MIR-1915 AND MIR-1225-5P REGULATE THE EXPRESSION OF CD133, PAX-2 AND TLR2 IN ADULT RENAL PROGENITOR CELLS

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INTRODUCTION AND AIMS

Adult renal progenitor cells (ARPCs), identified in the tubular compartment and in the Bowman's capsule, are positive for PAX2, CD133, CD24 and have multipotent differentiation ability (1-4). Recent studies indicate that microRNAs (miRNAs), a class of noncoding small RNAs that participate in the regulation of gene expression, may play a key role in stem cell self-renewal and differentiation. Distinct sets of miRNAs are specifically expressed in pluripotent stem cells but not in adult tissues, suggesting a role for miRNAs in stem cell self-renewal (5-6).

We compared miRNA expression profile of ARPCs with that of renal proximal tubular epithelial cells (RPTECs) and of mesenchymal stem cells (MSCs) with the aim to found distinct sets of miRNAs that may be specifically expressed both in tubular and glomerular ARPCs and to obtain the whole-genome miRNA expression profile of ARPCs.

METHODS

miRNA global expression profiles were obtained by Agilent microarray technology. Real time PCR was used for the validation of microarray results. miRNA targets were predicted by means of miRBase 17.0, TargetScan 5.2, PicTar and RNA22 1.0 algorithms. Mimic transfection, cell immunofluorescence, western blot and flow cytometric analysis were used for validation of miR-1915 and miR-1225-5p effect on CD133, PAX2 and TLR2 expression.

RESULTS

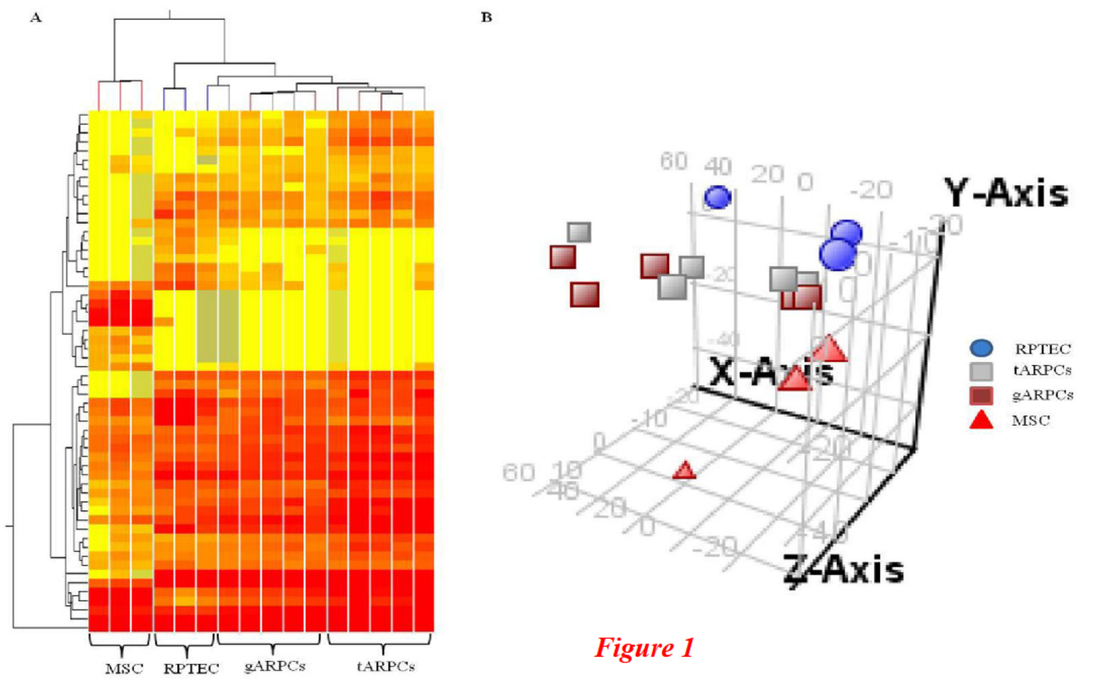


Figure 1

Figure 1. We found distinct sets of miRNAs that were specifically expressed both in tubular and glomerular ARPCs. Figure 1 shows unsupervised hierarchical clustering and principal component analysis (PCA) of miRNA expression profile. miRNA expression pattern of 5 tARPC and 5 gARPC, 3 RPTEC and 3 MSC different clones were examined using Agilent array composed of 1205 human miRNAs. A total of 327 miRNA resulted expressed between different cell types (false discovery rate < 0.01). (A) The 2-D hierarchical clustering and (B) the principal component analysis, PCA, showed that miRNA expression profile was different among MSC, RPTECs and ARPCs, whereas it was very similar between gARPCs and tARPCs.

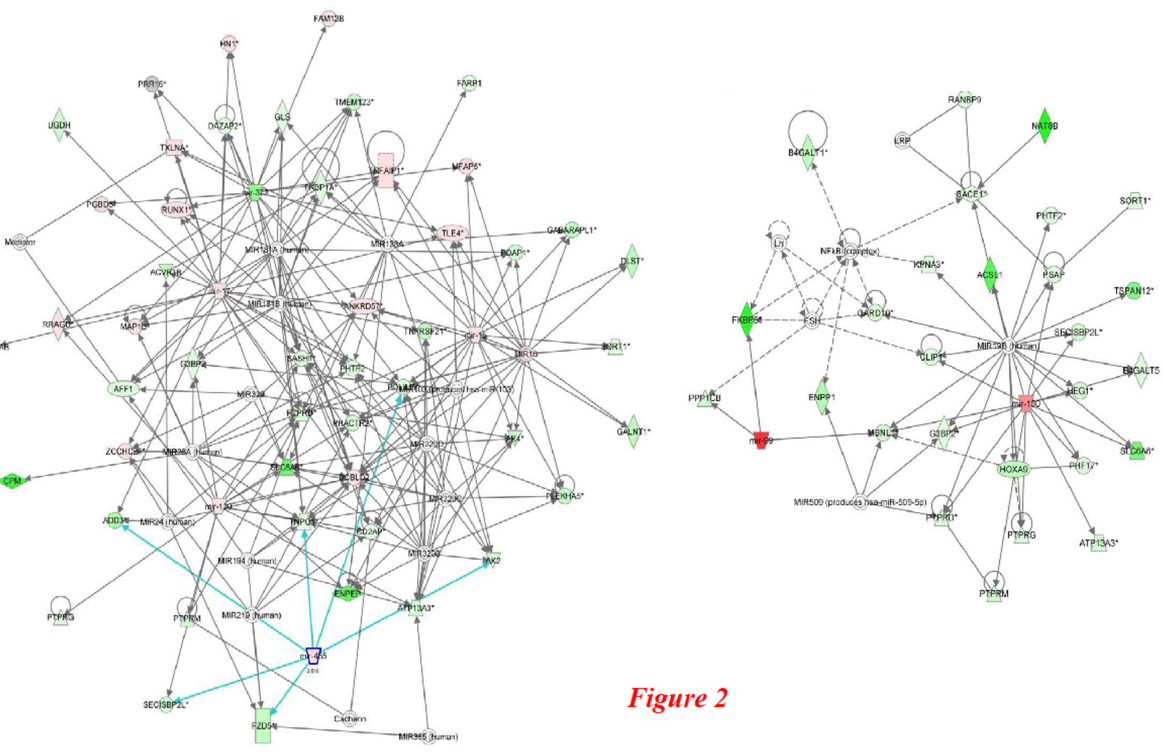
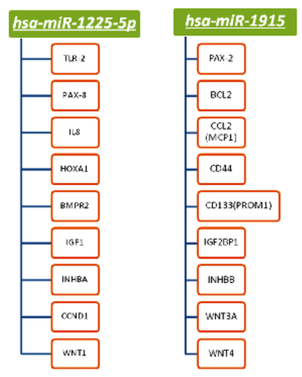


Figure 2

Figure 2. Network analysis of the top modulated miRNAs identified by microarray. Among miRNAs modulated specifically in ARPCs, we found several miRNAs that were predicted to target genes involved in the regulation of WNT/B-catenin signals, such as FZD5, SLC6A6, and HOXA9 and that could be one of the key regulators of the stem cell identity, including self-renewal and cell fate decisions. The network was algorithmically constructed on the basis of the functional and biological connectivity of miRNAs and genes. The network is graphically represented as nodes (miRNAs or genes) and edges (the biological relationship between miRNAs/genes). Merging miRNAs with ARPC modulated genes, a significant network was identified in which miR-130 regulated SLC6A6 and HOXA9 involved in the Frizzled signalling. Red and green shaded nodes represent up- and down-regulated genes/miRNAs, respectively.



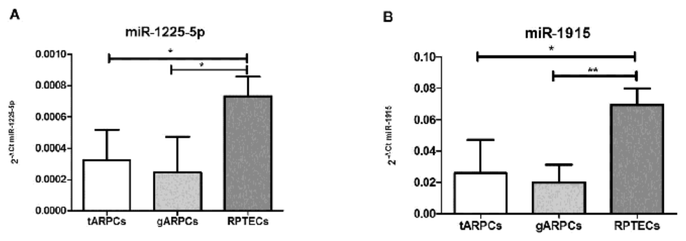


Figure 3

Figure 3 shows that miR-1915 and miR-1225-5p regulate the expression of important markers of renal progenitors, such as CD133 and PAX2, and important genes involved in the repair mechanisms of ARPCs, such as TLR2. Expression levels of miR-1225-5p and miR-1915, evaluated also by RT-PCR, were found significantly lower in tARPCs and gARPCs compared to RPTECs.

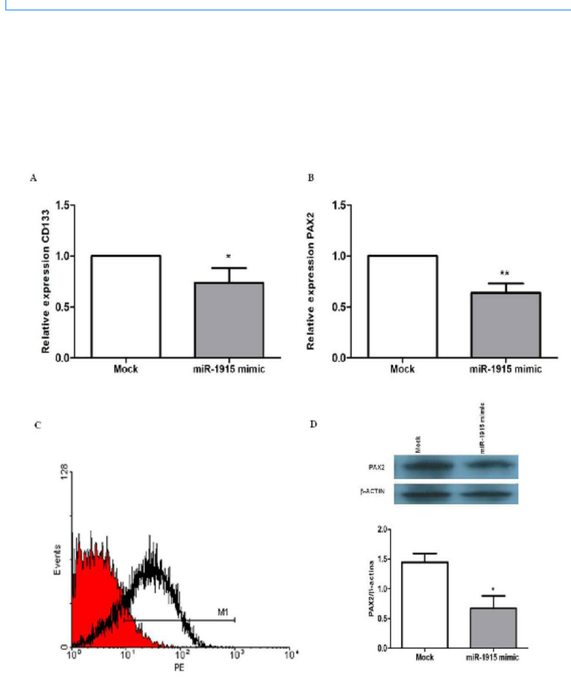


Figure 4

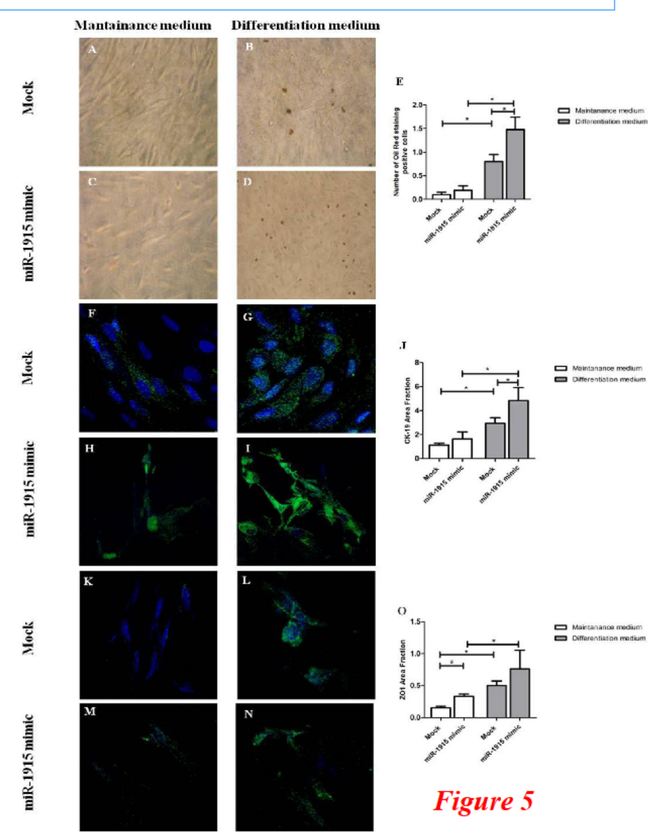


Figure 5

Figure 4 demonstrates that the expression of both the renal stem cell markers CD133 and PAX2 depends on lower miR-1915 levels and that the increase of miR-1915 levels improved capacity of tubular ARPCs to differentiate into adipocyte-like and epithelial-like cells. Finally, we found that the low levels of miR-1225-5p were responsible for high TLR2 expression in tubular ARPCs. miR-1915 regulates CD133 and PAX2 in ARPCs. (A-B) CD133 and PAX2 expression levels were analyzed by real-time PCR following transfection with miR-1915 mimic. Increasing the amount of miR-1915 within ARPCs resulted in a 1.5 fold reduction of both CD133 and PAX2 mRNA levels. Expression data were normalized on the housekeeping gene β-actin. (C) Surface marker expression of CD133, as measured by flow cytometry, resulted in a large reduction (8% vs 96% of mock transfection control) following transfection with 50 nM miR-1915 mimic. Red area represents the transfected condition. Data are representative of three independent experiments (D) Transfection of ARPCs with 100 nM miR-1915 mimic resulted in a 2 fold reduction of PAX2 protein expression, as shown by Western blot. β-actin was used as endogenous control. **Figure 5** shows that miR-1915 upregulation favors ARPC differentiation into adipocyte-like cells and (A-D) and epithelial-like cells (F-N).

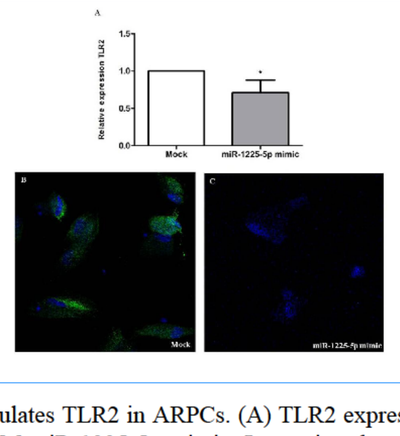


Figure 6

Figure 6 shows that miR-1225-5p regulates TLR2 in ARPCs. (A) TLR2 expression levels were analyzed by real-time PCR following transfection with 25 nM miR-1225-5p mimic. Increasing the amount of miR-1225-5p within ARPCs resulted in a 1.3 fold reduction of TLR2 mRNA levels 24 hours after transfection.
(B-C) TLR2 protein expression after transfection with 50 nM miR-1225-5p mimic. A strong reduction of TLR2 in ARPC was found after 3 days from transfection with miR-1225-5p mimic, as shown by immunofluorescence staining.

CONCLUSIONS

Together, miR-1915 and miR-1225-5p seem to regulate important traits of renal progenitors: the stemness and the repair capacity,. They could be used, in the future, to modulate the features of renal progenitors for therapeutic purposes.

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