Test inhibition of GAPDH expression through siRNA in rat mesenchymal stem cells

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Introduction

We isolate and apply mesenchymal stem cells (MSCs) from rat bone marrow for experimental therapeutic purposes, with particular reference to carotid restenosis prevention in a model of rat carotid arteriotomy. Target gene silencing with siRNAs in MSCs could allow a better comprehension of the mechanisms responsible for MSC effectiveness in vivo after injection.

Materials and methods

We isolated MSCs from rat bone marrow and we cultivated cells to passage 4, when they have been transfected with GAPDH-siRNA or with control scrambled Luc-siRNA or with control fluorescent Blokit-siRNA (Ambion) to check transfection efficiency.
Cells were examined at 48 hrs after transfection at fluorescence microscope to measure transfection efficiency and total RNA was also extracted from MSCs at the same time point to verify through RT-PCR the decrease of target GAPDH mRNA in comparison to control cells transfected with Luc-siRNA.

Experimental procedures / transfection protocol

We followed exactly the working instructions reported in the datasheet, on 300000 MSCs seeded in 6 multiwell plates.

Results and discussion

The transfection efficiency in MSCs was about 90%; the decrease of target GAPDH mRNA was about 90%.

Conclusion / summary

Metafectene produced very good results in target gene silencing in rat MSCs, especially considering the high level of expression of the target gene we selected.

References

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Appendix: Tables and/or figures

Cell code	Prim-ary	Class	Spec-ies	Organ	Type	Identification	Description	Reagent	Genetic Material	Efficiency	To-xicity
MSC	yes	Mam-malia	rat	Bone marrow	stroma	Mesenchymal stem/stromal cells	Male Wistar rats	METAFECTENE SI	siRNA	90%	N.A.