Diabetic Complications Consortium

Application Title: MicroRNA-mediated Mechanisms in Diabetic Glomerular Disease

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1. Project Accomplishments:

During the funding period we were able to show that miR-34 is a central mediator in podocyte injury in three murine models of podocytes injury (uninephretcomy/DOCA-salt, uninephrectomy/Angiotensin2 and radiation injury). In addition we identified candidate mRNAs that are regulated by miR-34 and mediate the podocytes injury. Furthermore, we determined that systemic administration of antisense-oligonucleotides (ASO) against miR-34 alters the transcriptomic response to kidney injury.

2. Specific Aims:

Aim 1: **Determine the role of loss or inhibition of miR-34a in murine model of DN.** Supporting our hypothesis we determined that mice deficient for miR-34a exhibit amelioration of podocytes loss in the uninephrectomy/deoxycorticosterone acetate (UN/DOCA)-salt (Fig.1) and UN/Angiotensin2 models. In both models increased reactive oxygen species (ROS) production contributes to podocytes loss, which is an important mechanism in diabetic nephropathy. Interestingly, other glomerular and tubule-interstitial changes detected in this model including mesangial expansion were not significantly altered in miR-34a-deficient mice compared to wild-type littermates.

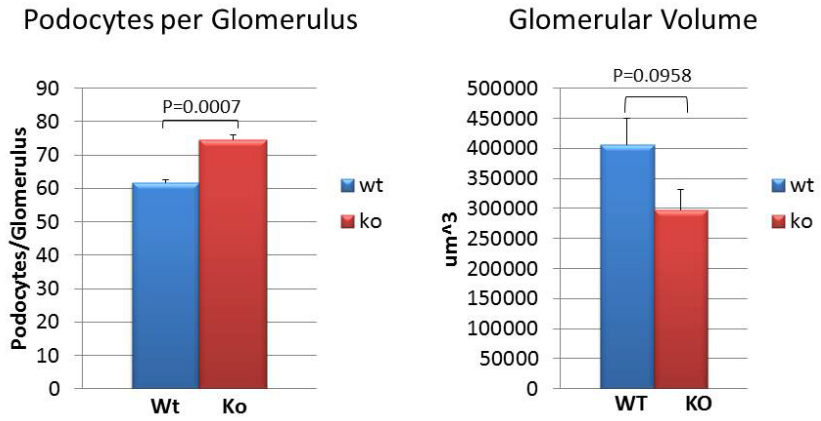

Figure 1: Loss of miR-34a is associated with increased podocytes number and decreased glomerular volume after uninephrectomy / DOCA-salt injury. No difference in podocytes number and glomerular volume between unchallenged miR-34a wildtype (WT) and knockout (KO) mice was detected.

To examine the efficacy of antisense oligonucleotides inhibiting miR-34a in mice after systemic administration, we first determined that transfection of human and mouse

podocytes with LNA-backbone-modified ASO (Exiqon) against miR-34a/b/c results in repression of miR-34a, b and c levels, quantified by qrt-PCR, and inhibits apoptosis induced by UV radiation. Next, we determined that total body gamma-radiation of mice results in a robust increase in miR-34a expression in the kidney and podocyte injury, assessed by quantitative analysis of electron-microscopy images, in wildtype mice within 24 hours. No difference of podocyte effacement was detected after administration of 20 mg/kg ASO against miR-34 versus scrambled control oligo, which has the same nucleotide composition in a different sequence. Nevertheless, unbiased transcriptomic profiling of kidney cortex using Affymetrix ST-chip arrays revealed significant changes in gene expression in mice treated with ASO against miR-34 versus control ASO. We are in the process of performing experiments using higher dose of ASO and will attempt to confirm observed transcriptomic changes.

Aim 2: **Identify miR-34a regulated genes in podocytes using PAR-CLIP.** To experimentally determine transcripts, which are targeted by miR-34 in podocytes we have applied Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation) PAR-CLIP method in cultured murine podocytes transfected with ASO against miR-34 or control-ASO. Despite the use of large amounts of cells the amount of total RNA that we could recover after transfection of ASO was significantly lower compared to untransfected cells. The data obtained from PAR-CLIP using a pool of multiple experiments revealed very low quality of input RNA and did not allow identifying microRNA binding sites. PAR-CLIP data from a sample of untransfected cultured podocytes processed in parallel generated good quality data and reproduced our previous results. Because we believe that transfection of cultured podocytes interferes with PAR-CLIP, we are in the process of generating stable miR-34a-deficient podocytes lines from primary podocytes cultures of miR-34 null and wildtype control mice.

Analysis of data from PAR-CLIP experiments in cultured human podocytes and tubular epithelial cells (TECs, HK2 cells) before and after exposure to TGF-beta identified a total 37 candidate microRNA binding sites on 3'-UTRs of 34 different transcripts using criteria as described [1]. Binding sites on other parts of the transcripts were not included in this analysis. Our preliminary analysis determined that 10 and 9 microRNA binding sites were unique to TECs or podocytes, respectively (Fig. 2). Furthermore, 10 sites were only detected in TGF-beta treated cells and 7 sites were present in all samples, independent of cell-type and TGF-beta. These data support the hypothesis that microRNA-mRNA interactions are cell-type and context specific. Further analysis of these data is in process.

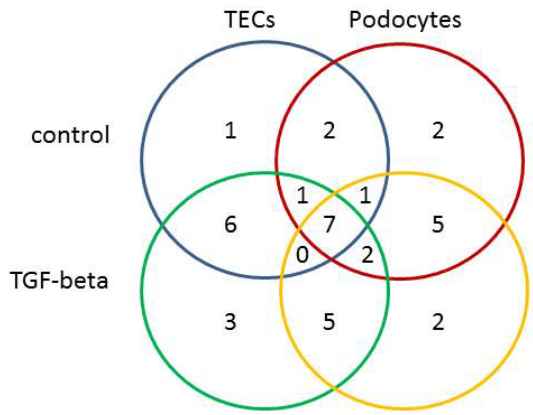

Figure 2: *Number of candidate microRNA binding sites in human tubular epithelial cells (TECs – blue and green circles) and human podocytes (red and yellow circles),+/- TGF-beta (green and yellow circles).* Candidate microRNA binding sites on 3'-UTRs of 34 different transcripts were identified. Binding sites on other parts of the transcripts were not included in this analysis.

3. Publications:

In preparation;

1. Hafner, M., et al., *Genome-wide identification of miRNA targets by PAR-CLIP*. Methods, 2012. **58**(2): p. 94-105.