



Circular RNAs as non-invasive urinary biomarker of kidney diseases

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Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Dr. Ying Zhao (Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China).

Response to: Moore JB 4th, Merchant ML, Uchida S. Circular RNAs as diagnostic tool for renal transplant patients with acute rejection. *Ann Transl Med* 2019;7:S302.

Submitted Jan 01, 2020. Accepted for publication Jan 29, 2020.

doi: 10.21037/atm.2020.01.115

View this article at: <http://dx.doi.org/10.21037/atm.2020.01.115>

We thank Moore *et al.* for their stimulating editorial commentary on our recently published article “Circular RNAs in Urine of Kidney Transplant Patients with Acute T Cell-Mediated Allograft Rejection.” (1).

Acute T cell-mediated allograft rejection episodes eventually lead to chronic allograft dysfunction with massive impact on patient outcome (2). Acute rejections are detected by elevated levels of serum creatinine and subsequent renal biopsy analysis. However, increases in serum creatinine are often delayed and renal biopsies bear high risks for complications. Correspondingly, we agree with Moore *et al.* that novel non-invasive diagnostic tools are of ultimate interest as timely application of immunosuppressants is crucial for successful allograft stabilization.

Circular RNAs (circRNAs) are single stranded and covalently closed circular RNA transcripts that were first discovered in 1976 as viroids with pathogenic activity towards certain higher plants (3). Thereupon, circRNAs were overlooked as mis-splicing events yielding solely low expression levels. In 2012 and 2013 though, Salzman *et al.*, Jeck *et al.* and Memczak *et al.* made the groundbreaking discovery of thousands of ubiquitously expressed circular RNAs using both advanced next generation sequencing and adapted bioinformatic algorithms (4). CircRNAs are formed by backsplicing when a 5' splice donor site is joined to an upstream 3' splice acceptor site. CircRNA generation is favored in genomic regions with long flanking complementary introns containing inverted repeat elements, such as Alu repeats. Backsplicing is further

processed by the canonical splicing machinery with the support of trans-acting RNA binding proteins (RBPs), such as FUS, the protein quaking, NF90 and NF110 (4). Of note, circRNAs represent molecular features that are particularly useful as biomarkers. First, resistance towards exonucleases make circRNAs stable in extracellular fluids, thereby yielding long half-lives exceeding 48 hours (4,5). Second, circRNAs are abundant in various human cell types. And third, circRNA expression patterns are diverse among different cell types (6). We thus hypothesized, that differential circRNA expression might be a direct surrogate of deteriorations in the kidney without the need of an invasive renal biopsy. In a first circRNA study we found *circular RNA sponge of miR-126 (ciRs-126)* highly increased in blood of patients with acute kidney injury (AKI) compared to controls. Further analysis revealed that *ciRs-126* acts additionally as an independent predictor of survival (7). In our next circRNA study, discussed in this letter to the editor, we tested whether circRNAs are detectable in urine as well and if differential expression of urinary circRNAs may identify patients with acute T cell-mediated renal allograft rejection. We measured increased levels of *hsa_circ_0001334* recognizing patients with acute rejections already at subclinical timepoints. In addition, the decrease of kidney function was positively correlated with elevated levels of *hsa_circ_0001334* one year after transplantation (1). As highlighted by the editorial authors, this novel diagnostic approach may prevent future invasive renal biopsies in transplant patients and may represent a rapid diagnostic

procedure.

As pointed out in the editorial commentary, our findings are based on a single-center study. We thus agree, that more patients in a multi-center setting are highly desired for future validations of *hsa_circ_0001334* as an urinary biomarker. With respect to the ideal molecular properties of circRNAs, we also suggest utilizing urinary circRNAs as novel non-invasive biomarkers of other kidney diseases.

As noted in the editorial commentary, the corresponding linear mRNA minichromosome maintenance complex component 2 has previously been reported as biomarker of renal tumors (8) and might thus indicate renal origin of *hsa_circ_0001334* as well. Our study demonstrated a close relation between acute rejections and urinary circRNA levels. Recent literature indicates that nucleic acids are primarily packaged into exosomes and are thereby released into the extracellular space. Exosomes are small vesicles between 40–100 nm. They are secreted by most cell types upon fusion of multivesicular endosomes with the cell membrane. Interestingly, RNA sequencing has shown that circRNAs are highly expressed in exosomes compared to their donor cells (9). In addition, exosomes have been found in renal tissue and in released urinary exosomes deriving from all types of epithelial cells along the nephron (10). CircRNAs in exosomes can thereby provide direct insights into the whole kidney. In contrast, renal biopsies solely represent one or more cylinder of the kidney. Further work on the exact release mechanisms of *hsa_circ_0001334* in our study and urinary circRNAs in general is thus highly suggested.

CircRNAs are expressed in a tissue-specific manner, are highly conserved between species (human and mouse) (4) and are differentially expressed upon disease induction (1,7) suggesting an important role in cell biology. Despite the fact that our study provided bioinformatic insights for potential microRNA bindings sites, we suggest, as mentioned by the editorial commentary, further studies addressing functional analyses. In general, the cellular location of circRNAs may determine their mechanism of action. Whereas the vast majority of exonic circRNAs are cytoplasmic, intronic RNAs are mostly expressed in the nucleus (4). Although the elucidation of circRNA function is still in its infancy, important cellular mechanisms have already been identified. First, circRNAs can act as miRNA sponges, thereby preventing target mRNAs repression. Second, circRNAs expressing motifs for RBP can sponge their protein function. Third, circRNAs are described as enhancer of protein function, as exemplified for the RNA polymerase II complex with the U1 small nuclear

ribonucleoprotein. Fourth, circRNAs can scaffold proteins by bringing enzymes and substrates into close proximity to regulate reaction kinetics, as demonstrated for phosphatases, acetylases and ubiquitin ligases. Fifth, circRNAs facilitate protein recruitment to specific cellular loci. Sixth, circRNAs containing internal ribosome entry site (IRES) elements and AUG sites can act as templates for translation, thereby generating specific peptides (4).

Taken together, our study established tools for the biomarker discovery of urinary circRNAs and specifically identified urinary *hsa_circ_0001334* as a novel non-invasive biomarker of acute T cell-mediated renal allograft rejection at subclinical timepoints. This study can be helpful for future collaborative work at the conjunction of clinical- and basic research using the promising potential of circRNAs as diagnostic tool.

Acknowledgments

Funding: We acknowledge the support of the Swiss National Science Foundation to JM Lorenzen and the National Centre of Competence in Research (NCCR) “Kidney. CH – Kidney Control of Homeostasis” to JM Lorenzen and M Kölling.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Kölling M, Haddad G, Wegmann U, et al. Circular RNAs

- in Urine of Kidney Transplant Patients with Acute T Cell-Mediated Allograft Rejection. *Clin Chem* 2019;65:1287-94.
2. Almond PS, Matas A, Gillingham K, et al. Risk factors for chronic rejection in renal allograft recipients. *Transplantation* 1993;55:752-6; discussion 756-7.
 3. Sanger HL, Klotz G, Riesner D, et al. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci U S A* 1976;73:3852-6.
 4. Kristensen LS, Andersen MS, Stagsted LVW, et al. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet* 2019;20:675-91.
 5. Memczak S, Papavasileiou P, Peters O, et al. Identification and Characterization of Circular RNAs As a New Class of Putative Biomarkers in Human Blood. *PLoS One* 2015;10:e0141214.
 6. Li H, Ning S, Ghandi M, et al. The landscape of cancer cell line metabolism. *Nat Med* 2019;25:850-60.
 7. Kölling M, Seeger H, Haddad G, et al. The Circular RNA ciRs-126 Predicts Survival in Critically Ill Patients With Acute Kidney Injury. *Kidney Int Rep* 2018;3:1144-52.
 8. Dudderidge TJ, Stoeber K, Loddo M, et al. Mcm2, Geminin, and KI67 define proliferative state and are prognostic markers in renal cell carcinoma. *Clin Cancer Res* 2005;11:2510-7.
 9. Li Y, Zheng Q, Bao C, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res* 2015;25:981-4.
 10. van Balkom BW, Pisitkun T, Verhaar MC, Knepper MA. Exosomes and the kidney: prospects for diagnosis and therapy of renal diseases. *Kidney Int* 2011;80:1138-45.

Cite this article as: Kölling M, Lorenzen JM. Circular RNAs as non-invasive urinary biomarker of kidney diseases. *Ann Transl Med* 2020;8(5):255. doi: 10.21037/atm.2020.01.115