

Comparison of transfection reagents from different suppliers in transfection of fluorescence-labelled control siRNA (siGlo from Dharmacon)

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Introduction:

The incitement of the experiment was the establishment of a protocol for transfection of rat cell lines (rat glomerular endothelial cells, RGE). Five different transfection reagents were compared in a single preliminary experiment.

Materials and methods:

In a 24well cell culture plate 100 000 cells per well were seeded and incubated in 500 μ l medium (DMEM with 5% FCS) over night. The next day transfection with fluorescence-labelled control RNA (siGloRNA; Dharmacon; 100nM) was carried out. For each transfection reaction 3 μ l of transfection reagent from four different suppliers were used. After incubation for 24 h the cells were trypsinized and resuspended in FACS buffer. Measurement of transfected cells and fluorescence intensity was carried out by using FACS (BectonDickensen).

Results and discussion:

No differences have been determined in the relative amount of transfected cells using the different transfection reagents (fig.1). On the other hand, the highest fluorescence intensity was measured in reactions using the metafectene pro transfection reagent (Fig.2)

Conclusion / summary:

Our single and therefore preliminary experiment revealed the usefulness of metafectene for transfection of rat glomerular endothelial cells with siRNA.

Appendix: Tables and/or figures:

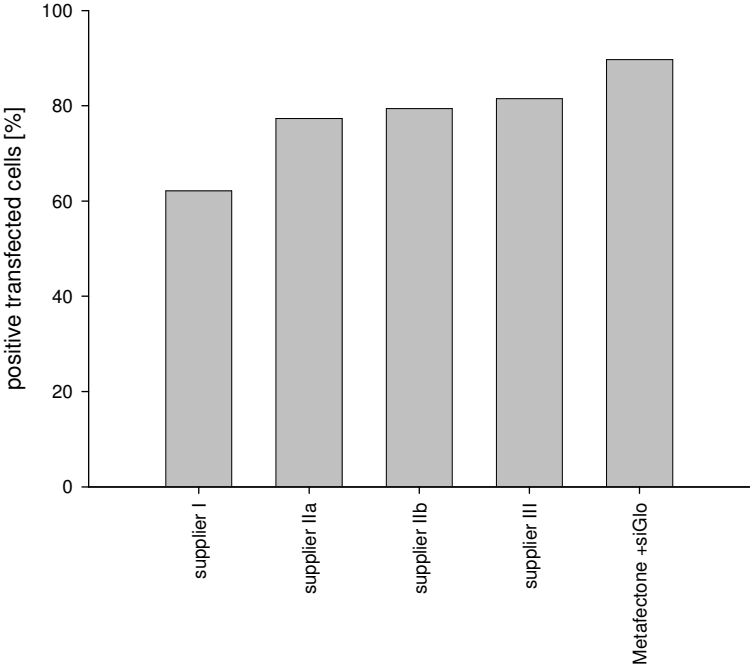


Fig.1: Relative amount of positiv transfected rat glomerular endothelial cells with fluorescence-labeled siRNA using transfection reagents from different suppliers.

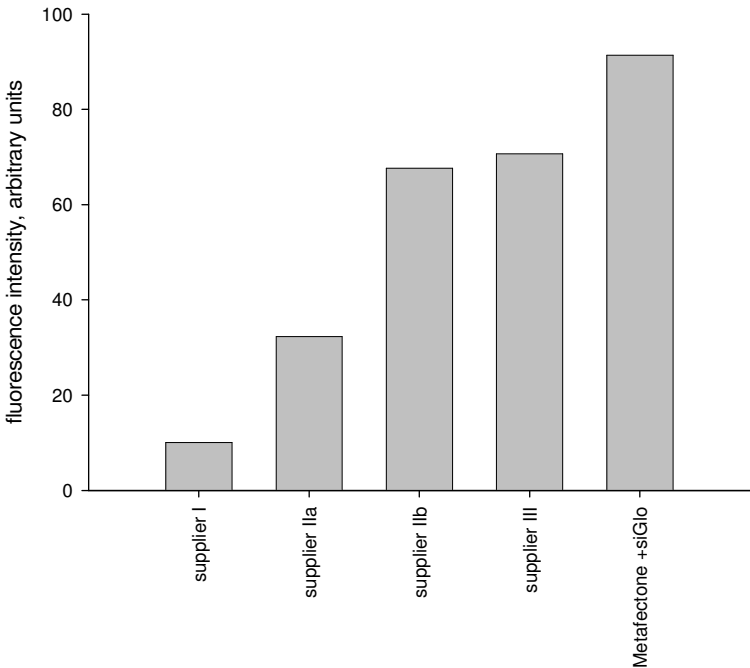


Fig.2: Fluorescence intensity measurements after transfection of rat glomerular endothelial cells with fluorescence-labeled siRNA using transfection reagents from different suppliers.