URINARY MARKERS FOR PROGRESSIVE RENAL FIBROSIS.

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Novel markers are required for diagnosis and monitoring of progression of chronic renal disease (CKD). Transforming growth factor-beta (TGF-b) is a central mediator in CKD underscored by progressive glomerulosclerosis and tubulo-interstitial fibrosis in Albumin-TGF-b1 transgenic (TG) mice, in which we had developed genetic markers for progressive renal fibrosis in this model ("predictor genes").

We examined the urine of TG mice prior to (2 weeks of age) and after the development of renal disease (4 weeks of age) for proteins using 1D PAGE/Western blotting, and 2D gel electrophoresis/Mass spectrometry (MS). In addition, we performed realtime-PCR on RNA from urinary cells. In order to facilitate a high through-put screening we are metabolically label mice with 15N/stable isotope allowing quantitative analysis of relative protein abundance in tissue, serum, and urine by mass spectrometry. Urinary excretion of candidate markers was correlated with intra-renal RNA and protein. For large-scale analysis, we established metabolically labeled mice with stable isotopes (N15) and used quantitative mass spectrometry to determine differences in urinary proteins.

We detected increased excretion of extracellular matrix components (collagenI/VII, Mmp2, and Timp1), differential excretion of members of the lipocalin (Mup1 & 3 decreased, lipocalin2 increased), and albumin gene family. Urinary excretion of predictor genes (RNA or protein) and non-coding RNAs was correlated with intra-renal expression.

The findings indicate that urine protein and RNA analysis can be used to monitor intra-renal gene expression. Metabolic labeling combined with quantitative mass spectrometry is a novel approach for quantitative proteomics in animal models. Studies determining correlations with renal fibrosis are underway.