Transfection of human HeLa cells with Metafectene SI and Metafectene EASY

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Introduction:

Aim of this study was to investigate and quantificate a) internalisation of different fluorescent plasmids after transfection with Metafectene EASY and b) downregulation of several "importins" after transfection of HeLa cells with siRNA using Metafectene SI. Furthermore results of importin knockdown should be compared to findings received with another transfection reagent.

Materials and methods:

Materials

Metafectene EASY and Metafectene SI (Biontex, Munich, Germany); siRNA for importins alpha 1, 3, 4, 5, 7 (Dharmacon and Qiagen); BLOCK-iT Alexa Fluor Red and BLOCK-iT Fluorescent Oligo (Invitrogen). Antibodies against importins were a kind gift of the Department of Biochemistry, University of Lübeck. Secondary antibodies were purchased from Daco.The GFP-coupled plasmid was self-composed in our lab.

Cells

The human cervical epithelial cancer cell line HeLa (ATCC, Rockville, MD) was cultured in Dulbecco's modified Eagle's MEM supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 µg/ml).

Experimental procedures / transfection protocol:

Cell viability

Cell morphology was evaluated by inverted phase contrast microscopy at 25x magnification. The number of viable cells used for the experiments was determined by Trypan Blue exclusion.

Transfection protocol

For transfection, HeLa (0,8 x 10^5 cells/ml or 7.0 x 10^5 cells/ml) were seeded in 0,5 ml of DMEM in 24-well culture plates for fluorecscence microscopy or in 2 ml medium in 6-well culture plates for siRNA-transfection. Transfection was performed following the manufacturers instructions. Transfection efficacy of fluorescent plasmids was evaluated after 24 hours by flourescence microscopy. Knockdown of importins was assessed by western blotting 48 hours after transfection with Metafectene SI. Protein concentration of cell lysates

was determined by the Bio-Rad BCA assay and 30 µg of protein was loaded per lane on a SDS-PAGE.

Results and discussion:

In HeLa cells, the cytotoxic effect of the Metafectene EASY and SI-mediated transfection was determined by inverted phase contrast microscopy. Cells showed no significant changes in morphology and no increased apoptosis after 24 or 48 hour of transfection compared to not transfected cells.
Transfection of cells seeded 7.0 x 105 cells/ml (as advised in the manufacturers manual) using Metafectene EASY resulted in a transfection efficiency less than 20%. Using a cell density of 0,8 x 10^5 cells/ml emerged to be more suited for transfection with Metafectene EASY. Fluorescence microscopy showed a good signal for GFP and red flourescent plasmid, comparable with signals achieved using Lipofectamine 2000 (Invitrogen) (data not shown). Importin knockdown using siRNA and Metafectene SI resulted in a decrease of importin protein of 60-90% (Fig.1). This knockdown efficiency was comparable to results of transfection of HeLa cells with RNAiMAX (Invitrogen) achieved at an earlier time (Fig.2).

Conclusion / summary:

Metafectene EASY and SI were highly effective in transfecting human HeLa cells with fluorescent plasmids and siRNA. Cytotoxicity emerged to be very low. Information in the manual regarding cell numbers using Metafectene EASY for fluorescence microscopy should be checked and be adapted to the experiment.
Transfection efficiancy was as high as compared to results achieved using transfection reagents of other manufacturers. Advantages of Metafectene EASY and SI are its low toxicity and the use of cell suspensions reducing experimental time by one day of growth period, normally necessary when transfecting adherent cells.

Appendix:

Table 1.

Cell code	Primary	Class	Species	Organ	Identi-fication	Reagent	Growth Properties	Genetic Material	Effi-ciency	Toxicity
HeLa	no	mammalia	human	cervical	epithelial	METAFECTENE SI, METAFECTENE EASY, Lipofectamin 2000, RNAiMAX	adherent	Plasmid, siRNA	60-90%	low

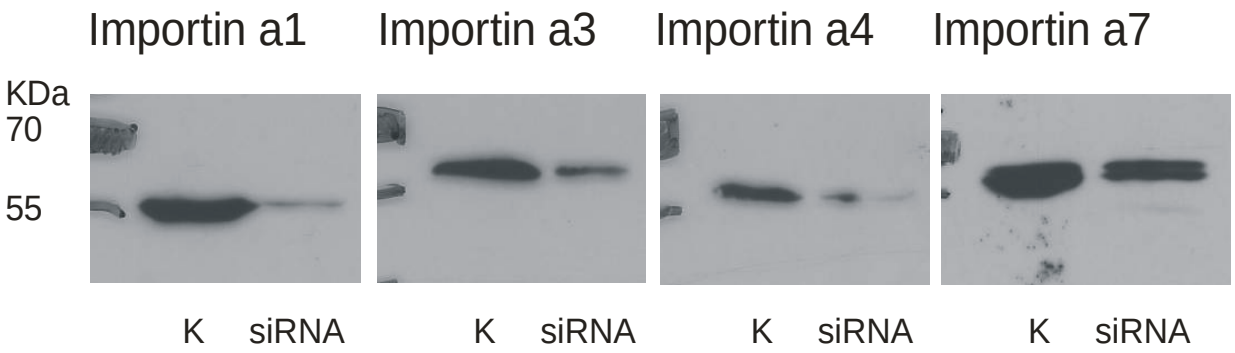

Fig. 1: Transfection of HeLa cells with siRNA raised against importins alpha 1, 4, 5 and 7 (Dharmacon) using Metafefectene SI.
Cells were transfected with Metafectene SI following instructions of the manufacturer's protocol. After 48 hours of incubation cells were lysed und 30µg of protein were loaded on a SDS-PAGE. Proteins were detected by Western Blotting using specific antibodies. K= Control; siRNA= siRNA transfected cells.

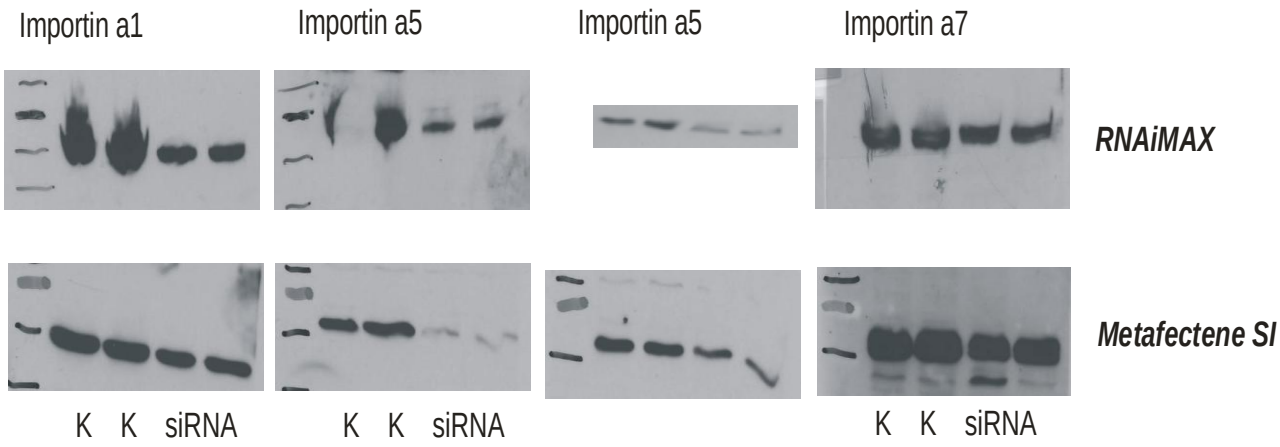

Fig. 2: Comparison of transfection efficiancy of RNAiMAX (Invitrogen) versus Metafectene SI in HeLa cells transfected with siRNA raised against importins alpha 1, 5 and 7 (Quiagen). Cells were transfected with Metafectene SI and RNAiMAX following instructions of the manufacturer's protocol. After 48 hours of incubation cells were lysed und 30µg of protein were loaded on a SDS-PAGE. Proteins were detected by Western Blotting using specific antibodies. K= Control; siRNA= siRNA transfected cells.