Endogenous controls for miRNA analysis in renal cells and tissue

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OBJECTIVES

Quantitative real-time PCR (RT-qPCR) is considered a highly sensitive method for the quantification of micro RNAs (miRNAs). To normalize the expression results across various conditions and to correct for variations in experimental procedures, the expression of the miRNA of interest is compared to stably expressed reference miRNAs. **The aim of this study was the identification of reference miRNAs in human renal tissue and in renal proximal tubule cells under commonly applied conditions.**

Table. RT-qPCR selection of candidate miRNAs

Ranking	Discovery cohort		Validation cohort	
	miRNA	SD	miRNA	SD
1	**hsa-miR-345-5p**	**0.101**	**hsa-miR-345-5p**	**0,101**
2	**hsa-miR-301a-3p**	**0.111**	**hsa-miR-301a-3p**	**0,110**
3	**hsa-miR-92a-3p**	**0.112**	**hsa-miR-92a-3p**	**0,112**
4	hsa-miR-200a-3p	0.114	hsa-miR-200a-3p	0,114
5	**hsa-miR-26a-5p**	**0.115**	**hsa-miR-26a-5p**	**0,115**
6	hsa-miR-320a	0.120	hsa-miR-320a	0,120
7	**hsa-miR-152-3p**	**0.123**	**hsa-miR-152-3p**	**0,123**
8	hsa-miR-186-5p	0.124	hsa-miR-186-5p	0.124
9	hsa-miR-106a-5p	0.124	hsa-miR-106a-5p	0.124
10	hsa-miR-19b-3p	0.125	hsa-miR-19b-3p	0.126
18	**hsa-miR-103a-3p**	**0.135**	**hsa-miR-103a-3p**	**0.136**

Figure 1. Expression of six candidate reference miRNAs in RPTECs under basal conditions and under stimulation with different ligands.

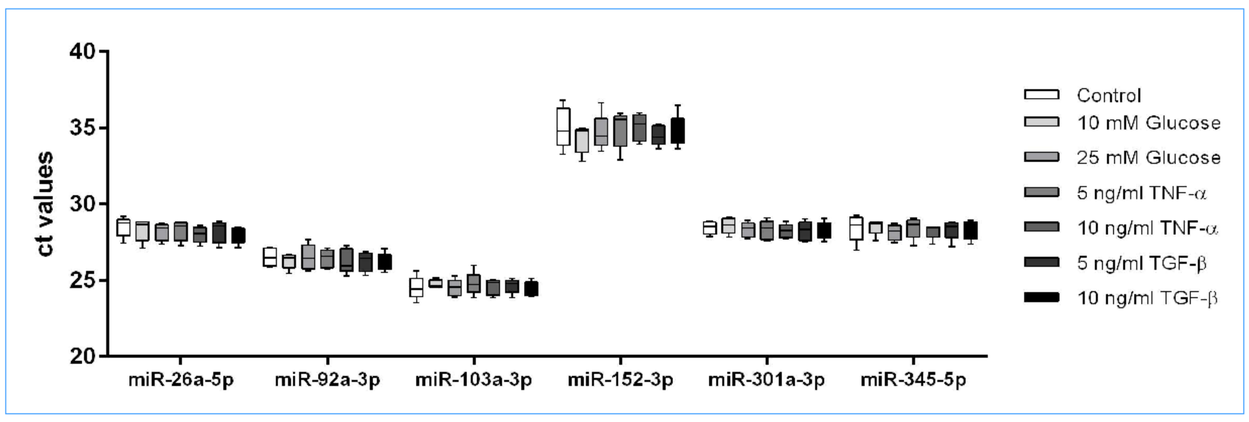
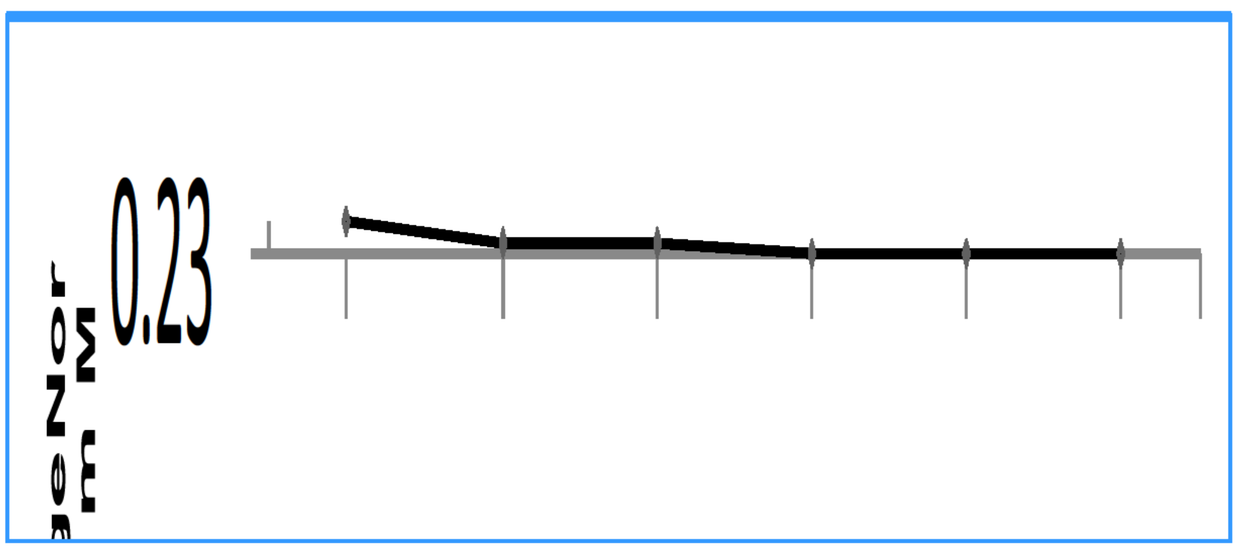


Figure 2. geNorm analysis of candidate reference miRNAs



METHODS

- Total RNA including miRNA was isolated from cryocut tissue sections from renal biopsy samples (n=97 patients and n=34 controls) using the miRNeasy® Mini Kit (Qiagen, Hilden, Germany). Total RNA was reverse transcribed using the Megaplex RT stem-loop primer pool (Life Technologies, Carlsbad, CA, USA), enabling miRNA specific cDNA synthesis of 755 different human miRNAs and small RNA controls. The cDNA was then pre-amplified by means of a 12-cycle PCR reaction with a miRNA specific forward primer and universal reverse primer. Finally, a dilution of pre-amplified miRNA cDNA was used as input for a 40-cycle RT-qPCR reaction with miRNA specific hydrolysis probes and primers (Life Technologies, Carlsbad, CA, USA)
- Human renal proximal tubular epithelial cells (RPTEC/ TERT1) were incubated with 10 mM, 25 mM Glucose, 5 ng/ml TNF-α and 10 ng/ml TGF-β respectively for a period of 24 hours. Untreated RPTECs served as control for all experiments
- For the incubation with 10 ng/ ml TGF-β an additional time course (0, 4, 24, 48, 72 hours) was generated
- Total RNA was extracted from RPTECs using the mirVana™ miRNA Isolation Kit (Ambion). cDNA synthesis was performed using the miRCURY LNA™ Universal cDNA synthesis Kit II (Exiqon). MicroRNA quantitative RT-qPCR was performed using ExiLENT SYBR® Green master mix and microRNA LNA™ PCR primer sets (Exiqon)
- We used geNorm analysis also for the evaluation of expression stability of reference miRNAs

RESULTS

We performed miRNA-profiling on a set of 131 renal biopsy samples from proteinuric glomerulopathies, and from healthy controls, respectively. From a list of top ranked stably expressed miRNAs, we selected **miR-345-5p, miR-301a-3p, miR-92a-3p, miR-26a-5p, miR-152-3p** and **miR-103a-3p** as best candidates for reference miRNAs (Table). We studied the expression of these 6 miRNAs in renal proximal tubule cells *in vitro* under basal conditions and under stimulation with glucose, TNF-a and TGF-ß (Figure 1). All six miRNAs showed stable expression as indicated by geNorm algorithm (M < 0.5) (Figure 2). However, **miR-26a-5p** showed highest stability of expression, while **miR-152-3p** showed least stable expression and also the highest Ct values. These results were confirmed under stimulation with TGF-β in a time course experiment.

CONCLUSIONS

We generated and validated a list of **stably expressed miRNAs** in both **human renal tissue** and in **renal proximal tubule cells**, which can serve as reference genes for quantification studies.

REFERENCES

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