Exploring shRNA-based therapy to prevent chemotherapy-induced cardiotoxicity

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Introduction

Topoisomerase IIα (TOP2A)

Doxorubicin chemotherapeutic treatment leads to cardiotoxicity through its effect on topoisomerase IIβ.

Cardiomyocyte-specific conditional Top2b knockout prevents doxorubicin cardiotoxicity.

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 eukaryotic 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.

RNA expression characterization: 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 weight 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


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