

Exploring oligonucleotide-based silencing strategies to prevent anthracycline-induced cardiotoxicity

Carolina Gómez-Moreno^{1,2}, Estíbaliz Fernández-Carro¹, Vira Sharko^{1,3},
Ana Rosa Remacha¹, Clara Alcaine^{3,4}, Angel Luis García-Otín^{5,6}, Laura Ordovás^{7,8,9},
Jesús Ciriza^{1,3,10}

¹ Grupo Tissue Microenvironment (TME) Lab, Instituto de Investigación en Ingeniería de Aragón (I3A), Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain.

² Grupo BSICoS, I3A, Instituto de Investigación Sanitaria de Aragón (IISA), 50009 Zaragoza, Spain.

³ Grupo TME Lab, I3A, IISA, 50009 Zaragoza, Spain.

⁴ Departamento de Fisiología y Enfermería, Facultad de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain.

⁵ Instituto Aragonés de Ciencias de la Salud (IACS), 50009, Zaragoza, Spain.

⁶ Unidad de Investigación Traslacional, Hospital Universitario Miguel Servet, IISA, 50009, Zaragoza, Spain.

⁷ Grupo BSICoS, I3A, Universidad de Zaragoza, 50009 Zaragoza, Spain.

⁸ Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), 50018 Zaragoza, Spain.

⁹ Grupo BSICoS, Consorcio Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, (CIBER-BBN), 50018 Zaragoza, Spain.

¹⁰ Grupo TME Lab, CIBER-BBN, 50018 Zaragoza, Spain.

e-mail: carolina.gomez@unizar.es

Abstract

Chemotherapy-induced cardiotoxicity is a major limitation of anthracycline-based treatments such as doxorubicin. Since TOP2B has been identified as a key mediator of DOXO-induced cardiotoxicity, this work explores and optimizes shRNA- and ASO-based silencing strategies targeting TOP2B. Preliminary results support the development of chemically modified ASOs with improved therapeutic potential.

Introduction

Cancer and cardiovascular disease are among the leading causes of death worldwide. However, several chemotherapeutic agents, including doxorubicin (DOXO), can induce cardiotoxicity, a severe side effect that limits their clinical use. DOXO exerts its antitumor activity through interaction with topoisomerase 2 α (TOP2A) [1], whereas interaction with the β isoform (TOP2B), predominantly expressed in cardiac tissue [2], has been associated with chemotherapy-induced cardiac damage. Importantly, previous *in vivo* studies demonstrated that cardiomyocyte-specific deletion of *Top2b* protects against DOXO-induced cardiotoxicity [3].

In recent years, oligonucleotide-based therapies have emerged as promising therapeutic tools due to their specificity and versatility, including applications in cardiovascular diseases [4]. Among the

oligonucleotide-based approaches, antisense oligonucleotides (ASOs) offer important advantages such as improved stability (compared to RNA) and compatibility with non-viral vector delivery approaches.

Therefore, the aim of this work was to develop and optimize oligonucleotide-based silencing strategies targeting TOP2B using both shRNA- and ASO-based approaches.

Materials and Methods

We designed two types of RNAi strategies to selectively silence *TOP2B* mRNA while avoiding inhibition of the highly homologous *TOP2A* isoform: three short hairpin RNA (shRNA) candidates and nine ASOs.

shRNA candidates were cloned into an inducible eukaryotic expression vector and evaluated in HEK293 cells following transient transfection (Lipofectamine 2000, Thermo), confirmed by fluorescence microscopy. TOP2B and TOP2A expression was assessed by immunofluorescence and quantitative PCR (qPCR).

ASO candidates were evaluated in transiently transfected HEK293 cells and induced cardiomyocytes derived from pluripotent stem cells (iCMs). Silencing efficiency was assessed by

immunofluorescence, quantitative fluorescence analysis and qPCR.

Results

Cloning and validation of the shRNA expression vectors were successfully achieved. Transient transfection assays in HEK293 cells showed efficient inducible tdTomato expression. Preliminary analyses revealed modest TOP2B inhibition for one shRNA candidate, although none achieved robust silencing efficiency.

Based on these results, ASO-based approaches were explored as an alternative. Initial evaluation of first-generation ASOs in HEK293 cells showed successful delivery and variable inhibitory effects among candidates. Immunofluorescence analyses revealed partial reductions in TOP2B protein expression up to 28% silencing (Fig. 1), whereas qPCR analyses revealed limited mRNA silencing, reaching a maximum reduction of 8%.

Subsequent evaluation in iCMs revealed differences in candidate performance between non-cardiac and cardiac cellular models, highlighting the importance of physiologically relevant systems (Fig. 1). Although some candidates performed better than others, none achieved efficient TOP2B inhibition. Consequently, a second generation of phosphorothioate-modified ASOs was designed and is currently under evaluation.

Conclusions

This work supports the continued optimization and development of oligonucleotide-based strategies targeting TOP2B for the prevention of anthracycline-induced cardiotoxicity. Although initial shRNA and ASO candidates showed limited silencing efficiency, the results obtained guided the rational design of a new generation of chemically modified ASOs with improved therapeutic potential.

References

- [1]. TEWEY, K.M., ROWE, T.C., YANG, L., HALLIGAN, B.D. and LIU, L.F. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science*. 1984, vol. 226, pp. 466–468.
- [2]. CAPRANICO, G., TINELLI, S., AUSTIN, C.A., FISHER, M.L. and ZUNINO, F. Different patterns of gene expression of topoisomerase II isoforms in differentiated tissues during murine development. *Biochimica et Biophysica Acta*. 1992, vol. 1132.
- [3]. ZHANG, S., LIU, X., BAWA-KHALFE, T., LU, L.S., LYU, Y.L., LIU, L.F., et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine*. 2012, vol. 18, no. 11, pp. 1639–1642.
- [4]. GABISONIA, K., PROSDOCIMO, G., AQUARO, G.D., CARLUCCI, L., ZENTILIN, L., SECCO, I., et al. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. *Nature*. 2019, vol. 569, no. 7756, pp. 418–422.

Acknowledgements

This work was supported by grant PID2022-139859OB-I00 funded by MICIU/AEI/10.13039/501100011033 and FEDER/UE. The authors are also grateful for the support of the Government of Aragón (DGA T62_23R).

Carolina Gómez-Moreno was funded in 2024 through an internship scholarship for the development of her MSc thesis at I3A in collaboration with the Chairs of Industrial Transformation and URBASER. In 2025, she was funded with a predoctoral scholarship by the Government of Aragón (CPB_27_24).

The authors would like to acknowledge the technical support provided by the Servicios Científico Técnicos del CIBA-IACS, particularly the Proteómica and Secuenciación y Genómica funcional, as well as the Servicios de Apoyo a la Investigación of I3A-NANBIOSIS, in particular the Unidad de Caracterización Tisular.

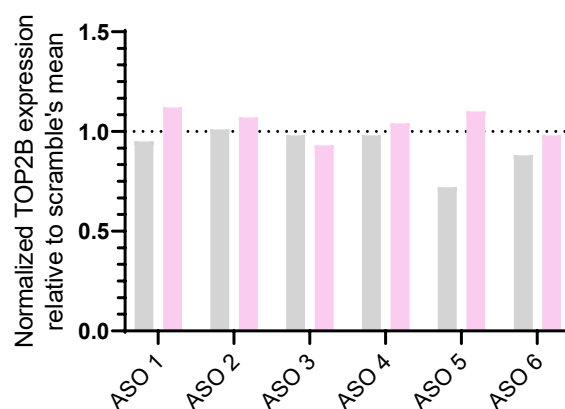


Fig 1. TOP2B expression after ASO treatment in HEK293 cells (grey) and iCMs (pink). TOP2B protein expression was normalized relative to scramble-treated cells. Quantification was performed by immunofluorescence analysis.