miRNA21 EXPRESSION IN URINE AND RENAL TISSUE IN RATS WITH UNILATERAL URETERAL OBSTRUCTION

Marina Parastseva, Olga Beresneva, Mikhail Zaraisky, Ivan Kayukov, Galina Ivanova,
Anatoly Kucher, Mokhamad Khasun, Alexey Smirnov
First Pavlov State Medical University
St.-Petersburg, Russia

Objectives:

TexmiRNAs are endogenous non-coding RNAs that are ~22 nucleotides in length and can have structural, enzymatic and regulatory functions. miRNAs play important roles in the progression of renal fibrosis. miRNA21, through a feed-forward loop and a downstream mediator of transforming growth factor-β (TGF-β), amplifies TGF-β signaling and promotes fibrosis. miRNA21 is high on the list of non-coding, small, regulatory RNAs that promote renal fibrosis and emerges as a serum biomarker for kidney diseases, but many questions await answers. In this regard we attempted to evaluate the expression miRNA21 in urine and renal tissue in rats with unilateral ureteral obstruction (UUO) – the classical model of experimental tubulointerstitial fibrosis

Methods:

Experiment was performed on ten male Wistar rats. One day before the operation in experimental animals, placed in a metabolic chamber, a control sample of urine (C) was collected. Under general anesthesia ligation of the left ureter was performed. On the ureter two ligatures were applied using silk 2/0 Silkam. Ureter cut between the ligatures. Fourteen days after ligation rats again placed in metabolic chamber and urine was collected (urine from intact kidney – I). The animals take out from the experiment. Urine from pelvis of kidney with UUO (urine from obstructed kidney – O) received by syringe. Expression of micro RNA21 in all three samples of urine ($UmiRNA21_C$, $UmiRNA21_I$, $UmiRNA21_O$; respectively) and in tissue of kidney with UUO ($KmiRNA21_O$) or intact kidney ($KmiRNA21_I$) were determined. MiRNA21 and reference RNA U6 cDNA was prepared based on StemLoop-technology. Expression was examined using semiquantitative RT-PCR protocol. Calculation of the relative gene expression level of miRNA21 was done according to the standard procedure $2^{-\Delta Ct}$. All data are presented as median [interquartile range]. Wilcoxon pairs test and rank Spearmen correlation coefficient were used

Results:

$UmiRNA21_I$ (3.78[2.0-5.28]) and $UmiRNA21_O$ (3.78[3.25-3.82]) were significant higher than $UmiRNA21_C$ (1.15[0.71-1.74]; P=0.0125 and P=0.0069, respectively). miRNA21 expression in urine from intact kidney and organ with ureteral obstruction were practically identical (P=0.953). In kidney with UUO the tissue levels of miRNA21 expression (19.22[4.92-45.25]) was more than in intact organ (9.38[0.66-27.86]). However this difference did not achieve statistical significance (P=0.0926). There was manifest direct correlation between tissue levels of miRNA21 expression in kidney with UUO and $UmiRNA_I$ (RS=0.770, P=0.0092). On the other hand any significant associations between $UmiRNA21_I$ and $UmiRNA21_O$ (RS=-448; P=0.1943), $KmiRNA21_I$ and $UmiRNA21_I$ (RS=-0.236, P=0.5109), $KmiRNA21_O$ and $UmiRNA21_O$ (RS=0.117, P=0.7484) or $KmiRNA21_O$ and $UmiRNA21_I$ (RS=-0.212, P=0.5563) were not revealed.

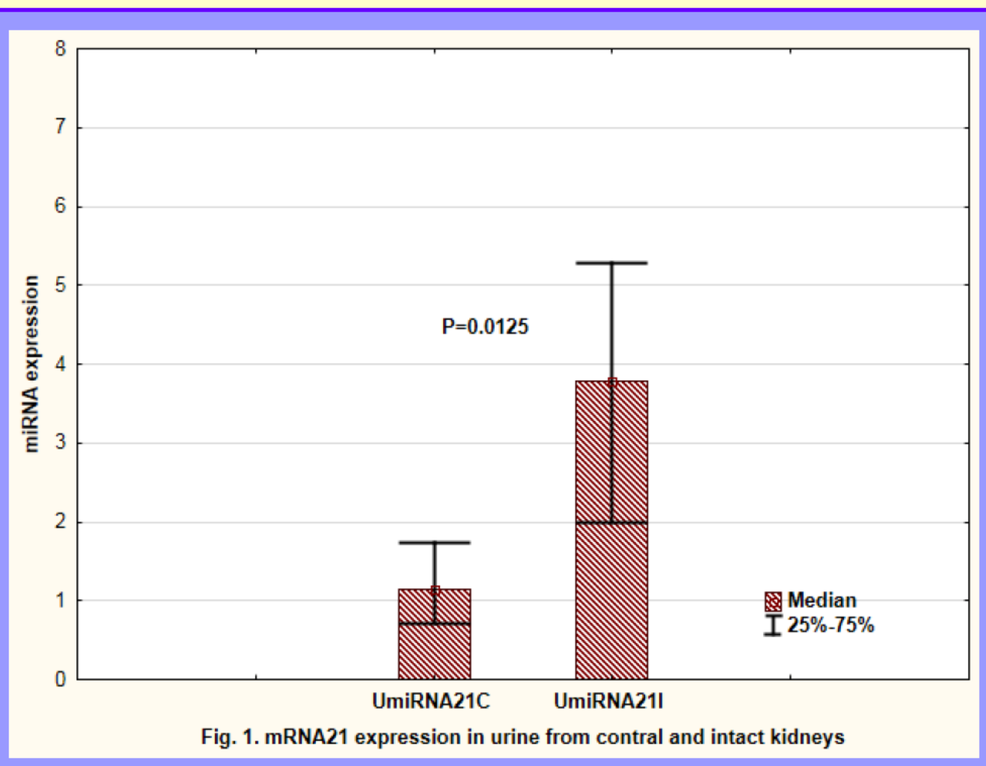

Fig. 1. mRNA21 expression in urine from contral and intact kidneys

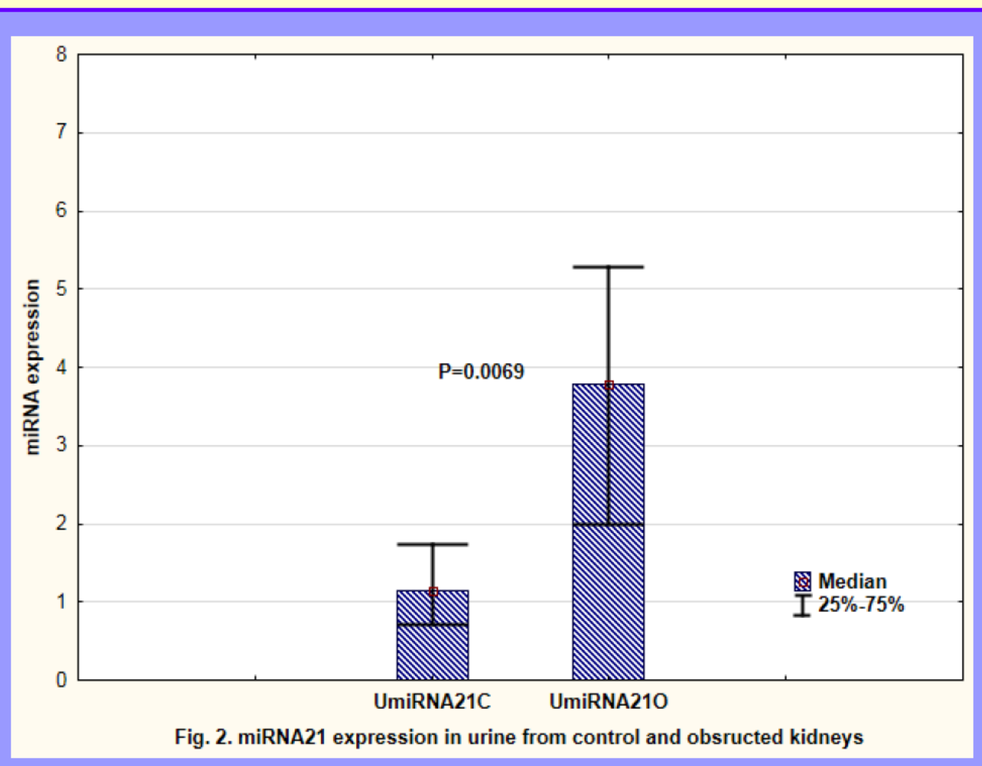

Fig. 2. miRNA21 expression in urine from control and obsructed kidneys

Conclusions:

Obtained results, in at least do not contradict the assumption that the UMO can activate the expression of miRN21s in the kidney tissue. Perhaps in these conditions this miRNA from kidney with obstruction released into systemic circulation and further in contralateral organ. The intact kidney accelerates urinary excretion of miRNA21. Thus, OOM can cause peculiar changes in the expression, distribution and excretion of micro RNA, but its role in the development of renal tubulointestitsial fibrosis requires further studies.

DOI: 10.3252/pso.eu.53era.2016