DiaComp Pilot & Feasibility

Identifying genomic pathways associated with fibrosis in diabetic nephropathy
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Increased transcription and a permissive epigenetic modification at profibrotic renal tubulointerstitium genes in BTBR ob/ob model of type 2 diabetic nephropathy.

The BTBR mouse strain with the ob/ob leptin-deficiency mutation develops severe type 2 diabetes and diabetic nephropathy (DN). BTBR ob/ob mice develop a constellation of abnormalities that closely resemble advanced human disease (*1-2*). Connective tissue growth factor (CTGF) and transforming growth factor-beta1 (TGFβ1) are key mediators of fibrogenesis in DN (*3-4*). We postulated that increased expression of CTGF and TGFβ1 genes in DN is transcriptionally-mediated and associated with epigenetic changes at these loci. We used Matrix ChIP-MeDIP epigenetic platform (*6*) to compare profiles of RNA polymerase II, along with permissive and repressive chromatin marks at the pro-fibrotic CTGF and TGF-β genes in the BTBR wild-type (BTBR-WT) and the diabetic BTBR-ob/ob mice. PCRCrunch and GraphGrid software was used to acquire, analyze and visualize the ChIP-MeDIP-qPCR data (*5*).

We used renal tubulointerstitium isolated from 24 weeks old diabetic BTBR-ob/ob mice and age matched control. At this time point the BTBR ob/ob mice have several of the major physiologic and structural hallmarks of DN (*1-2*). There were higher levels of Pol II at the CTGF and TGFβ1 genes in the renal tubulointerstitium isolated from BTBR ob/ob diabetic mice compared to the controls (Fig.1, *row 1*). At the COX2 and FSP1- genes Pol II levels were not different. These results indicate higher transcription rates at the fibrogenic CTGF and TGFβ1 genes in the diabetic mice. The levels of the histone permissive modification, H3K9,14Ac (*row 2*), resembled Pol II profile, suggesting these changes are functionally related. The level of H3K4m2 (*row 3*) permissive mark was higher at the CTGF gene in BTBR ob/ob mice but these differences did not reach statistical significance. There were no significant differences in H3K4m3 (*row 4*) permissive mark. There was a small decrease in the silencing modification H3K27m3 (*row 5*) at sites examined but these difference were not statistically significant. Histone H3 levels (*row 6*) were not different in the BTBR ob/ob compared to the BTBR wild-type mice suggesting that the diabetic milieu does not alter nucleosome density. The levels of DNA methylation (5mC, *row 7*)) were lower in the BTBR ob/ob mice but again these differences did not rich statistical significance.

Compared to the robust epigenetic changes seen under high ambient glucose condition in cultured renal cells (*7-8*) the differences between BTBR ob/ob and BTBR wild-type mice were smaller. The levels of epigenetic changes observed here were also smaller than those seen in models of acute kidney injury (*5, 9*). However, the magnitude of differences induces by diabetes seen in BTBR strains are similar to transcription and epigenetic changes seen in other models of DN (*5-6, 10-11*). Given that DN is a slow chronic process these relatively modest changes are not unexpected but nonetheless are likely detrimental contributing to the disease.

In sum, we have demonstrated to ability to define epigenetic differences at key renal pro-fibrotic genes in the BTBR model of DN. The diabetes-induced epigenetic changes are consistent with the pathologic increase in the expression of CTGF and TGFβ1 genes. Studies are in progress to identify chromatin enzymes bound to these genes that mediated these and other diabetes-induced chromatin modification. The translational impact of identifying chromatin modifying enzymes tethered to genomic sites is that they provide identifiable points for potential drug interventions to mitigate DN.

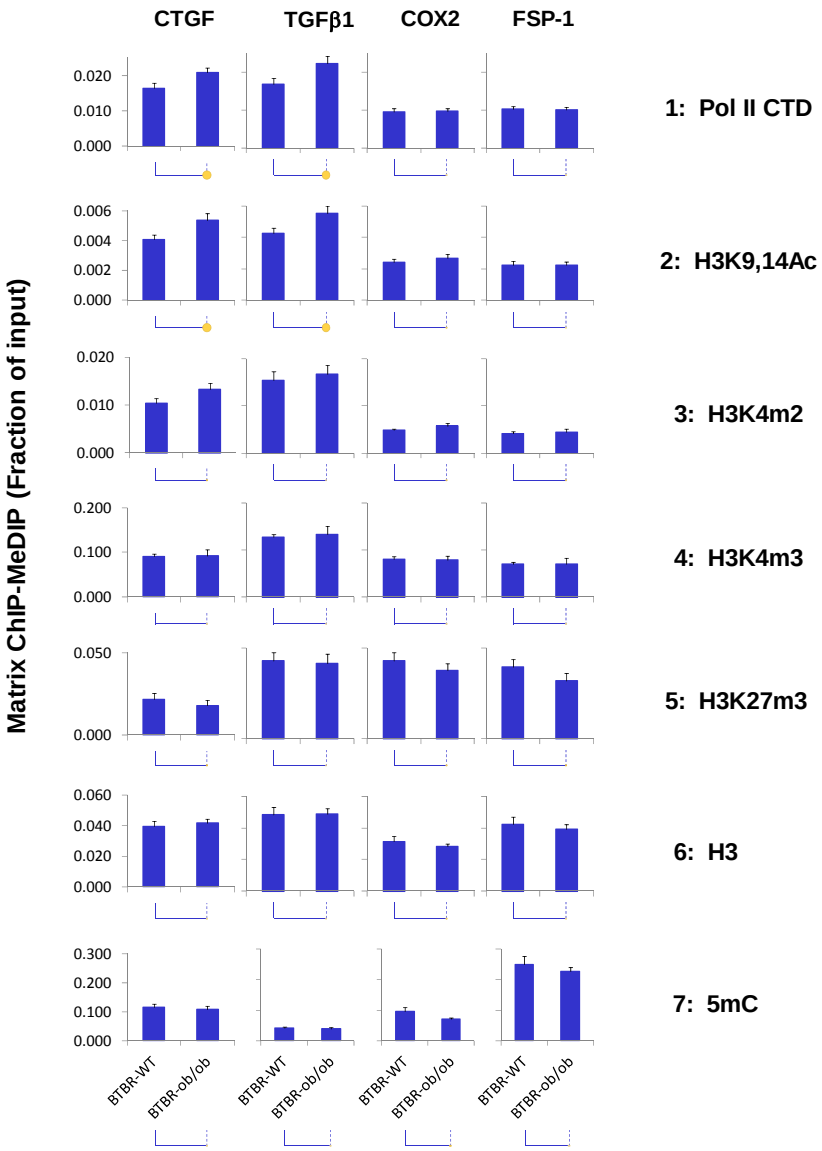

Fig.1. Matrix ChIP-MeDIP analysis of RNA polymerase II, histone H3 modifications and DNA methylation levels at CTGF, TGFβ1, COX2 and FSP-1 genes in renal tubulointerstitium in wild-type and ob/ob BTBR mice. Renal tubulointerstitium was isolated from 24 weeks old diabetic BTBR-ob/ob mice and age matched controls, BTBR-wt (n=8), cross-linked with formaldehyde and sheared (Bioruptor). Sheared chroamtn was assayed using Matrix ChIP-MeDIP 96-well platform and the following antibodies RNA polymerase II C-terminal domain (Pol II CTD) (*row 1*), H3K9,14Ac (*row 2*), H3K4m2 (*row 3*), H3K4m3 (*row 4*), H3K27m3 (*row 5*), total H3 (*row 6*) and 5-methylcytosine (5mC, *row 7)*. ChIP DNA was analyzed using primers at the start of the genes using real-time PCR (384-well plates, ABI7900). Data were acquired, analyzed and graphed using in-house generated PCRCrunch and GraphGrid software(*5*). Statistical significance is indicated in the grid below the graphs. Each vertical line and its attached horizontal component is associated with the bar above it. The p-value of the t-test between the two groups is indicated by the size of the solid circle at the intersection of their respective lines. Data represents Mean±SEM expressed as fraction of input (n=8 preparations for each group). The circles below each bar indicate statistical significance (are represented by circles ● **p<0.05** ● **p<0.01**)(*5*).

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