Diabetic Complications Consortium

Application Title: Establishing miRNome Expression Profiles of Renal Function Decline in T1D.

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1. Project Accomplishments:

This Pilot and Feasibility project has successfully analyzed the expression profile of the human miRNA genome (miRNome) in a well-characterized cohort of patients from the Joslin Kidney Study (JKS). Over the past year, we have performed miRNome expression profiling in pooled RNA isolated from baseline plasma specimens from several subgroups of Type 1 diabetic (T1D) patients enrolled in the JKS using a highly-sensitive quantitative real-time PCR (qRT-PCR) based approach. As proposed, we performed miRNome profiling in T1D patients who progressed rapidly from normal renal function (CKD stages 1 or 2 at baseline) to ESRD over the course of 7-20 years of follow-up (i.e., CKD1/2 rapid-progressors; n=38) and those who maintained normal renal function over this same time frame despite persistent proteinuria (i.e., CKD1/2 non-progressors; n=40). Pooled RNA was also isolated and assayed from plasma from a reference panel of T1D patients with persistent normoalbuminuria (n=40). In addition to these 3 subgroups, miRNome expression profiling of pooled plasma was also performed in 2 additional T1D subgroups that were not part of our original proposal: patients who progressed from impaired renal function to ESRD rapidly over the course of follow-up (CKD3 rapid-progressors; n=40) and a panel of T1D patients with persistent normoalbuminuria and more than 35 years duration of T1D (i.e., long-duration normoalbuminurics; n=43). Data from comparisons made to date were used to select candidate miRNAs for screening in RNA isolated from individual baseline plasma specimens as well as in urine specimens collected at this same time point.

2. Specific Aims:

Specific Aim 1. To determine miRNA expression profiles in plasma and urinary RNA from rapid progressors, non-progressors, and patients with normoalbuminuria.

Results:

Using Qiagen's Human miRNome miScript miRNA PCR Array (V16.0, 384-well), we performed expression profiling of 1,066 miRNAs in pooled plasma samples from participants of the JKS. In total, miRNome profiles from pooled specimens from 5 subgroups were determined: 1) T1D patients who progressed rapidly from normal renal function to ESRD over the course of 7-20 years of follow-up (i.e., CKD1/2 rapid-progressors; n=38), 2) those who maintained normal renal function over this same time frame (i.e., CKD1/2 non-progressors; n=40), 3) a reference panel of T1D patients with persistent normoalbuminuria (n=40), 4) patients who progressed from impaired renal function to ESRD rapidly over the course of follow-up(CKD3 rapid-progressors;

n=40), and 5) a panel of T1D patients with persistent normoalbuminuria and more than 35 years duration of T1D (i.e., long-duration normoalbuminurics; n=43). A summary of the number of detectable miRNAs from each pooled sample is presented in Table 1.

Pooled Sample	Expressed miRNA (Ct>30)
CKD1/2 RP	327
CKD1/2 NP	761
Normoalbuminurics	326
CKD3 RP	274
Long-duration Normoalbuminurics	415

Table 1. Summary of Expressed miRNAs in Each Subgroup.

Comparisons of relative miRNA expression profiles between each of these 5 subgroups have been performed. Our major efforts have focused on identifying miRNAs that are differentially expressed between CKD1/2 rapid progressors and Normoalbuminurics. Among these 2 subgroups, a total of 367 miRNAs were detectable in both pooled specimens. Fold-change analyses among expressed miRNAs in these 2 subgroups identified 72 miRNA with a fold-difference either > 2.5 or < 0.40 (Figure 1). Moreover, a total of 37 miRNA had fold-differences either > 2.0 or < 0.50 and were highly statistically different between these 2 subgroups (P<0.01; Figure 2). Overall, the vast majority of miRNAs we identified were over-expressed in CKD 1/2 rapid progressors relative to Normoalbuminurics.

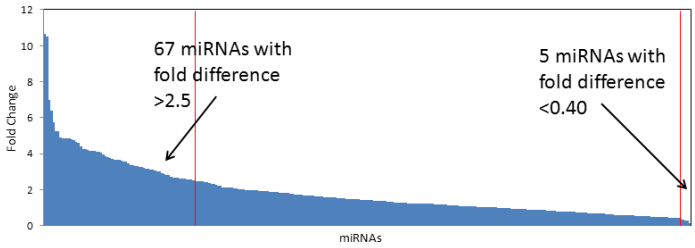

Figure 1. Distribution of miRNA expression fold-change among CKD1/2 rapid progressors and Normoalbuminurics.

Based on these analyses, we selected the top 20 differentially expressed miRNAs (i.e., candidate miRNAs) for expression analysis in RNA isolated from individual baseline plasma specimens from CKD1/2 rapid progressors, Normoalbuminurics, and CKD1/2 non-progressors, as well as in RNA isolated from urine specimens collected from these same individuals at the same baseline time points.

Specific Aim 2. To determine the role of miRNAs in renal function decline and progression to ESRD in T1D.

Results:

Group-wise comparisons of differences in candidate plasma miRNA expression between CKD1/2 rapid progressors, Normoalbuminurics, and CKD1/2 non-progressors were assessed by nonparametric Kruskal-Wallis and Mann-Whitney tests, as appropriate (Table 2).

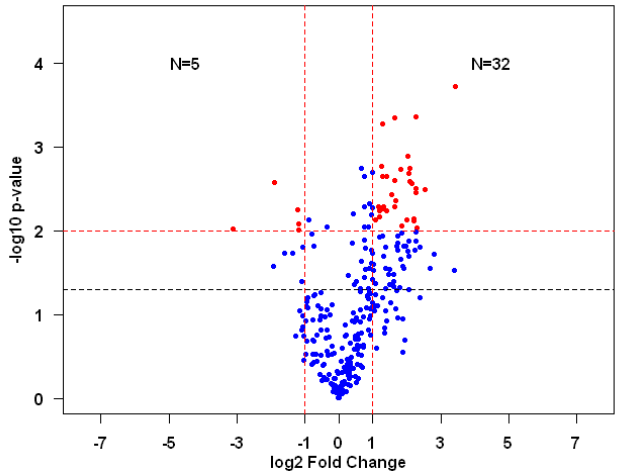

Figure 2. Volcano plot of expression fold- change (log2 fold change) and statistical significance (-log10 P-values) of expressed miRNAs among CKD1/2 rapid progressors and Normoalbuminurics.

The relative differential expression levels of 10 candidate miRNAs identified in our pooled samples (miR-375, miR-194-

5p, miR-146-5p, miR-16-2-3p, miR-26b-5p, miR-15b-3p, miR-3940-5p, miR-373-5p, miR-3200-5p, and miR-3911) were confirmed in individual samples from CKD1/2 rapid progressors and Normoalbuminurics ($P \leq 0.05$). The relative expression of 6 of these miRNAs (miR-375, miR-194-5p, miR-146-5p, miR-16-2-3p, miR-26b-5p, and miR-15b-3p) also differed among Normoalbuminurics and CKD1/2 non-progressors ($P \leq 0.05$). Interestingly, while a subset of these candidate miRNAs differed in their expression among CKD1/2 non-progressors and CKD1/2 rapid progressors, several had similar expression levels among these 2 subgroups. Three of these miRNAs (miR-26b-5p, miR-3200-5p, and miR-3911) showed either increased or decreased expression as disease severity increased among each of the 3 subgroups; suggesting that these miRNA may have distinct roles at various stages in this disease process.

	Log2 Relative Expression Levels (Mean±SD)*				Mann-Whitney *U* Test *P*-values		
miRNA	Normoalbuminurics (NA)	CKD1/2 Non-Progressors (NP)	CKD1/2 Rapid Progressors (RP)	Kruskal-Wallis ANOVA *P*-values	NA vs. CKD1/2 NP	NA vs. CKD 1/2 RP	NP CKD 1/2 vs. RP CKD 1/2
miR-375	-13.1±1.2	-11.4±1.2	-11.8±1.2	<0.0001	<0.0001	**0.0001**	0.07
miR-7-5p	-14.7±1.6	-12.3±2.1	-13.8±2.5	<0.0001	<0.0001	0.07	0.006
miR-194-5p	-15.5±1.2	-14.0±1.6	-14.0±1.9	0.0003	0.0002	**0.0007**	0.97
miR-146-5p	-5.9±2.4	-2.9±2.0	-4.1±1.6	<0.0001	<0.0001	**0.0003**	0.002
miR-16-2-3p	-14.9±1.6	-12.0±1.8	-12.7±1.8	<0.0001	<0.0001	**<0.0001**	0.02
miR-3653	-8.0±1.6	-6.2±1.8	-7.2±1.8	0.0002	<0.0001	0.06	0.02
miR-26b-5p	-11.9±1.7	-9.1±2.2	-10.6±2.1	<0.0001	<0.0001	**0.004**	0.005
miR-10b-5p	-10.9±2.2	-8.4±2.4	-10.6±1.7	<0.0001	<0.0001	0.31	<0.0001
miR-15b-3p	-14.6±1.8	-13.2±2.1	-10.7±2.1	<0.0001	0.001	**<0.0001**	<0.0001
miR-451a	-9.4±2.2	-6.9±2.8	-9.3±2.1	<0.0001	0.0001	0.74	0.0002
miR-199a-5p	-13.4±2.1	-11.9±2.5	-14.2±1.8	0.0002	0.008	0.09	0.0001
miR-3940-5p	-11.3±1.6	-12.0±2.0	-12.9±1.4	0.0003	0.12	**<0.0001**	0.03
miR-181c-5p	-11.8±1.0	-11.2±1.1	-12.1±1.1	0.0024	0.02	0.19	0.001
miR-663a	-10.6±2.0	-12.5±1.5	-6.7±1.3	<0.0001	0.0002	<0.0001*	<0.0001
miR-373-5p	-14.2±1.7	-13.6±1.7	-15.2±1.8	0.0016	0.14	**0.02**	0.0007
miR-199b-5p	-11.3±1.7	-8.4±1.6	-9.1±1.6	<0.0001	<0.0001	<0.0001*	0.02
miR-3200-5p	-11.7±1.3	-11.5±1.4	-12.6±1.1	0.0004	0.25	**0.004**	0.0002
miR-4738-3p	-16.0±2.1	-13.4±1.9	-13.8±1.9	<0.0001	<0.0001	<0.0001*	0.23
miR-3911	-8.2±4.5	-9.9±4.3	-12.4±4.0	0.0008	0.14	**0.0004**	0.009
miR-3907-p	-14.5±1.8	-11.5±1.9	-12.9±1.9	<0.0001	<0.0001	0.0008*	0.001

Table 2. Summary of candidate miRNAs expression among Normoalbuminuric, CKD 1/2 Rapid Progressor, and CKD 1/2 Non-Progressor Patients in individual plasma specimens.
*miRNAs that were in the opposite direction as those identified in pooled specimens.

Eight of the 20 candidate miRNAs were found to be expressed in urine specimens from CKD1/2 rapid progressors, Normoalbuminurics, and CKD1/2 non-progressors (Ct>30) and considered in group-wise comparisons between these subgroups. Three miRNAs (miR-3940-5p, miR-663a, and miR-3200-5p) were statistically different among these 3 groups (P<0.05), with miR-3940-5p not only having the strongest association (P=0.0006), but also being consistent with results observed in plasma specimens. Similar to its relative expression in plasma, miR-3940-5p's is increased in urine in Normoalbuminurics (log2 relative expression = -8.0) relative to CKD1/2 non-progressors and CKD1/2 rapid progressors and its expression is further reduced

in those with more advanced disease (CKD1/2 non-progressors log2 relative expression = -9.5 and CKD1/2 rapid progressors log2 relative expression = -10.1). Interestingly, miR-3940-5p targets *COL1A1*, a gene regulated by TGF-β and involved in TGF-β-induced fibrosis. Together, these data suggest that miR-3940-5p is a novel miRNA deregulated in diabetic nephropathy and one that may have therapeutic potential in the treatment of this disease.

3. Publications:

Data generated as part of this Pilot and Feasibility project has been selected for a platform presentation at the American Society of Nephrology's annual Renal Week meeting to be held in Philadelphia, PA in November 2014. Additionally, a manuscript detailing these findings is currently in preparation.