Exosomal miRNAs: potential biomarkers and therapeutic vehicles for CKD

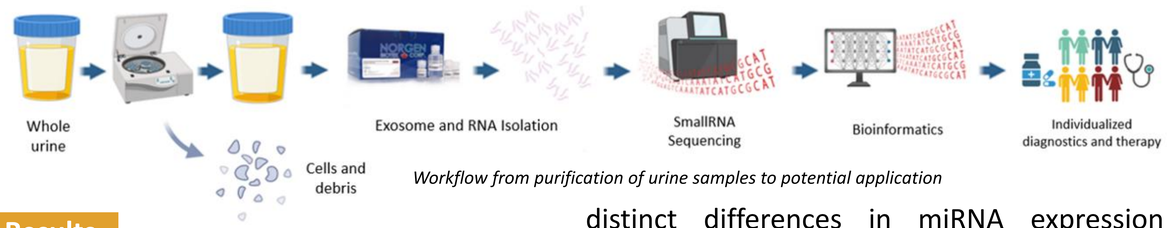
GERHARD DOMAGK Nachwuchsförderprogramm

Universitätsmedizin GREIFSWALD

Chronic kidney disease (CKD) affects about 10 % of the world's population and is expected to be among the top five causes of years of life lost by 2040.[1] Since current diagnostic parameters mainly detect CKD in its advanced stages, there is an urgent need for new biomarkers. Recent studies have shown **altered miRNA expression patterns in urinary exosomes**, endosome-derived extracellular vesicles, of CKD patients.[2] Due to their **stability** and **accessibility**, urinary exosomal miRNAs are considered **promising biomarkers**. Additionally, exosomes have gained attention as **therapeutic vehicles** because of their **low cytotoxicity** and **limited immunogenicity**.[3] Moreover, selective engineering allows optimization of exosomal cargo and the modulation of the targeting process, ensuring protective and efficient delivery to target cells. Encapsulated miRNAs remain functional and therefore act as posttranscriptional regulatory elements in recipient cells.[4] With these characteristics, exosomes can be considered as a promising tool for diagnosis and therapy of CKD, paving the way for personalized medicine in nephrology.

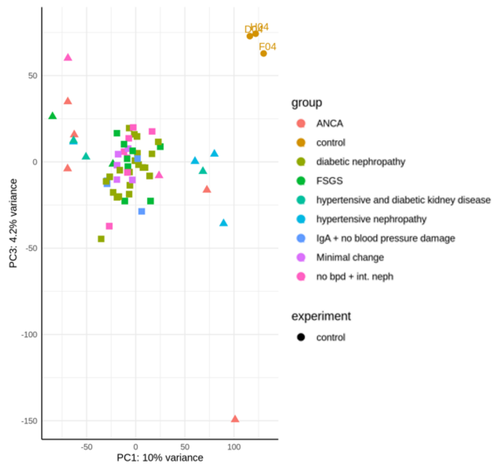
Exosomal miRNA in diagnostics

Methods Urine samples of 69 CKD patients and 5 controls were collected and processed to isolate exosomal small RNAs, which then were sequenced to analyse miRNA expression patterns. Clinical data was gathered and subgrouping performed depending on CKD entity.



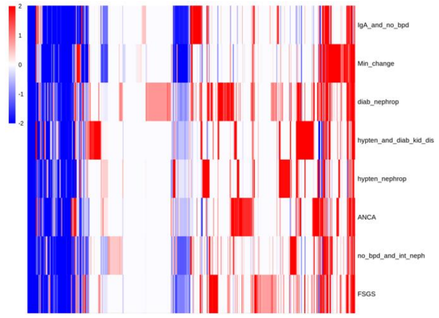
Workflow from purification of urine samples to potential application

Results



PCA showing a separation of controls and diseases

Principle component analysis (PCA) revealed a separation of controls and diseases based on differentially expressed small RNAs levels. However, no clear separation was observed among the various CKD entities.
Hierarchical clustering of miRNA expression data across different CKD entities showed distinct differences in miRNA expression patterns. Certain miRNAs clustered based on similar expression profiles, with specific miRNAs showing significantly differential expression levels among the various CKD entities.



Heatmap showing expression levels of miRNAs across different CKD entities

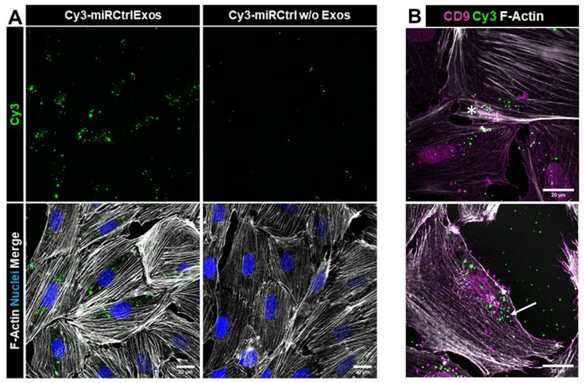
What's next?

- **Evaluation of differentially expressed miRNAs** between control and disease as well as between disease entities
- **Correlation** between miRNA expression patterns and **clinical parameters**

Exosomes as therapeutic vehicles for small RNAs

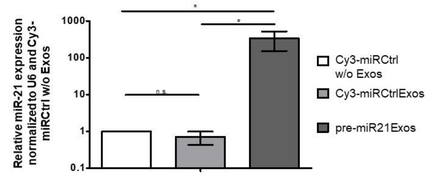
Methods Exosomes from cultured murine podocytes were isolated and transfected with Cy3-labeled miRNA or siRNA and then co-cultured with podocytes. Exosomal cargo uptake was characterized using fluorescence microscopy. Additionally, exosomes were transfected with pre-miR-21 or Filamin A (FlnA)-siRNAs. To assess transfection efficiency, RT-qPCR and Western blot analysis were performed on co-cultured podocytes.

Results



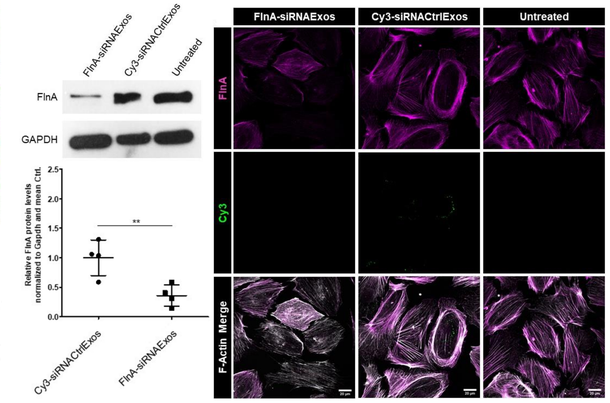
A - Podocytes treated with transfected exosomes showed intracellular Cy3-signals
B – Intracellular Cy3-signals detected in CD9-positive and negative spots

Cultured podocytes internalised cargo of exosomes loaded with Cy3-miRNA. Staining for the exosomal marker protein CD9 and other endosome markers illustrate endocytotic exosomal cargo uptake. Treatment with pre-miR-21 loaded exosomes led to upregulation of miR-21 in podocytes.



Transfection with pre-miR-21 loaded exosomes upregulates miR-21 expression

The treatment of cultured podocytes with exosomes transfected with FlnA-siRNA resulted in internalisation and subsequent downregulation of FlnA protein expression.



Exosomes loaded with FlnA-siRNA down-regulate FlnA expression in podocytes

Conclusion

- Exosome loading with fluorescently-labelled small RNAs is **suitable for exosome tracking approaches**.
- Transfection of exosomes with small RNAs **enables functional transfer**, making them a potential therapeutic vehicle.

Luzia Maron · Institut für Anatomie und Zellbiologie, AG Endlich · Universitätsmedizin Greifswald

[1]Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. Lancet (London, England) 2018; 392(10159):2052–90.
[2]Lange T, Artelt N, Kindt F, Stracke S, Rettig R, Lendeckel U et al. MiR-21 is up-regulated in urinary exosomes of and kidney disease patients and after glomerular injury. J Cell Mol Med 2019; 23(7):4839–43.
[3]Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y et al. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. J Nanobiotechnology 2022; 20(1):279.
[4]Munir J, Yoon JK, Ryu S. Therapeutic miRNA-Enriched Extracellular Vesicles: Current Approaches and Future Prospects. Cells 2020; 9(10).