

Evaluation of Metafectene Pro, Oligofectamine, and Lipitoids for knockdown of β -TRCP

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Introduction:

Effective demonstration of E3 ubiquitin ligase activity towards a substrate requires the efficient siRNA-mediated knock-down of the ligase and concomitant increases in levels of the substrate. Therefore, using a well-characterized siRNA against the F-box protein β -TRCP (targeting both β -TRCP1 and 2), we investigated the transfection efficiency of a variety of cationic liposome reagents by examining the stabilization of the β -TRCP substrates, β -catenin, Claspin, and Cdc25A(2, 3).

Materials and methods:

Metafectene PRO was obtained from Biont^{ex} Laborat^ories GmbH. Oligofectamine (Invitrogen) and lipitoid reagent were also utilized(4). The β -TRCP oligo was previously developed and available from Dharmacon(1). HeLa and T98G cells were cultured in DMEM supplemented with 10% bovine serum (Invitrogen) and 1X Pen-Strep-Glutamine (Gemini Biosciences). The following antibodies were utilized: rabbit anti-CUL1 (Zymed), mouse anti-Cdc25A (Santa Cruz), mouse anti- β -catenin (BD Biosciences), and mouse anti-Claspin(3).

Experimental procedures / transfection protocol:

HeLa or T98G cells were plated in six well plates in DMEM with 10% serum one day prior to transfection. At the time of transfection, each well was 50-60% confluent. Prior to transfection, the DMEM was removed, the wells were washed with PBS. And 1mL of OptiMEM (Invitrogen) was added. For each transfection reagent, 5 μ l of a 20 μ M stock of siRNA was mixed with 100 μ l OptiMEM. A range (from 6 μ l to 18 μ l) of Metafectene PRO was diluted in 100 μ l OptiMEM, mixed with the diluted siRNA, and incubated for 25 minutes prior to addition to cells. For Oligofectamine, 12 μ l of reagent was mixed with 50 μ l of OptiMEM, incubated five minutes, combined with the diluted siRNA, and incubated 25 minutes prior to addition to cells. Lipitoid reagent (4 μ l in 100 μ l Optimem) was mixed with the diluted siRNA and incubated 25 minutes prior to addition to cells. Cells were incubated with transfection reagent for 4 hours before washing with PBS and replacement of the media with DMEM supplemented with 10% serum. After 24 hours, the transfection protocol was repeated, and at 72 hours post-transfection, cells were harvested. Cells were lysed in 50mM Tris (pH 7.5), 150mM NaCl, 1mM EDTA, 50mM NaF, 0.5% Triton X-100, and standard protease and phosphatase inhibitors. The indicated amounts of whole cell lysate were separated by SDS-PAGE, transferred to PVDF membrane, and western blotted.

Results and discussion:

In HeLa cells, both Metafectene PRO and Oligofectamine were able to effectively stabilize multiple β -TRCP targets compared to mock transfection, while the lipitoid reagent failed to stabilize β -TRCP targets. Notably despite effective transfection with both Metafectene PRO and Oligofectamine, Metafectene PRO was more efficient in stabilizing β -TRCP targets (Cdc25A and Claspin) than Oligofectamine. In T98G cells, Metafectene PRO,

Oligofectamine, and lipitoid reagent were similarly effective in stabilizing β -TRCP targets compared to mock transfection.

Conclusion / summary:

Metafectene PRO was more effective than either Oligofectamine or lipitoid reagents in transfection of HeLa cells and equally effective in T98G cells.

References:

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2. **Nakayama, K. I., and K. Nakayama.** 2006. Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer* **6**:369-81.
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Appendix: Tables and/or figures:

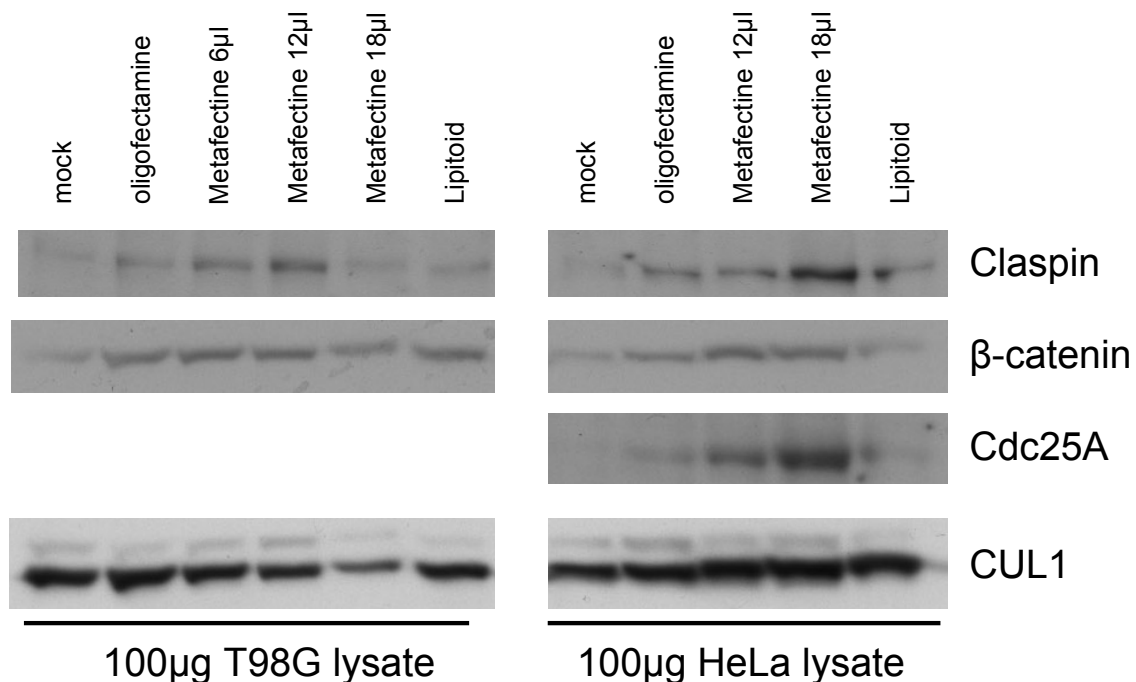


Figure 1: T98G or HeLa cells were transfected with an siRNA against Beta-TRCP using Oligofectamine, Metafectene PRO, and lipitoid reagent. One hundred micrograms of lysate from each sample was separated by SDS-PAGE and western blotted as indicated.