

Towards advanced cardiac miRNA therapies through DNA-based nanostructures

Natalia Hernández-Bellido^{1*}, Alejandro Postigo^{2*}, Carol Orrite³, Elisa Garrido-Huésca¹,
Laura García-Mendivil¹, Esther Pueyo⁴, Silvia Hernández-Ainsa⁵ Laura Ordovás

(1) (a) I3A-University of Zaragoza, (b) IIS Aragón, BSICoS group, Zaragoza, Spain

(2) (a) INMA-CSIC-University of Zaragoza, CLIP group, Zaragoza, Spain

(3) (a) INMA-CSIC-University of Zaragoza, CLIP group (b) I3A-University of Zaragoza, BSICoS group, Zaragoza, Spain

(4) (a) I3A-University of Zaragoza, (b) IIS Aragón (c) CIBER-BBN, BSICoS group, Zaragoza, Spain

(5) (a) ARAID, (b) INMA-CSIC-University of Zaragoza, CLIP group, Zaragoza, Spain

(6) (a) ARAID, (b) I3A-University of Zaragoza, (c) IIS Aragón, BSICoS group, Zaragoza, Spain

*Equally contributed (nhbellido@unizar.es, apostigo@unizar.es)

Introduction

Cardiac microRNAs (miRNAs) have been found to be dysregulated in cardiac disease and aging both in animal models and humans. Thanks to their pleiotropic effects, miRNAs are spotlighted as promising therapeutic targets to treat cardiac conditions. However, their low stability and potential off-target effects *in vivo* difficult the development of effective and safe miRNA therapies. Nanotechnology enables the fabrication of biocompatible carriers to perform targeted delivery of both conventional and advanced therapies. In particular, nanostructures built with DNA (DNS) have been successfully developed for miRNA therapies in cancer, but they have not been applied in cardiac therapy yet [1].

Purpose

The main goal of this study is to demonstrate the capacity of DNS to deliver miRNA therapies in human cardiomyocytes.

Methods

Our group has identified MIR24-2 upregulated with age in the human left ventricle and miR24-2-5p to interact with genes related with the cardiac function, such as *SERCA2* [2]. Different versions of DNS were designed and generated by self-assembly of DNA strands containing anti-miR24-2-5p capture segments. Physicochemical characterization of DNS was then conducted to determine their auto-assembling capacity, size, morphology and thermal stability. The characterization of the biological effect of the DNS was also conducted *in vitro* and in cell culture of HEK293 and human induced pluripotent stem cells (iPSC)-derived cardiomyocytes (iCM) to assess their cytotoxicity and internalization capacity.

Results

DNS were assembled correctly and reproducibly into carriers of around 30-50 nm. *In vitro*, the DNS showed thermal stability at physiological temperature, proper stability in serum and they disassembled specifically in the presence of the target miR24-2-5p sequence. In HEK293 and iCM cultures, the DNS showed lack of cytotoxicity demonstrating *in vitro* biocompatibility. DNS were efficiently internalized by HEK293, but the uptake capacity of DNS by iCM was remarkably lower.

Conclusions

We created and characterized biocompatible anti-miR-loaded DNS that effectively captured the target miR-24-2-5p *in vitro*. DNS internalized in iCM, but with lower efficiency than in HEK293 cells. Our results indicate that DNS are suitable candidates to carry miRNA therapies to human cardiac cells *in vitro*, but the observed cell-type specific differences suggest that future efforts need to be directed towards developing DNS functionalization capable of promoting efficient and specific carrier uptake *in vivo* by primary cardiac cells *in vivo*.

References

- [1] B. R. Madhanagopal, S. Zhang, E. Demirel, H. Wady, and A. R. Chandrasekaran, "DNA Nanocarriers: Programmed to Deliver," *Trends Biochem. Sci.*, vol. 43, no. 12, pp. 997–1013, Dec. 2018, doi: 10.1016/J.TIBS.2018.09.010.
- [2] E. Ramos-Marquès *et al.*, "Chronological and biological aging of the human left ventricular myocardium: Analysis of microRNAs contribution," *Aging Cell*, vol. 20, no. 7, Jul. 2021, doi: 10.1111/ACEL.13383.