Diabetic Complications Consortium

Application Title: **The epigenome maps of human diabetic kidney disease**

Principal Investigator: Katalin Susztak MD, PhD

1. Project Accomplishments:

Goals: Map chromatin modification patterns (H3K4me1/2/3, H3K36me3, H3K27me3, H3K27ac and CTCF) and gene regulatory regions in (A) **normal human** kidney cortical tubule cells (n=5) and (B) in patients with **stage 2 diabetic CKD** (n=5), DM (n=5) or HTN (n=5) but no detectable renal disease and (C) compare gene regulatory region in control and **stage 2 diabetic CKD** (GFR range 60-90 cc/min) (D) understand the **association between epigenetic modifications and transcript levels and downstream clinical and histological phenotype in patients.**

Accomplishments: We have collected more than 40 human kidney samples, including more than 5 from subjects with diabetes, 5 controls 5 from patients with hypertension and 5 with diabetic kidney disease. Samples have been cross-linked and sonicated into 200 bp fragments. Chromatin immunoprecipitation has been performed with 7 different histone tail antibodies. The precipitated DNA from the first sample has been submitted for Next Generation Sequencing analysis. We are in the process of analyzing the results. Our initial scan indicates that the results obtained with CTCF and H3K4me2 are not optimal, while the other5 antibodies indicated high quality ChIP enrichment.

We have generated high quality ChIP results from several samples, we are finalizing the data analysis.

Here are some of the preliminary observations.

Figure1. The chromatin immunoprecipitation generated good quality data. Figure 1. shows low duplication read in the data, enrichment of tags on transcription start sites and significant correlation between H3K4me1 and H3K27ac; two different enhancer marks.

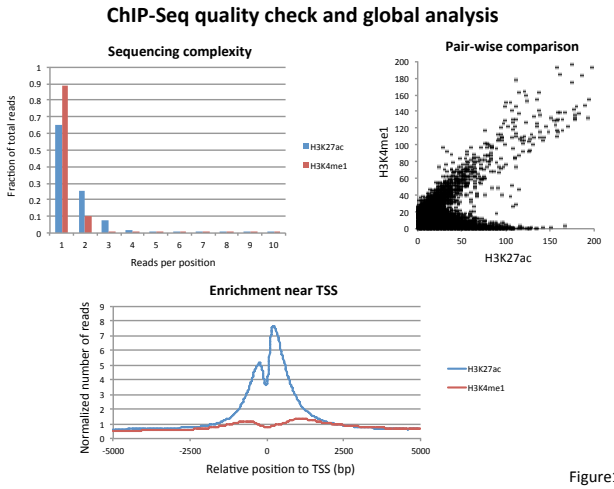
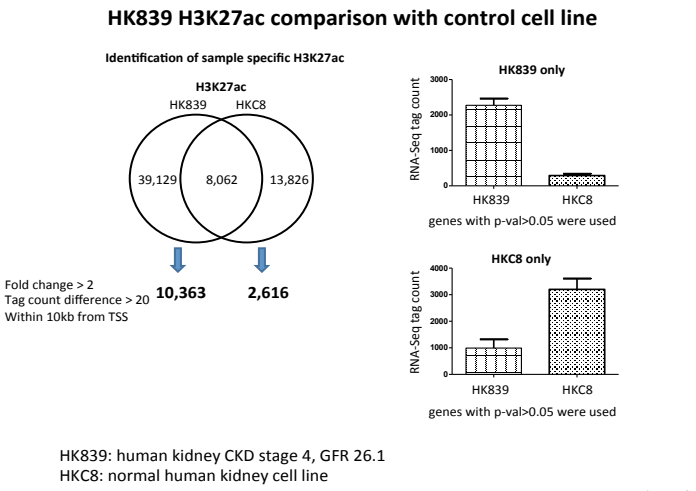


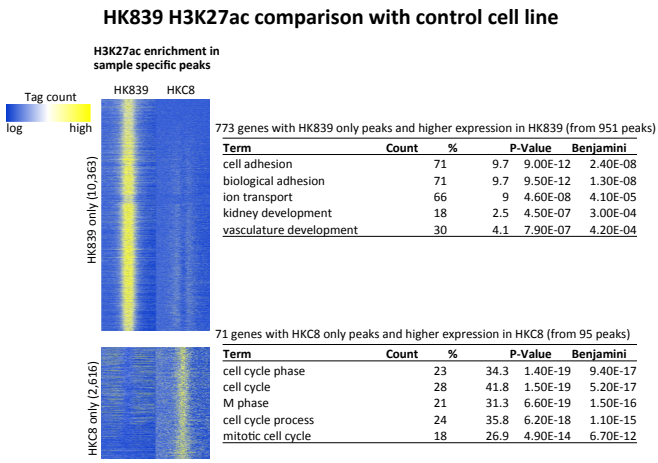
Figure1

Figure2. We have compared H3K27ac marks in control human cell line HKC8 and HK839; a human kidney sample obtained from a patient with stage 4 CKD (GFR; 26 cc/min/1.73m2). This analysis has identified significant overlap between human kidney samples and cultured human tubule samples, with transcripts and enhancers that are specifically expressed either in CKD tubule kidneys or cultured cells.



HK839: human kidney CKD stage 4, GFR 26.1
HKC8: normal human kidney cell line

Figure2



773 genes with HK839 only peaks and higher expression in HK839 (from 951 peaks)

Term	Count	%	P-Value	Benjamini
cell adhesion	71	9.7	9.00E-12	2.40E-08
biological adhesion	71	9.7	9.50E-12	1.30E-08
ion transport	66	9	4.60E-08	4.10E-05
kidney development	18	2.5	4.50E-07	3.00E-04
vasculature development	30	4.1	7.90E-07	4.20E-04

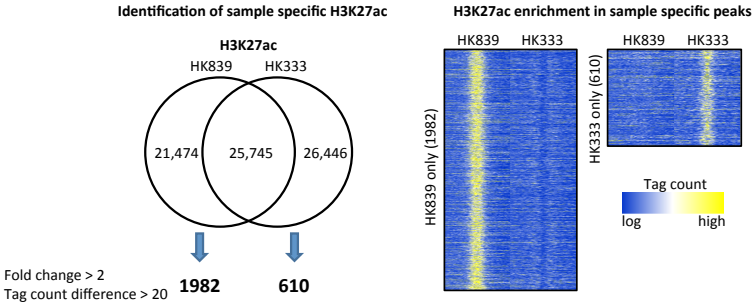
71 genes with HKC8 only peaks and higher expression in HKC8 (from 95 peaks)

Term	Count	%	P-Value	Benjamini
cell cycle phase	23	34.3	1.40E-19	9.40E-17
cell cycle	28	41.8	1.50E-19	5.20E-17
M phase	21	31.3	6.60E-19	1.50E-16
cell cycle process	24	35.8	6.20E-18	1.10E-15
mitotic cell cycle	18	26.9	4.90E-14	6.70E-12

Figure3

Figure3 shows that cultured cells were likely dividing and therefore they showed multiple peaks in cell cycle associated genes. On the other hand sample obtained from fibrotic kidneys were enriched for kidney developmental genes and for genes that function in cell adhesion.

Figure4 shows histone modification differences between a sample with normal kidney function HK333 compared to one obtained from a patient with CKD. The result indicates significant epigenetic changes in CKD samples.



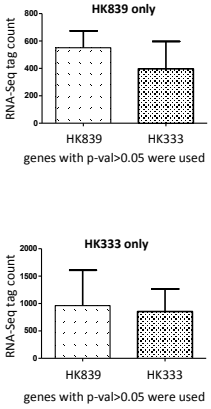
ID	Non tum Dx	CKD stage	% glom sclerosis	% Fibrosis	Interstitium - pathology comment	GFR
HK333	DM/HTN		2	5	15 protein	75.52515
HK839	CKD		4			26.09723

Figure4

Figure5 shows functional analysis and list of differentially expressed genes in control and CKD samples, including expression of specific genes and their fold

Figure5

H3K27ac comparison analysis between different patients



Genes with HK839 only peaks and higher expression in HK839

HK839 only	# of tag in HK839	# of tag in HK333	Log2 FC	P-value
LTBP2	1276	326	1.97	0.0444
FMO2	720	177	2.02	0.0401
CD4	368	77	2.26	0.0425
ADAM8	305	63	2.28	0.04185
FOXP2	161	33	2.29	0.02365
IKZF1	551	109	2.34	0.0345
CCL21	1020	197	2.37	0.02235
HIC1	303	55	2.46	0.04915
MOXD1	1471	263	2.48	0.0108
CCND2	749	126	2.57	0.0025
ADRA2A	259	40	2.69	0.0396
CD6	271	38	2.83	0.01545
CCDC80	1248	171	2.87	0.03045
CD200R1	89	11	3.02	0.03275
HLA-DQB1	2062	247	3.06	0.0002
NRARP	172	20	3.10	0.0471
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PLD4	238	27	3.14	0.0116
CAMK4	170	18	3.24	0.0208
NTM	107	11	3.28	0.0152
CTHRC1	126	11	3.52	0.0273
FAM159A	16	1	4.00	0.00005
IRF4	111	2	5.79	0.02915

Genes with HK333 only peaks and higher expression in HK333

HK333 only	# of tag in HK839	# of tag in HK333	Log2 FC	P-value
GSTT1	7	2886	-8.6875	0.00135
SLC5A9	48	1059	-4.46352	0.0002
LDHC	16	110	-2.78136	0.00675
RGS3	1406	3536	-1.33052	0.0161

change values.

Specific Aims:

The hypothesis of the proposal is that epigenetic changes **play key role in diabetic kidney disease development. Here we propose to** map chromatin modification patterns (H3K4me1/2/3, H3K36me3, H3K27me3, H3K27ac and CTCF) and gene regulatory regions in (A) **normal human** kidney cortical tubule cells (n=5) and (B) in patients with DKD (n=5), DM (n=5) and HTN (n=5) but no detectable renal disease and (C) define differences in stage 2 diabetic CKD (D) understand the **association between epigenetic modifications, transcript levels and downstream clinical and histological phenotype development in DKD.**

2. Publications:

Poster presentation ASN Renal Week 2014
Manuscript in preparation.