Evaluation of Metafectene Pro, Oligofectamine, and Liptoids 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 Biontex Laboraties 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, 5µl of a 20µM stock of siRNA was mixed with 100µl OptiMEM. A range (from 6µl to 18µl) of Metafectene PRO was diluted in 100µl OptiMEM, mixed with the diluted siRNA, and incubated for 25 minutes prior to addition to cells. For Oligofectamine, 12µl of reagent was mixed with 50µl of OptiMEM, incubated five minutes, combined with the diluted siRNA, and incubated 25 minutes prior to addition to cells. Liptoid reagent (4µl in 100µl 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, transfered 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 stablizing 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 regeants in transfection of HeLa cells and equally effective in T98G cells.

References:

1. **Guardavaccaro, D., Y. Kudo, J. Boulaire, M. Barchi, L. Busino, M. Donzelli, F. Margottin-Goguet, P. K. Jackson, L. Yamasaki, and M. Pagano.** 2003. Control of meiotic and mitotic progression by the F box protein beta-Trcp1 in vivo. Dev Cell **4:**799-812.
2. **Nakayama, K. I., and K. Nakayama.** 2006. Ubiquitin ligases: cell-cycle control and cancer. Nat Rev Cancer **6:**369-81.
3. **Peschiaroli, A., N. V. Dorrello, D. Guardavaccaro, M. Venere, T. Halazonetis, N. E. Sherman, and M. Pagano.** 2006. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. Mol Cell **23:**319-29.
4. **Utku, Y., E. Dehan, O. Ouerfelli, F. Piano, R. N. Zuckermann, M. Pagano, and K. Kirshenbaum.** 2006. A peptidomimetic siRNA transfection reagent for highly effective gene silencing. Mol Biosyst **2:**312-7.

Appendix: Tables and/or figures:

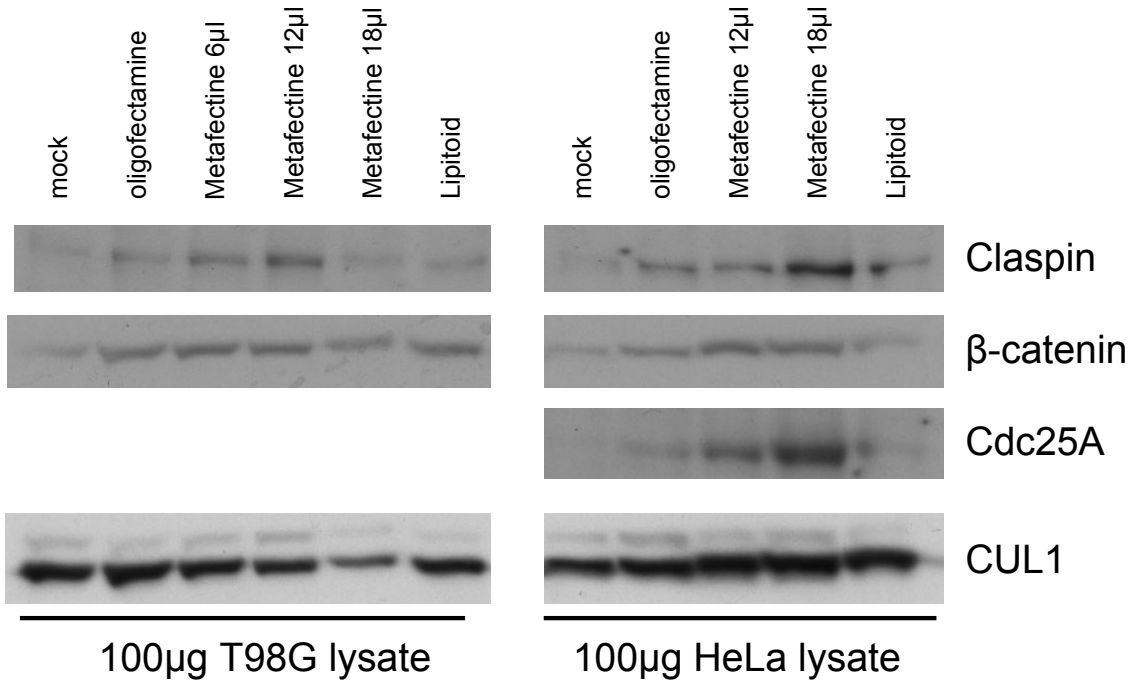

Figure 1: T98G or HeLa cells were transfected with an siRNA against Beta-TRCP using Oligofectamine, Metafectene PRO, and lipitoid reagent. One hundred micrograms of lysate from each sample was separated by SDS-PAGE and western blotted as indicated.