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TGF-β and Diabetic Nephropathy: Lessons Learned Over the Past 20 Years

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Twenty years ago and today, diabetic nephropathy remains the leading cause of renal failure in the United States. In the 1990s, there was a recognition that the growth factor transforming growth factor-β (TGF-β) may play an important role in the production of extracellular matrix proteins (ECM) that constitute fibrosis in the diabetic kidney.[1] Increased levels of TGF-β were found in kidneys from diabetic patients,[2] but exactly how hyperglycemia led to increased TGF-β was unknown.

To answer this question, Daniels, McClain and Crook investigated the role of the hexosamine biosynthesis pathway (HBP) in glucose-mediated TGF-β transcription.[3] The HBP converts fructose-6-phosphate into glucosamine-6-phosphate, and excessive hexosamines had been implicated in growth factor regulation in vascular smooth muscle cells.[4] The authors used a plasmid containing the promoter region of TGF-β1, the isoform most commonly associated with renal fibrosis,[5] as well as the luciferase reporter gene. They transfected rat proximal tubules, mesangial cells and vascular smooth muscle cells with this TGF-β/luciferase vector. In all 3 cell types, glucosamine induced a more potent and durable TGF-β transcriptional response, measured by a luciferase assay, than did glucose. Why did glucose and glucosamine, both triggers of the HBP, have such different effects on TGF-β transcription? Glucose and glucosamine both raise levels of UDP-N-acetyl glucosamine, a product of the HBP that inhibits the rate-limiting enzyme for this pathway, glutamine:fructose-6-phosphate amidotransferase (GFAT). As glucosamine enters the HBP distal to GFAT, it would not be affected by this negative feedback loop. The authors suggested that this may explain the more sustained and pronounced TGF-β response to glucosamine compared to glucose.[3]

The paper by Daniels et al nicely shows how metabolites in the HBP directly stimulate TGF-β transcription in 3 cell types important in diabetic nephropathy. This paper also raises important questions about TGF-β and diabetic nephropathy which remain relevant today. One such question is how do glucose and glucosamine directly stimulate TGF-β transcription? The authors speculate about a glucose or hexosamine response element in the

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TGF-β1 promoter but also acknowledge that the HBP can regulate the transcription factors cAMP response element (CRE) and Sp1 which have been implicated in TGF-β signaling. A few years later, another group found that increased HBP (through GFAT) stimulated expression of upstream stimulatory factors (USF) in human mesangial cells.[6] USF-1 or USF-2 then bound to a glucose response element (GlRE) in the TGF-β1 promoter, thus providing a mechanism whereby the HBP transcriptionally regulates TGF-β1 expression.[6] However, this same group had also identified 2 AP-1 binding sites in the human TGF-β1 promoter also activated by hyperglycemia.[7] These studies underscore the fact that glucose- and HBP-mediated transcriptional regulation of TGF-β1 is complex and likely affected by multiple transcription factors and promoter binding sites.

In addition to glucose and HBP-mediated transcriptional regulation of TGF-β, the past 20 years have revealed other mechanisms by which the diabetic milieu stimulates TGF-β activity. All 3 mammalian isoforms of TGF-β (TGF-β1, -β2, -β3) are secreted in a latent form, rendered as such by noncovalent binding to the latency-associated peptide (LAP). This latent complex is stored in the ECM by latent TGF-β binding proteins. Many activators of the latent TGF-β, acting through proteolytic cleavage from the LAP or conformational change, are upregulated in diabetes, providing other mechanisms for high glucose-induced TGF-β activity. Once activated, TGF-β ligands bind to the TGF-β type II receptor (TβRII) which then heterodimerizes with the type I receptor to phosphorylate Smads 2/3 and other signaling proteins that mediate TGF-β-dependent effects.[8,9] The matricellular protein thrombospondin-1 (TSP-1), a potent activator of TGF-β, is upregulated in diabetic patients and animal models.[10,11] Furthermore, activation of the HBP can lead to O-glycosylation (O-GlcNAcylation) protein modifications which mediates glucose-induced upregulation of TSP-1.[12] MMP-2 and MMP-9 are potent activators of TGF-β through proteolytic cleavage, and these MMPs are implicated in the pathophysiology of diabetic nephropathy.[13,14] There are many other proteins (e.g., angiotensin II, reactive oxygen species) that may also be involved in diabetes-induced TGF-β activity though whether the HBP is involved with these or in MMP-dependent TGF-β activation is not clear.

Another question raised by this manuscript is how does HBP-mediated TGF-β1 alter cellular responses in the diabetic kidney? Twenty years ago, TGF-β's ability to stimulate ECM proteins such as collagen I and fibronectin was well-recognized and these responses were implicated both in wound repair and fibrosis.[15,16] TβRII is nearly ubiquitously expressed on cells, and there is increased appreciation that the cellular effects of TGF-β vary greatly depending upon the target cell type and microenvironment. In addition to effects on mesenchymal cells like myofibroblasts and mesangial cells, TGF-β can induce apoptosis and dedifferentiation in epithelial cells (e.g., podocytes and proximal tubules).[17] Although TGF-β-induced dedifferentiation may initially be an adaptive response to injury, prolonged dedifferentiation impairs renal function and may contribute to tubulointerstitial fibrosis.[18]

TGF-β mediates a wide range of cellular responses, not all of which are detrimental. Impaired autophagy, the auto-degradation of damaged organelles, is implicated in the pathophysiology of diabetic nephropathy.[19] TGF-β promotes autophagy and, while excessive autophagy is destructive,[20] some TGF-β-induced autophagy may be adaptive. TGF-β may also mediate beneficial effects on the tubules in the context of diabetes. Diabetic

patients have increased levels of serum aldosterone which stimulates epithelial sodium channel (ENaC) activity in the distal tubule.[21] TGF-β activity suppresses aldosterone and other steroids (e.g., cortisol) as well as directly reduces ENaC activity leading to less sodium reabsorption.[22,23] TGF-β also decreases the activity of sodium/glucose cotransporters (SGLT) in the proximal tubule.[24] Although this was shown in cultured cells and needs to be further studied *in vivo*, it is intriguing given the recent studies showing that SGLT2 inhibitors preserve renal function in diabetic patients.[25] In addition to tubular effects, TGF-β has divergent effects on inflammation, an important component of renal injury including diabetic nephropathy. TGF-β has potent immunosuppressive effects and can induce Treg differentiation, but in certain microenvironments may also promote a proinflammatory T17 response.[26] However, diabetic mice that lack the Smad3 signaling protein (Smad3 KO db/db mice) had a significant reduction in inflammation compared to those diabetic mice with Smad3 intact, indicating that TGF-β signaling through Smad3 is proinflammatory in diabetes.[27]

TGF-β mediates many different cellular effects, some of which may promote the progression of diabetic nephropathy and others that may be adaptive. Yet, most of the animal models in which TGF-β signaling is systemically modified suggest that, overall, this growth factor contributes to diabetic nephropathy pathophysiology. Diabetic mice (Akita mutation) with genetically reduced TGF-β1 expression have reduced glomerulosclerosis and albuminuria.[28] In addition, treating db/db diabetic mice with a monoclonal antibody to TGF-β protected against renal insufficiency.[29] However, when diabetic patients were treated with a monoclonal antibody to TGF-β1, there was no improvement in renal function.[30] There are many potential explanations for this negative result. However, given TGF-β's pleiotropic responses, future therapeutic approaches should focus on approaches that modify, rather than completely block, this important signaling pathway.

In summary, the publication by Daniels showed the importance of the HBP and its metabolites in the stimulation of TGF-β transcription across 3 different cell types. This finding and others spurred on numerous investigations into the many ways that the diabetic environment augments TGF-β activity as well as the diverse TGF-β-dependent cellular responses exhibited by these diverse cell types. Furthermore, the HBP and its associated O-GlcNAcylation of proteins remains a vibrant area of research in diabetes.[31] In addition to TGF-β, the HBP has been linked to diabetes-induced upregulation of plasminogen activator inhibitor 1 (PAI-1) in mesangial cells and angiotensinogen in tubule cells.[32,33] Although much of the beneficial effects of SGLT2 inhibitors is attributed to hemodynamic effects and restored tubuloglomerular feedback, amelioration of glucose toxicity in the proximal tubule may also contribute.[34] Whether some of the beneficial effects of SGLT2 inhibitors may be due to blocking the HBP in the proximal tubule or surrounding endothelial cells requires further research. Although 20 years after the work by Daniels, we know more about the role of TGF-β and the HBP in diabetic nephropathy, a clear answer about how to target these pathways therapeutically remains elusive.

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