

Allograft outcome: the application of machine perfused fluid miRNAs

Mohammadreza Ardalan, Sepideh Zununi Vahed

Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence to: Sepideh Zununi Vahed. Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Email: sepide.zununi@gmail.com.

Comment on: Dos Santos VG, Laura García BM, Ruiz HM, *et al.* MicroRNAs in Kidney Hypothermic Machine Perfusion Fluid as Novel Biomarkers for Graft Function. Have Changes in Normalization Guidelines Support Previous Results? *Transplant* 2018;102:S59-60.

Received: 06 November 2018; Accepted: 26 November 2018; Published: 27 November 2018.

doi: 10.21037/ncri.2018.11.04

View this article at: <http://dx.doi.org/10.21037/ncri.2018.11.04>

Evaluation of allograft quality before engraftment is becoming an important task in the realm of organ transplantation. Prediction of renal allograft outcome for making the final decision to do the transplant or not, is not a well-addressed issue (1). This necessity becomes more obvious as the shortage of organs has increased the acceptance rate of risky organs retrieved from 'extended criteria' donors (ECD) and donation after circulatory death (DCD) (2,3). These kidney allografts harbor more susceptibility to delayed graft function (DGF) and require more elaborate tools to preserve and predict the graft viability (2,3). Hypothermic machine perfusion (HMP) is not only a reliable preservation tool but also a novel method to investigate the allograft status (2,4). A key question is which parameters can be applied during HMP to predict the allograft quality. Currently, the accepted signs of kidney allograft viability are renal flow rate and vascular resistance measurement during HMP (5,6). Elevated perfusion resistance during HMP has been shown to be a risk factor for future graft failure. Additional parameter includes *ex vivo* assessment of oxygen consumption during HMP; higher oxygen consumption is positively correlated with better allograft function (2). Measurement of metabolomics activity in HMP perfusate using NMR (nuclear magnetic resonance spectroscopy) is another proposed method (7,8). Although some biochemical and ischemic injury markers have been specified in the renal allograft effluent, they are not sufficiently established since their role in allograft function prediction *in vivo* is yet disputable (5,6).

microRNAs (miRNAs) are small noncoding RNAs that regulate gene expression and control physiological and pathological processes (9). miRNAs are considered to be

pertinent to kidney injury and promising novel biomarkers due to their stability in body fluids and their correlations with pathological processes. Cell-free or circulating miRNAs in perfused solution during HMP can also offer a valuable pre-transplantation biomarkers for evaluation of organ viability (3). Gómez-Dos-Santos *et al.*, identified a panel of circulating microRNAs (miR-144-5p, miR-486-5p, miR-142-5p, miR-144-3p) in perfusion fluid during HMP that was correlated with future DGF development (3).

Quantitative PCR (qPCR) is an accurate and sensitive method for quantifying circulating miRNAs, but harbors great limitation that originates from its normalization methodology considering the inter-individual variability (10). Cell-free miRNAs are subjected to a set of pre-analytical factors including the amount of the sample, the collection and storage conditions, and the efficacy of miRNA isolation and reverse-transcription (RT) that all can alter the total miRNA levels (10,11). The normalization of RT-qPCR data is essential to diminish these technical bias presented during the entire experimental process. Several methods have been developed and used to normalize circulating miRNAs that hinder the possibility of comparing results from different studies. In order to normalize RT-qPCR data, endogenous miRNAs (housekeeping genes) (12), exogenous synthetic oligonucleotides (spiked-in method) (13), and the geometrical mean of all the expressed miRNAs (3,13) are used, each having their own pros and cons.

microRNAs along with small nuclear and nucleolar RNAs have been reported to be stable cellular housekeeping genes. Since these controls are affected by similar sources

of variability during the experimental processes as other target genes, they remain reliable normalization genes. However, evidence indicates that these RNAs are highly variable and are not stably detectable in body fluids (14). In order to overcome this issue, synthetic oligonucleotides have been used. Since synthetic oligonucleotides are added prior to miRNA isolation, they follow the same processes and experimental biases as endogenous miRNAs do. This normalization method eliminates the variability in RNA extraction and RT reaction and it is more accurate; however, it entails some limitations. Some technical variables and errors that are existed before these processes and even differ in several types of body fluids and diseases, cannot be normalized by this method. To solve this issue, a combination of endogenous and synthetic oligonucleotides is used (11). Gómez-Dos-Santos and coworkers concluded that the mean expression value presents a better normalization strategy for the evaluation of miRNAs in perfusion fluid during HMP (3).

Because of the present heterogeneity in normalization strategies, it is impossible to define universally accepted reference genes for circulating miRNAs quantification. Therefore, it is required to focus on standardizing methods to select case-specific reference genes. Identifying and validating the most stable miRNAs for each experimental setting and selecting them as reference genes seem to be the best normalization strategy so far. In situations where the data is larger, besides selecting the most stable genes, global mean value can be advantageously utilized since all the miRNAs used as reference genes will be considered as target genes.

Acknowledgments

The authors wish to express their gratitude to Dr. Fatemeh Zununi Vahed.

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Dr. Qiyang Dai (Metrowest Medical Center, Framingham, USA).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/ncr.2018.11.04>). 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.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Jochmans I, Pirenne J. Graft quality assessment in kidney transplantation: not an exact science yet! *Curr Opin Organ Transplant* 2011;16:174-9.
2. De Deken J, Kocabayoglu P, Moers C. Hypothermic machine perfusion in kidney transplantation. *Curr Opin Organ Transplant* 2016;21:294-300.
3. Gómez-Dos-Santos V, Ramos-Muñoz E, García-Bermejo ML, et al. MicroRNAs in Kidney Machine Perfusion Fluid as Novel Biomarkers for Graft Function. Normalization methods for miRNAs profile analysis. *Transplant Proc* 2018. doi: 10.1016/j.transproceed.2018.09.019
4. Jochmans I, O'Callaghan JM, Pirenne J, et al. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transpl Int* 2015;28:665-76.
5. Jochmans I, Moers C, Smits JM, et al. The prognostic value of renal resistance during hypothermic machine perfusion of deceased donor kidneys. *Am J Transplant* 2011;11:2214-20.
6. Moers C, Varnav OC, van Heurn E, et al. The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. *Transplant* 2010;90:966-73.
7. Guy AJ, Nath J, Cobbold M, et al. Metabolomic analysis of perfusate during hypothermic machine perfusion of human cadaveric kidneys. *Transplant* 2015;99:754-9.
8. Hall IE, Bhangoo RS, Reese PP, et al. Glutathione S-transferase iso-enzymes in perfusate from pumped kidneys are associated with delayed graft function. *Am J Transplant* 2014;14:886-96.
9. Trionfini P, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. *Nat Rev Nephrol* 2015;11:23-33.
10. Faraldi M, Gomasca M, Banfi G, et al. Free Circulating miRNAs Measurement in Clinical Settings: The Still Unsolved Issue of the Normalization. *Adv Clin Chem* 2018;87:113-39.
11. Sourvinou IS, Markou A, Lianidou ES. Quantification of

- circulating miRNAs in plasma: effect of preanalytical and analytical parameters on their isolation and stability. *J Mol Diagn* 2013;15:827-34.
12. Schwarzenbach H, da Silva AM, Calin G, et al. Data Normalization Strategies for MicroRNA Quantification. *Clin Chem* 2015;61:1333-42.
13. Vigneron N, Meryet-Figuere M, Guttin A, et al. Towards a new standardized method for circulating miRNAs profiling in clinical studies: Interest of the exogenous normalization to improve miRNA signature accuracy. *Mol Oncol* 2016;10:981-92.
14. Marabita F, de Candia P, Torri A, et al. Normalization of circulating microRNA expression data obtained by quantitative real-time RT-PCR. *Brief Bioinform* 2016;17:204-12.

doi: 10.21037/ncri.2018.11.04

Cite this article as: Ardalan MR, Zununi Vahed S. Allograft outcome: the application of machine perfused fluid miRNAs. *Non-coding RNA Investig* 2018;2:65.