POSSIBLE ROLE OF microRNAs IN CKD PROGRESSION

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Introduction – Objectives

miRNAs are short non coding RNAs that regulate mRNA expression. Similar to other diseases, miRNAs have been found to be modified in renal tissue and urine during kidney disease and therefore could serve as markers or provide information on the pathophysiology of disease. In this study, in the context of the European project iMODE-CKD, we aimed to correlate changes in urinary miRNA abundance to progression of chronic kidney disease (CKD).

Workflow – Clinical Data

Discovery Phase

Extract RNA

Discovery Cohort
70 samples
-35 progressors
-35 non progressors

Next Generation Sequencing

Data analysis

63 Differentially expressed miRNAs

Differentially expressed proteins from the same samples (collaboration in the iMODE-CKD consortium)

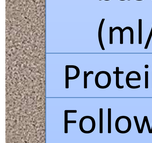
Prioritization of miRNAs

Data process
- **Ingenuity Pathway Analysis (IPA):** stringent filters (no cancer pathways, experimentally observed or high confidence binding of miRNA and mRNA-target)
- **statistical analysis**

8 miRNAs targeting 17 proteins from DE protein list

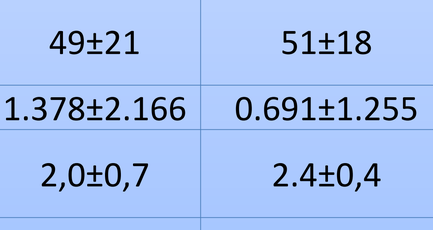
- **hsa-miR-324-5p**
- **hsa-miR-301b-3p**
- **hsa-miR-145-5p**
- **hsa-miR-410-3p**
- **hsa-miR-34c-5p**
- **hsa-miR-136-5p**
- **hsa-miR-449c-5p**
- **hsa-miR-708-5p**

Validation Phase



Extract RNA

Validation Cohort
52 samples
-28 progressors
-24 non progressors



TaqMan Low Density Array (TLDA)
microcards containing primer sets for the 8 selected miRNAs.

Data Analysis

Urinary miRNA candidate biomarkers for CKD progression

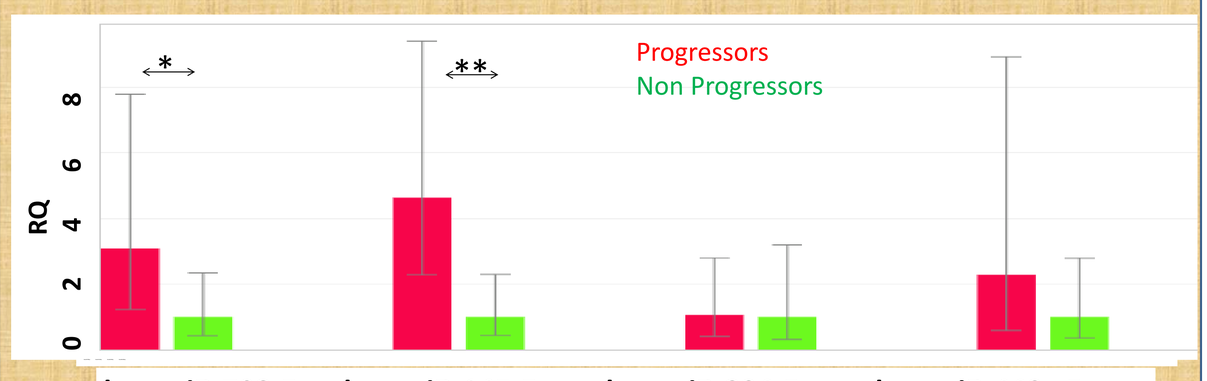
Table 1: Clinical data of the samples used in the study

Discovery Cohort	All n=70	Non progressors n=35	Progressors n=35	Significance
Gender (F/M)	23/47	11/24	12/23	P = 1.0000
Age (years)	65±15	63±15	67±15	P = 0.0964
Diabetes (Y/N)	25/45	11/24	14/21	P = 0.6179
baseline eGFR (ml/min/1.72)	44±16	47±16	41±16	P = 0.1154
Proteinuria (µg/µl)	0.471±0.866	0.158±0.260	0.784±1.120	P = 0.0285
Follow up duration (years)	2.6±0.4	2.6±0.4	2.6±0.4	P = 0.5213
%slope/year	-6±9	0.7±1.4	-13±8	P = 0.00001
Validation Cohort	**All n=52**	**Non progressors n=24**	**Progressors n=28**	**Significance**
Gender (F/M)	16/36	7/17	9/19	P = 0.8274
Age (years)	58±16	60±16	57±17	P = 0.5324
Diabetes (Y/N)	42/10	6/18	4/24	P = 0.3399
baseline eGFR (ml/min/1.72)	49±21	51±18	47±23	P = 0.4627
Proteinuria (µg/µl)	1.378±2.166	0.691±1.255	1.967±2.596	P = 0.0099
Follow up duration (years)	2,0±0,7	2.4±0,4	1.6±0,7	P = 0.0010
%slope/year	-13,4±21,8	0.8±1,9	-26.1±23,7	P = 0.0001

Results

Table 2: Abundance in NGS and TLDA for the 8 selected miRNAs

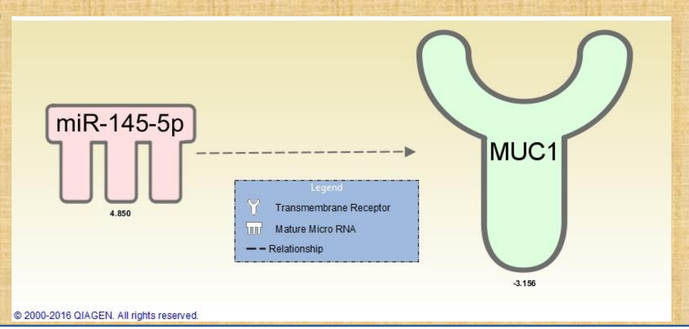
	Discovery Phase (NGS) 70 samples			Validation Phase (TLDA) 52 samples		
miRNA	**% of samples detected**	**Fold Change (Prog vs Non)**	**P-value**	**% of samples detected**	**Fold Change (Prog vs Non)**	**P-value**
hsa-miR-34c-5p	37,1 (26)	8,743	4,56E-21	15,4 (8)	4,508	0,332
hsa-miR-410-3p	37,1 (26)	1,734	4,64E-06	11,5 (6)	3,955	0,174
hsa-miR-324-5p	41,4 (29)	0,301	3,93E-05	21,2 (11)	1,062	0,517
hsa-miR-301b-3p	32,9 (23)	3,377	3,81E-04	3,8 (2)	0,698	-
hsa-miR-708-5p	**22,9 (16)**	**0,809**	**7,32E-04**	**25,0 (13)**	**3,941**	**0.040**
hsa-miR-145-5p	**14,3**	**4,850**	**1,39E-03**	**40,4 (21)**	**5,055**	**0,016**
hsa-miR-136-5p	37,1 (26)	0,973	1,84E-03	1,9 (1)	-	-
hsa-miR-449c-5p	34,3 (24)	4,108	3,93E-03	21,2 (11)	2,461	0,145



Boxplots of the Relative Quantity (RQ) of the targets per biological group in the validation study.

Reference group : Non Progressor (green)
Normalization : hsa-miR-30a

Strongest possible connection revealed in this study in relation to CKD



Conclusions

✓hsa-miR-145-5p and hsa-miR-708-5p were validated to be modified in urine of CKD patients with progressive disease.

✓Limited number of miRNAs were validated →

- Variability in the samples?
- Change in technology?
- Change in normalization?
- Change in cohort?

Future Plans

➢Treat renal cells with miRNA antagomirs for miR-708 and miR-145 and investigate the possible changes in their targets with LC-MS/MS → possibly reveal the direct regulation of the miRNAs to the proteins and enlighten prospects of the CKD progression.

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