Exploring shRNA-based therapy to prevent chemotherapy-induced cardiotoxicity

Carolina Gómez-Moreno[1,2], Natalia Hernández-Bellido[1,3], Laura García-Mendívil[3], Marcos Sánchez-Barat[1,3], Laura Paz-Artigas[4], Jesús Ciriza[2,4], Laura Ordovás[1,3,5].

[1] Grupo BSICoS, Instituto de Investigación en Ingeniería de Aragón (I3A), 50009 Zaragoza, Spain
[2] Grupo TME Lab, Instituto de Investigación en Ingeniería de Aragón (I3A), 50009 Zaragoza, Spain
[3] Grupo BSICoS, Instituto de Investigación Sanitaria Aragón (IISA), 50009 Zaragoza, Spain
[4] Grupo TME Lab, Instituto de Investigación Sanitaria Aragón (IISA), 50009 Zaragoza, Spain
[5] Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), 50018Zaragoza, Spain
Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain.
Tel. +34-976762707, e-mail: *800406@unizar.es*

Abstract

Chemotherapy-induced cardiotoxicity is an often fatal consequence of some cancer treatments, such as anthracyclines like doxorubicin (DOXO), for which there is currently no preventive treatment. Since the cardiotoxic mechanism of DOXO is due to its action on topoisomerase 2 β (TOP2B), some studies have described the protective effects of its cardiomyocyte-specific inhibition. In this work we have develop and characterized an RNA interference (RNAi) strategy to inhibit *TOP2B*. Although our preliminary results show low inhibition of *TOP2B in vitro*, they encourage future development of more efficient shRNAs.

Introduction

Cancer and cardiovascular disease are the leading causes of death in western countries. But in addition, some chemotherapeutic agents used to treat cancer also induce cardiotoxicity, a fatal side effect that limits the use of potentially effective therapies.

In recent years, gene silencing therapy with RNAi has emerged as a promising treatment for several pathologies, including cardiac diseases, with proven efficacy in preclinical and clinical phases [1, 2]. Since chemotherapy-induced cardiotoxicity is generally related to the drug's mechanism of action on cancer cells, which is also present in pathways from non-tumor cells, its prevention and treatment will likely require interventions that selectively protect the heart.

DOXO is one of the most effective and widely used chemotherapeutic treatments for cancer. DOXO acts on topoisomerase 2 (TOP2) [3] and exerts its antitumor effect by acting on the α isoform (TOP2A), which is expressed on highly proliferative cells. Unfortunately, its interaction with the β isoform, which is expressed on post-mitotic cells and the only isoform present on the adult heart [4], leads to cardiotoxicity. *In vivo* studies have shown that cardiomyocyte-specific conditional knock-out of *Top2b* prevents DOXO-induced cardiotoxicity [5].

Taken together, we aimed to develop an *in vitro* RNAi-mediated gene silencing strategy to inhibit TOP2B expression so that future studies can determine its cardioprotective effect.

Materials and Methods

To this end, we designed a short hairpin RNA (shRNA)-based RNAi strategy to selectively silence *TOP2B* and not the highly homologous *TOP2A* isoform.

First, three shRNA candidates were cloned into a eukaryotic inducible expression vector, downstream of a tdT fluorescent reporter gene. The recombinant vectors were confirmed by enzymatic digestion and Sanger sequencing.

Next, we evaluated their activity in routine HEK293 cells by transient transfection, confirmed by fluorescence microscopy. The effects of the hairpins on TOP2B and TOP2A were studied at the level of protein expression (Western blot and immunofluorescence) and mRNA expression (quantitative PCR).

Results

After designing three shRNA-TOP2B candidates, their cloning into a eucaryotic inducible expression vector was highly efficient.

The efficiency of the transient transfection of HEK293 cells with the confirmed recombinant vectors was adequate. Inducible expression of the tdT reporter gene was highly efficient, with no signal detected by immunofluorescence on the uninduced sample, and high expression detected on samples induced with doxycycline. Preliminary results show a qualitative reduction in TOP2B expression in samples transfected with shRNA-TOP2B candidate 3 (Fig. 1). Sadly, the protein expression of TOP2A and TOP2B couldn't be determined by Western Blot due to the lack of sensitivity of the antibody and/or the low transfer efficiency of high molecular weight proteins on nitrocellulose membrane. Further optimization is needed for this technique.

After confirming the specificity of the primers to determine RNA expression by conventional PCR, preliminary qPCR studies showed that only shRNA-TOP2B candidate 3 modestly inhibited *Top2b* expression, but with slight effect on *Top2a.*

Conclusions

Preliminary results show one shRNAs candidate with limited inhibition of TOP2B at the mRNA and protein levels. Further studies are required to investigate the activity of these candidates in both routine and cardiac cells of human origin.

References

[1] Gabisonia, Khatia, Giulia Prosdocimo, Giovanni Donato Aquaro et. al. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. *Nature* [online]. 2019, **569**(7756), 418–422. ISSN 14764687. Dostupné z: doi:10.1038/s41586-019-1191-6

[2] FERENCE, Brian A. *The Potential Clinical Benefit of Lowering Lipoprotein(a)* [online]. B.m.: American Medical Association. 3. květen 2022. ISSN 15383598. Dostupné z: doi:10.1001/jama.2022.5333

[3] TEWEY, K. M., T. C. ROWE, L YANG, B. D HALLIGAN a L.F LIU. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. [online]. 1984, **Sciencie 226**, 466–468. Dostupné z: doi:10.1126/science.6093249

[4] CAPRANICO, Giovanni, Stella TINELLI, Caroline A AUSTIN, Mark L FISHER' a Franco ZUNINO. *Different patterns of gene expression of top0isomerase II isoforms in differentiated tissues during murine development*. 1992.

[5] ZHANG, Sui, Xiaobing LIU, Tasneem BAWA-KHALFE, et. al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine* [online]. 2012, **18**(11), 1639–1642. ISSN 10788956. Dostupné z: doi:10.1038/nm.2919

Acknowledgements

This work was supported by grant PID2022-139859OB-I00 funded by MICIU/AEI/10.13039/501100011033 and FEDER/UE.

Carolina Gómez-Moreno was funded by Internship scholarships for the development of the TFM (Final Master Thesis) of the Aragon Engineering Research Institute (I3A) in collaboration with the Chairs of Industrial Transformation and URBASER.

The authors would like to acknowledge the Servicios Científico Técnicos del Centro de Investigación Biomédica de Aragón (CIBA) and the Servicio de Apoyo a la Investigación de la Universidad de Zaragoza (SAI) for their technical support. In particular, to the services of Secuenciación y genómica funcional and Proteómica.

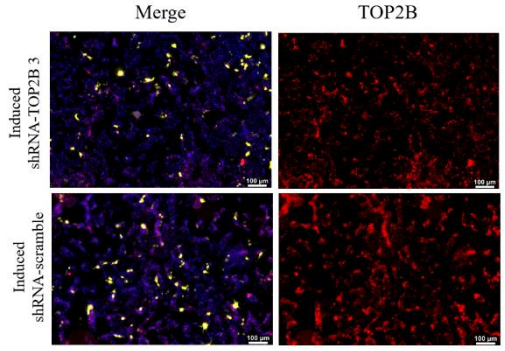

Fig. 1. Transfected HEK293 with recombinant vector expressing tdT and shRNA-TOP2B 3 by fluorescence microscopy. Note: tdT (yellow), nuclei (blue) and TOP2B (red).