Neuro2A cells transfection with METAFECTENE SI

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INTRODUCTION

The aim of this report is to set up the conditions for silencing of Neuro2A cell line (mouse neuroblastoma) using Metafectene SI as transfection reagent.

MATERIALS AND METHODS

Neuro2A 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 a 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 Neuro2A 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.
We followed the transfection procedures indicated by manufacturer and we prepared lipoplexes for each well by adding in a tube:

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

All components were mixed by gentle agitation and were incubated for 15 min at room temperature. The transfection mix and 1,8 ml of DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 µg/ml streptomycin were added to the cells. Cell were then incubated in a 5% CO_2 incubator at 37°C.
48 hours after transfection, cells were lysed, and lysates were separated by SDS-PAGE and then transferred onto PVDF membrane. The PVDF membrane was blocked in 5% milk in PBS for 30 minutes at room temperature, incubated with a primary antibody (anti-Rab7 for the bottom of the membrane and anti-tubulin for the top of the membrane), washed in 5% milk, 0.1% Tween in 1x PBS, and then incubated with a secondary antibody conjugated with horseradish peroxidase. Bands were visualized by the enhanced chemoluminescence system (ECL). Protein levels were quantified by densitometry using the ImageJ software. The level of abundance of the Rab7 protein was normalized against tubulin.

RESULTS AND DISCUSSION

After 48 hours of transfection, Rab7 knockdown using siRNA and Metafectene SI resulted in a decrease of Rab7 protein about of 80 % (Fig. 1).

CONCLUSION

Neuro2A cells were silenced by transfecting Rab7 siRNA using Metafectene SI reagent and the decrease of Rab7 expression level that we obtained was high (about 80 %) after 48 hours.

APPENDIX AND FIGURES

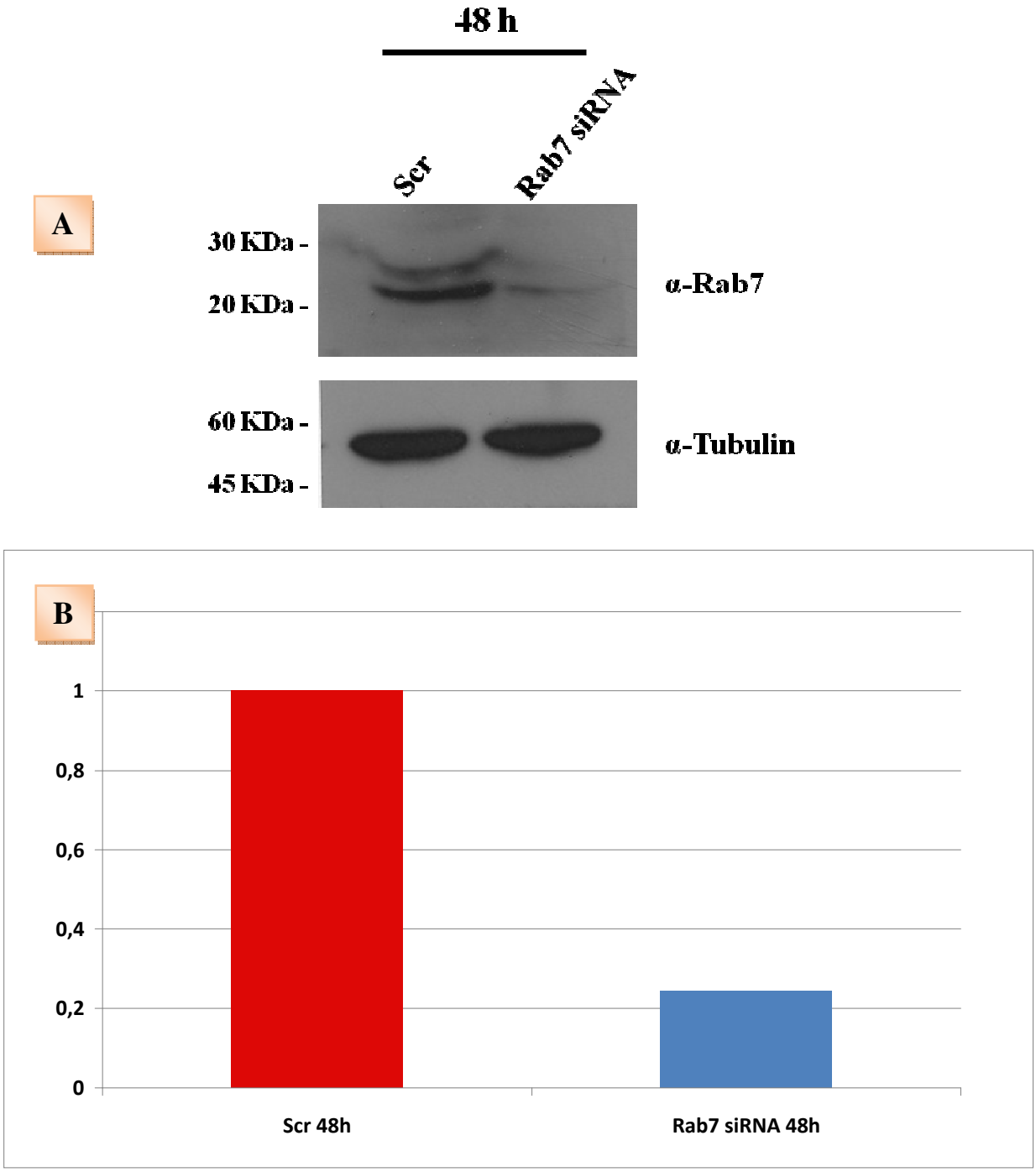

Fig. 1

A Neuro 2A cells were transfected 48 hours with control RNA (scr) or with Rab7 siRNA using Metafectene SI. Cells were then 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 80 %.