Exploring shRNA-based therapy to prevent chemotherapy-induced cardiotoxicity

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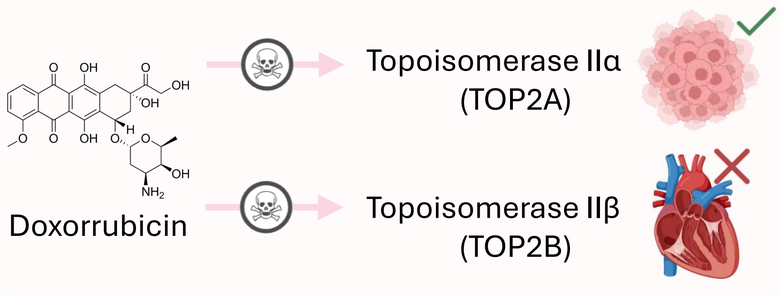
Introduction



Doxorrubicin chemotherapeutic treatment leads to **cardiotoxicity** through its effect on **topoisomerase IIβ** [1]

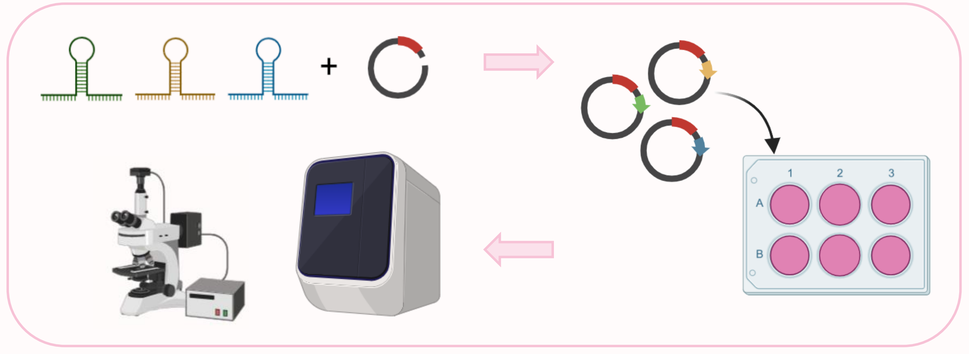
Cardiomyocyte-specific conditional ***Top2b*** **knockout prevents** doxorubicin **cardiotoxicity** [2]

Goals

1. Development of a short hairpin RNA (**shRNA**) based approach for RNA interference-mediated gene **silencing** of **TOP2B.**
2. **Characterization** of the specificity and efficacy of the shRNA-TOP2B candidates

Materials and methods

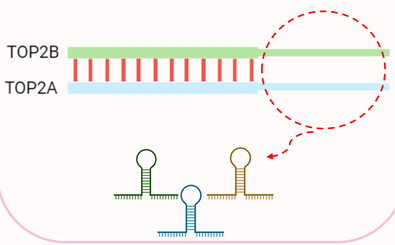
- **Design** of three **shRNA-TOP2B candidates** by sequence analysis.
- **Cloning** of the shRNA-TOP2B candidates into an **eucaryotic inducible expression vector**, downstream of a tdT reporter gene.
- Transient **transfection** of the cloned shRNA-TOP2B vectors into **HEK293** cells.
- **Characterization** of the specificity and efficacy of the shRNA-TOP2B candidates at the level of RNA expression (**quantitative PCR**) and protein expression (**Western blot** and **immunofluorescence**)



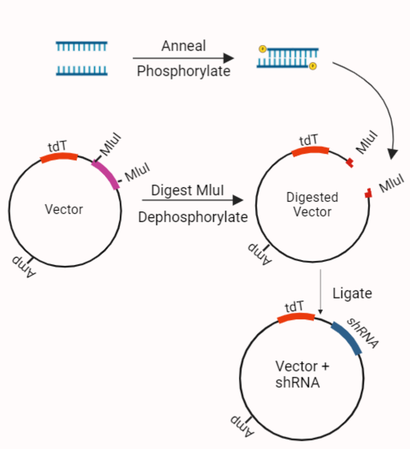
Results

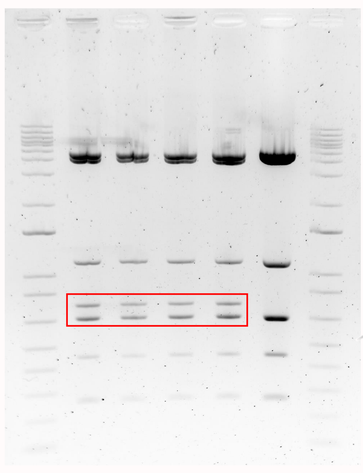
Design of shRNA-TOP2B candidates

Three shRNA-TOP2B candidates were designed and selected in sequence regions of low homology with TOP2A .



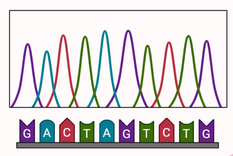
Cloning of shRNA-TOP2B candidates





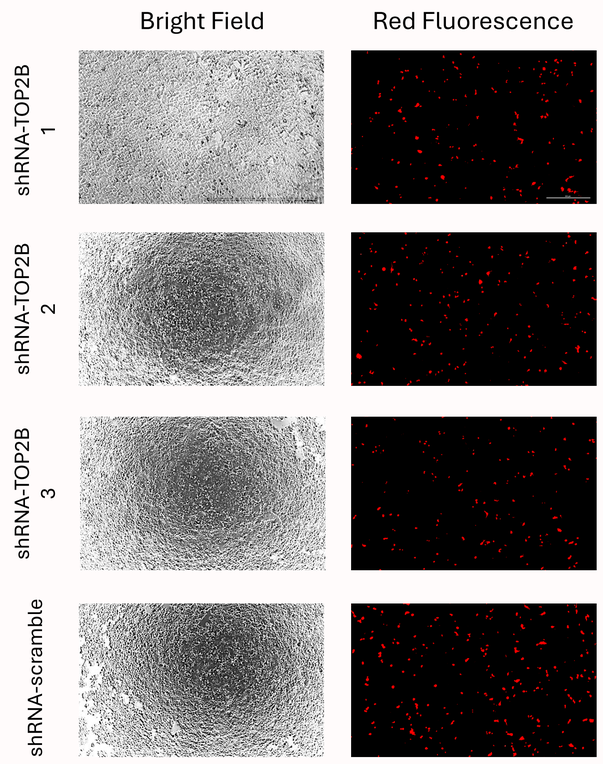
Recombinant vectors were confirmed by enzymatic digestion with SpeI and HindIII.

Recombinant vectors were sequenced.



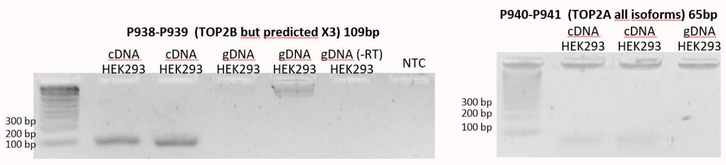
Transfection of recombinant shRNA-TOP2B vector into HEK293 cells

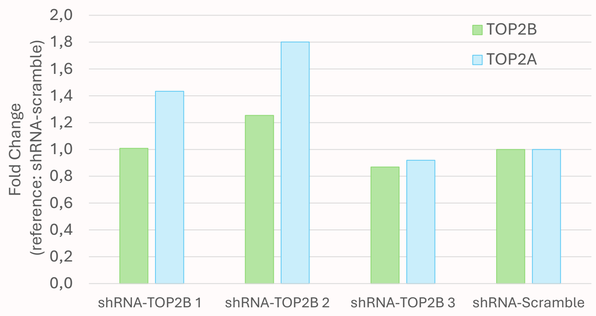
Doxycycline induction leads to tdT expression upstream of the shRNAs in the transient transfection.



RNA expression characterization: qPCR

Primers have been designed for the specific amplification of *TOP2B* and *TOP2A* by qPCR.

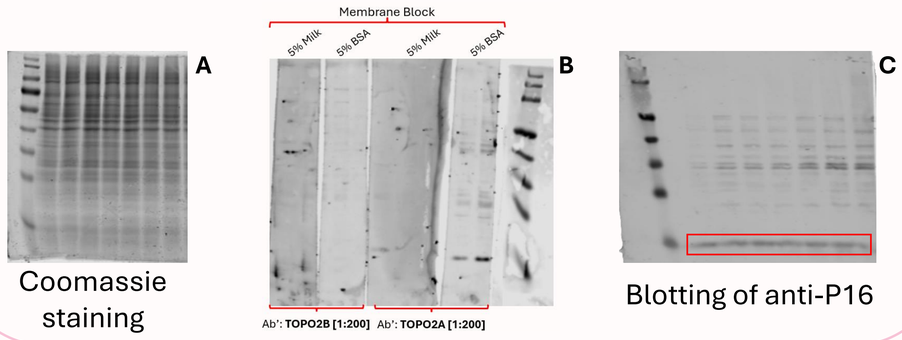




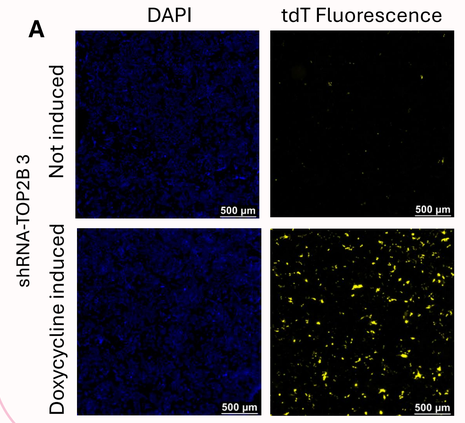
Only shRNA 3 mildly inhibits *TOP2B* with some effect on *TOP2A*. Further candidates need to be tested.

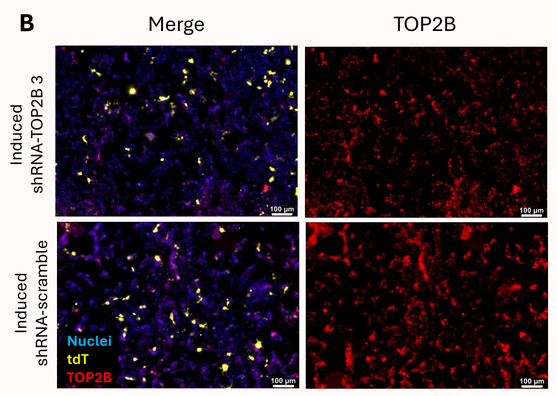
Optimization of TOP2A and TOP2B quantification

Protein extracts were confirmed by Coomassie staining (A). Blotting with antibodies against TOP2A and TOP2B confirms no specific detection of these high molecular weight proteins (B), while low molecular wight detection is confirmed by anti-P16 antibody (C).



Protein expression characterization: Immunofluorescence





The efficiency of the induction was assessed by the tdT reporter signal using fluorescence microscopy (A).

Immunofluorescence with anti-TOP2B antibody shows a qualitative reduction of TOP2B with the shRNA-TOP2B3 candidate (B).

Conclusions

- **Sequence analysis** identifies regions of low similarity with *TOP2A* to target TOP2B-specific shRNAs.
- The **cloning strategy is effective** for the cloning of shRNA in an expression vector
- Preliminary results show **shRNA-TOP2B 3** to be the only candidate with moderate inhibitory capacity of *TOP2B*.
- **Further studies** are required to fully characterize the shRNA candidates or design additional ones to achieve significant silencing of *TOP2B*.

References

[1]Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D. & Liu, L. F. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* **226**, 466–468 (1984). [2] Zhang, S. *et al.* Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat. Med. 2012 1811* **18**, 1639–1642 (2012).

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