

## The role of serum microRNAs in the pathogenesis of IgA nephropathy

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IgA nephropathy is the most common form of glomerulonephritis worldwide with approximately 30% of patients progressing to end stage renal disease within 10 years of diagnosis, and requiring renal replacement therapy. Diagnosis requires an invasive kidney biopsy and currently there is no reliable way to predict progression early on. MicroRNAs (miRs) are short non-coding nucleotides that post transcriptionally regulate gene expression and have been shown to be dysregulated in various diseases including IgAN. The aim of this study was to investigate a potential role of serum miRs in IgAN progression.

Blood samples were collected from IgAN patients who were clinically defined as progressors (IgANp) and non-progressors (IgANnp), as well as membranous nephropathy (MN) patients (CKD positive controls) and healthy subjects. Next Generation Sequencing (NGS) was performed on processed sera. An independent set of sera were used to validate identified miRs using (RT-qPCR). Exosomes from serum were isolated using the Total Exosome RNA and protein Isolation Kit and exosome number was quantified using the EXOCET Quantification Kit.

MiRs which exhibited a  $\geq 1.5$  fold change in expression between IgAN and healthy subjects and MN were selected from the NGS data. Nine candidate miRs met these criteria; miRs -223-3p, -425-5p, 143-3p, -29a-3p and -339-5p, -122-5p, -483-5p, -144-5p and -96-5p. However, none of the nine miRs retained this significant difference following validation by RT-qPCR. Two of the miRs (miRs -122-5p and -483-5p) exhibited a 1.5 fold difference in expression between IgANp and IgANnp, healthy subjects and MN in the discovery cohort, which remained significantly differentially expressed between IgANp compared to IgANnp and healthy subjects but not compared to MN following validation. However, expression levels of miRs -122-5p and -483-5p in serum-derived exosomes were, significantly differently expressed in IgANp compared to IgANnp and healthy subjects and this time also compared to MN. ROC curves revealed that the area under the curve (AUC) between IgANp vs IgANnp for miR-483-5p was 1.00 ( $p=0.0004$ ) and for miR-122-5p was 0.92 ( $p=0.018$ ). Results also revealed that IgAN patients sera (both IgANp and IgANnp) contained 1.7 fold more exosomes compared to healthy subjects ( $p=0.02$ ) and 1.6 fold more than MN patient sera ( $p=0.04$ ) but there was no significant difference in exosome number between IgANp vs IgANnp. Moderate correlations were observed between serum exosomal miR-483-5p and proteinuria (U<sub>PCR</sub>) ( $R^2=0.310$ ,  $p=0.01$ ), and weak but significant correlations were found between exosomal miR-483-5p expression and IgAN-associated serum analytes TNFR1 ( $R^2=0.2462$ ,  $p=0.0159$ ) and CD27 ( $R^2=0.1107$ ,  $p=0.0385$ ), miR-122-5p correlated with TNFR1 levels ( $R^2=0.1191$ ,  $p=0.0414$ ).

The data suggest that exosomal miRs could serve as biomarkers predicting IgAN progression and may also be involved in the pathogenesis of IgAN.