Podocyte-specific JAK2 overexpression worsens diabetic kidney disease in mice

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Activation of JAK-STAT signaling has been implicated in the pathogenesis of diabetic kidney disease. An increased expression of JAK-STAT genes was found in kidney glomerular cells, including podocytes, in patients with early diabetic kidney disease. However, it is not known whether increased expression of JAK or STAT isoforms in glomerular cells can lead to worsening nephropathy in the setting of diabetes. Therefore, we overexpressed JAK2 mRNA specifically in glomerular podocytes of 129S6 mice to determine whether this change alone could worsen diabetic kidney disease. A 2-3 fold increase in glomerular JAK2 expression, an increase similar to that found in humans with early diabetic kidney disease, led to substantial and statistically significant increases in albuminuria, mesangial expansion, glomerulosclerosis, glomerular fibronectin accumulation, and glomerular basement membrane thickening, and a significant reduction in podocyte density in diabetic mice. Treatment with a specific JAK1/2 inhibitor for 2 weeks partly reversed the major phenotypic changes of diabetic kidney disease and specifically normalized expression of a number of downstream STAT3-dependent genes implicated in diabetic kidney disease progression. Thus, moderate increases in podocyte JAK2 expression at levels similar to those in patients with early diabetic kidney disease can lead directly to phenotypic and other alterations of progressive diabetic glomerulopathy. Hence, inhibition of these changes by treatment with a JAK1/2 inhibitor suggests that such treatment may help retard progression of early diabetic kidney disease in patients.

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Diabetic kidney disease (DKD) is the most common cause of end-stage renal disease in the United States and in most other countries today.[1] Its incidence continues to increase dramatically despite some slowing in the rate of DKD progression to kidney failure.[1] In spite of the unquestionable salutary impact of blood pressure control and pharmacologic inhibition of the renin-angiotensin-aldosterone system, additional powerful interventions are needed in order to make any real headway in the fight against progression of DKD. One of the roadblocks in developing such interventions has been the lack of availability of a small animal model of DKD that fully recapitulates the disease in humans.[2] Our group has taken the tack of comparing transcriptomic profiles of humans with progressive DKD to those of mouse DKD models to determine both similarities[3] and differences[4] in these expression patterns, as both could give clues to pathways important in the pathogenesis of human DKD. One striking difference between human and mouse gene expression profiles is in the enhanced expression of Janus Kinase–Signal Transducer and Activator of Transcription (JAK-STAT) family members in humans with early and progressive DKD that are not recapitulated in current mouse models.[3,4] This increase in JAK-STAT gene expression in human DKD suggested that this pathway might be important in the more robust kidney disease that humans experience compared with that in mouse models.

JAK-STAT proteins transduce signals from many different types of non–tyrosine kinase plasma membrane receptors, including many cytokine and chemokine receptors,[5] whereby JAK-STAT activation transmits inflammation signals. We found that JAK1, 2, and 3 as well as STAT1 and STAT3 were expressed at levels in diabetic kidney that were several-fold higher than those in normal subjects.[4] The pattern of this increased expression was revealing. In subjects with early DKD, with low levels of albuminuria and normal kidney function, the increases in JAK-STAT expression were confined to glomerular cells, including podocytes. In subjects with progressive DKD and reduction in kidney function, the increases in JAK-STAT expression were mainly in tubulointerstitial cells.[4] The apparently sustained and chronic elevation of JAK-STAT mRNA and protein expression first in glomeruli and subsequently in cortical tubulointerstitium in

humans with DKD corresponds well with the natural progression of the disease, which initially damages glomeruli and subsequently tubulointerstitial tissues.[4] These findings suggest that the increases in JAK-STAT expression in glomeruli might help drive the severity and progression of early DKD. Therefore, we determined the effect of 1 of the early DKD changes, namely the increase in podocyte JAK2 expression, on DKD severity by overexpressing JAK2 specifically in podocytes in a mouse DKD model.

RESULTS

In order to generate cell type–specific enhanced JAK2 expression, a loxP-stop-loxP mouse JAK2 construct was inserted into the ROSA26 gene locus using the pBigT/pROSA26PA targeting system.[6] We then generated mice with cell type–specific overexpression of the *Jak2* transgene by crossing the mice with tissue-specific Cre recombinase mice (Figure 1). Because of the moderately enhanced sensitivity of the 129S6 mouse strain to DKD compared with C57BL/6 mice,[7] the targeted mutation was bred onto this background, and NPHS2 (podocin) Cre mice[8] were also established on the same background by a marker-assisted speed congenic strategy.

Podocyte-specific JAK2 expression was confirmed in male $Jak2^{loxP/loxP}$ NPHS2-Cre/+ (podocyte JAK2) mice by JAK2 immunoblotting of glomerular lysates from the homozygote podocyte JAK2 mice as well as the $Jak^{loxP/loxP}$ control mice. While this experiment did not prove that JAK2 expression was increased only in podocytes, NPHS2-Cre/+ mice have been demonstrated previously to be completely specific for podocyte expression.[8] Glomerular JAK2 expression was 2- to 3-fold greater in the podocyte JAK2 mice than in the control mice (Figure 2), an increase that was similar to that found in glomeruli from humans with early DKD compared with living kidney transplant donors.[4] Podocyte JAK2 mice were born at predicted ratios in litters of normal size. They appeared normal and had no baseline albuminuria, mesangial expansion, or other phenotypic or morphological differences from the floxed mouse controls or from wild-type mice (Table 1).

To determine whether the podocyte JAK2 mice developed a greater degree of DKD than control mice, both were bred to $Ins2^{Akita/+}$ (Akita) mice on the same 129S6 genetic background. Four groups of male mice were used for the remainder of the studies in this report: nondiabetic control (control), Akita diabetic control (control diabetic), nondiabetic podocyte JAK2 (JAK2), and Akita diabetic podocyte JAK2 mice (JAK2 diabetic). To accelerate pathologic changes, all 4 groups were implanted with subcutaneous mini-pumps that provided a daily dose of angiotensin (Ang) II for a total of 4 weeks. At the end of the 4-week Ang II infusion, all groups developed similarly elevated blood pressures (Table 1), and the 2 diabetic groups had similar degrees of hyperglycemia as well as other baseline characteristics (Table 1). Kidney weights were significantly greater in the diabetic mice compared with nondiabetic controls, and were greater in the JAK2 diabetic mice than in the control diabetic mice (Table 1). Glomerular STAT3 phosphorylation was assessed as a marker of JAK2 activation in the 4 groups. STAT3 phosphorylation was increased moderately in the JAK2 glomeruli but much more so in the JAK2 diabetic glomeruli (Figure 3).

Ang II infusion by itself did not significantly affect albuminuria or mesangial expansion in this model

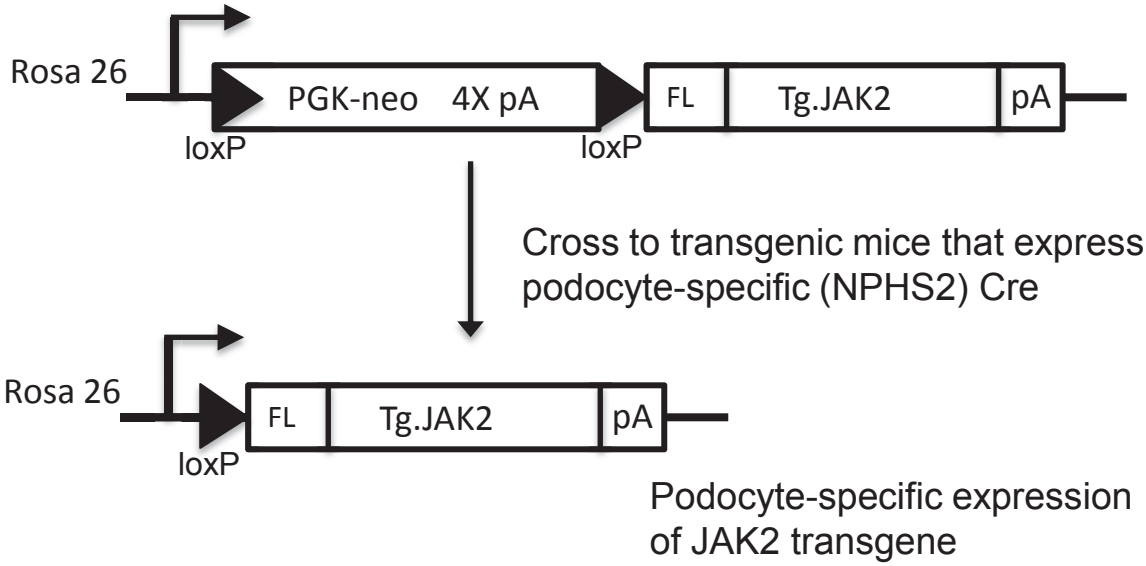

Figure 1 | Podocyte-specific JAK2 transgene strategy. A loxP-stop-loxP JAK2 construct was inserted into the ROSA26 gene locus using the pBigT/pROSA26PA targeting system and electroporated into W4 mouse ES cells derived from 129S6 mice. A gene targeted ES cell clone was established and used to produce germline transmitting ES cell-mouse chimeras. Mice carrying the loxP-stop-loxP JAK2 allele were then bred to podocin (NPHS2) Cre mice on a 129S6 background. Podocyte-specific Cre recombinase then excised the floxed stop cassette containing the quadruplicate polyadenylation sequence (4X pA), resulting in transcription of the recombinant JAK2 cDNA driven by the endogenous ROSA26 promoter.