PAI-1), extracellular matrix proteins, proinflammatory cytokines including interleukins and tumor necrosis factor, adhesion molecules including monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and lipid peroxidation and glycoxidation, processes which play a role in the pathogenesis and progression of diabetic kidney injury. Our studies in ZDF rats indicate that *in vivo*, accumulation of lipids in the kidney is associated with a) increased expression of TGF-b1, VEGF, PAI-1, b) increased expression of collagen and fibronectin, c) reduced expression of podocyte markers including podocin, ZO-1, and d) mesangial expansion. These functional and structural changes likely contribute to the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. We provide evidence indicating that rosiglitazone decreases lipid accumulation in ZDF kidney by 1) prevention of fatty acid biosynthesis by suppression of nuclear SREBP-1 protein abundance; 2) induction of fatty acid oxidation via PPAR, ACO and CPT-1; 3) prevention of increased cholesterol biosynthesis by suppression of nuclear SREBP-2 protein abundance; 4) prevention of LDL uptake via inhibition of elevated ox-LDLR expression; and 5) augmentation of cholesterol efflux via increased expression of ABCA1. The prevention of renal lipid accumulation was coupled with i) simultaneous decreases in the expression of profibrotic growth factors and proinflammatory cytokines including TGF, VEGF, and IL-6, ii) prevention of extracellular matrix protein accumulation, and iii) prevention of podocyte injury and loss. These result in the significant amelioration of the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria.

PPAR agonists (thiazolidinedione or TZDs) have been shown to protect against the development of diabetic nephropathy in both human and animal models. Nevertheless, the molecular mechanism underlying the TZD-mediated renal protection has not been fully characterized. Although effective normalization of hyperglycemia and hyperlipidemia by TZD treatment may play an important role in the prevention of renal complications of diabetes, several lines of evidence also support a direct role for TZDs on the kidney. For example, renal glomerular mesangial cells express PPAR receptors and PPAR agonists have anti-fibrotic action in both *in vivo* and *in vitro* studies In addition, PPAR agonists have been shown to be renal protective in models of type I diabetes, independent of any alterations in systemic blood glucose or lipid levels. In the streptozotocin diabetic rat treatment with troglitazone was shown to prevent the increased expression of TGF, fibronectin and type IV collagen. Troglitazone prevented the increase in glomerular diacylglycerol (DAG) content, protein kinase C (PKC) activity and ERK2 phosphorylation while inducing an increase in DAG kinase activity. Troglitazone and pioglitazone also had similar effects in cultured mesangial cells, as they both prevented the high glucose induced increases in DAG, PKC and ERK2 phosphorylation. In another study in the streptozotocin diabetic rat troglitazone prevented the increased expression of PAI-1. Troglitazone has also been shown to be protective against glomerulosclerosis and proteinuria in the 5/6 nephrectomy model of nondiabetic renal disease, by preventing the increased expression of PAI-1 and TGF. Altogether, these studies therefore indicate that in addition to their systemic effects, PPAR agonists also have direct renal effects and modulate diabetic and non-diabetic renal disease by multiple cellular mechanisms, including modulation of renal lipid metabolism

as supported by our current study. Another intriguing finding of the current study is the demonstration of the significant lipid accumulation in the podocytes and the concomitant reduction of podocyte markers podocin and ZO-1 in the ZDF rat kidney, and the corrective effect of rosiglitazone. Podocyte injury is closely related to development and progression of diabetic nephropathy in humans. We demonstrated that podocin and ZO-1 expression was markedly reduced suggesting that decrease in podocin or ZO-1 may be the determinants of increased glomerular permeability and urinary protein loss.

Augmentation of podocyte proteins including podocin, nephrin, and ZO-1 by rosiglitazone demonstrates an important mechanism for the PPAR mediated decrease in proteinuria and renal protective effect in the setting of diabetes mellitus. In summary, we conclude that ZDF rats exhibit a primary alteration in renal lipid metabolism. The accumulation of triglyceride and cholesterol in the kidney glomerular and tubular cells is mediated via simultaneous increase in fatty acid synthesis and decrease in fatty acid oxidation, increase in cholesterol synthesis and uptake, and decrease in cholesterol efflux. The increase in lipid deposition is also associated with podocyte injury and increased expression of TGF-M, VEGF, PAI-1, IL-6, accumulation of extracellular matrix proteins, and proteinuria, suggesting the existence of renal lipotoxicity. Treatment of ZDF rats with the PPAR agonist rosiglitazone depletes the ectopic deposition of excess lipids in the kidney, and significantly ameliorates lipotoxicity-associated renal pathological abnormalities.

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