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Background and objectives The authors have previously used miRNA analysis in the peripheral blood of patients with SLE to identify novel molecular pathways contributing to immune response deregulation. As disease phenotype results from gene expression changes not only in the immune effector cells but also in the target organs, the authors sought to identify novel genes within the kidney in lupus nephritis.
Materials and methods The authors isolated RNA from renal biopsy samples of 12 subjects with proliferative or membranous lupus nephritis and five healthy controls. MiRNA expression was analysed by TaqMan Low Density Arrays (TLDA human miRNA v1.0). Two different bioinformatic algorithms (TargetScan, PicTar) were used for the prediction of the gene targets of selected differentially expressed microRNAs. Experimental validation of the miRNA-gene target interaction was performed by luciferase assay and real-time PCR analysis. MicroRNA expression levels were monitored in kidney extracts from 2 months and 6 months lupus-prone (NZB/W F1) mice by real-time PCR.
Results A 24-microRNA signature defines human lupus nephritis with 9 microRNAs up-regulated and 15 microRNAs down-regulated compared to normal tissue. Among them, miR-422a exhibited the highest up-regulation (17.2-fold) relative to control tissues. Bioinformatic analysis predicted that miR-422a has a binding site in the 3'UTR of kallikrein 4 (KLK4) gene. Overexpression of miR-422a suppressed by 65% KLK4 luciferase activity and by 82% KLK4 mRNA levels in 293 cells. In order to monitor miR-422a/KLK4 expression during lupus nephritis progression, the authors used NZB/W F1 lupus prone mice. During early stages (2 months old NZB/W F1 mice) miR-422a was 4.1-fold up-regulated, while KLK4 mRNA levels were 3.4-fold down-regulated while at later stages (6 months) miR-422a was 9.4-fold up-regulated and KLK4 mRNA levels were 7.6-fold down-regulated. Experiments using antagomirs for miR-422 to further define its contribution to nephritis together with the construction of gene networks -by combining peripheral blood and renal microRNA data, are in progress.
Conclusions The kallikrein family of genes has an important role in regulating inflammation, apoptosis, coagulation and fibrosis in the kidneys. Our data implicate regulation of KLK4 by miR-422a as a key pathogenetic event in lupus nephritis and suggest a potentially protective role of these genes in immune-mediated renal disease.

7. Genetics and regulation of immune response

1 IDENTIFICATION OF A NOVEL MICRORNA-GENE CIRCUIT IN HUMAN LUPUS NEPHRITIS: EVIDENCE FOR MODULATION OF KALLIKREIN GENES BY MIR-422A

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