HeLa cells transfection with METAFECTENE SI

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INTRODUCTION

The aim of this report is to set up the conditions for silencing of HeLa cell line (line derived from cervical cancer) using Metafectene SI as transfection reagent.

MATERIALS AND METHODS

HeLa cells were grown in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 µg/ml streptomycin, in a 5% CO_2 incubator at 37°C.
The transfection reagent used in this study was Metafectene SI (Biontex Laboratories GmbH).
For silencing we used Rab7 siRNA (Rab7 is a small GTPase expressed in all tissues, that controls transport to endocytic degradative compartments).

EXPERIMENTAL TRANSFECTION PROTOCOL

2 ml of a HeLa cell suspension were plated one day before transfection into 6-well tissue culture plates at a density of 8.0 x 10^4 cells/ml.
Lipoplexes were prepared by adding to a tube the following reagents:

- 180 µl 1X SI Buffer
- 12 µl Metafectene SI
- 180 pmol siRNA

After gentle agitation the mixture was incubated for 15 min at room temperature.
The transfection mix was laid onto cells previously washed with 1xPBS, and then 1.8 ml of DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 µg/ml streptomycin was added to the cells. The cells were then incubated in a 5% CO_2 incubator at 37°C.
After 24 or 48 hours of transfection, cells were lysed, and lysates were separated by SDS-PAGE, and transferred onto PVDF membrane. The PVDF membrane was blocked in 5% milk in PBS for 30 minutes at room temperature, incubated with primary antibody (anti-Rab7 for the bottom of the membrane and anti-tubulin for the top of the membrane), washed in 5% milk in Tween 0,1% in 1xPBS and then incubated with a secondary antibody conjugated with horseradish peroxidase. Bands were visualized by the enhanced chemoluminescences system. Protein levels were quantified by densitometry using the ImageJ software. The level of the Rab7 protein was normalized against tubulin.

RESULTS AND DISCUSSION

After 24 hours Rab7 knockdown using Rab7 siRNA and Metafectene SI resulted in a decrease of the Rab7 protein of about of 35 %, while after 48 hours the decrease was about 95% (Fig. 1).

CONCLUSION

HeLa cells were silenced very efficiently using Metafectene SI reagent. Indeed, Rab7 was almost not anymore detectable 48 hours after transfection. Similar results (about 95% of Rab7 silencing) were obtained with Oligofectamine following manufacturer instructions, but only 5 days after transfection (data not shown). Thus transfection of Rab7 siRNA with Metafectene SI reagent results in a more rapid and effective silencing of Rab7.

APPENDIX AND FIGURES

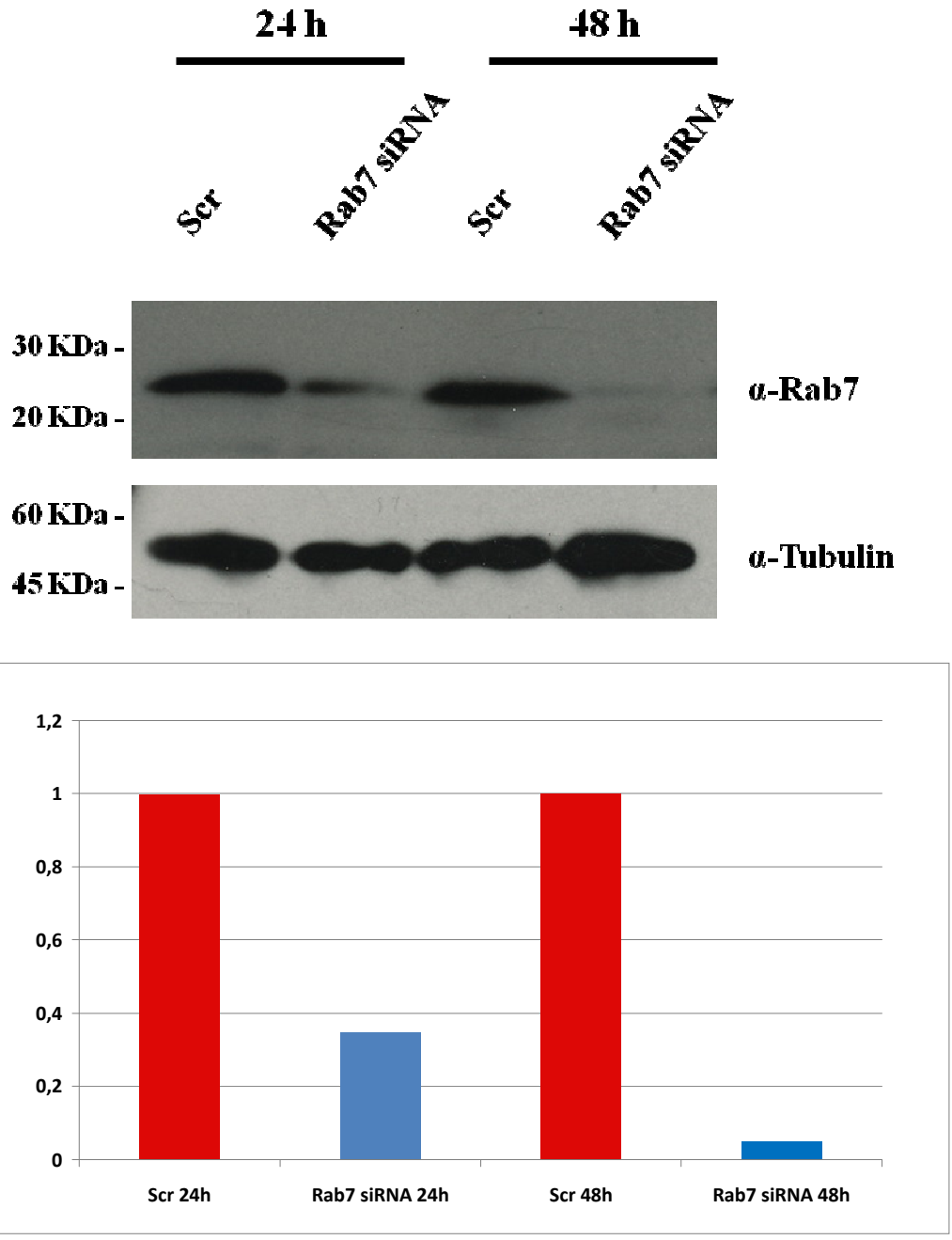

Fig. 1

A => HeLa cells were transfected with control RNA (scr) or Rab7 siRNA using Metafectene SI. Cells were lysed, and lysates were subjected to SDS-PAGE and immunoblotting analysis using an anti-Rab7 antibody. An anti-tubulin antibody was used to verity equal loading.

B => Quantification of Rab7 abundance (relative to tubulin). The decrease of Rab7 expression was about 35 % after 24h and about 95% after 48h of transfection. Quantification has been made on three different experiments.