SiRNA-transfection of SKOV-3-Luc cells with the Biontex K2® Transfection System

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This study aimed at comparing knockdown efficacies upon transfection of stably luciferase expressing SKOV-3 ovarian carcinoma cells with chemically non-modified siRNAs, using the K2® transfection reagent at different amounts. The following experiment was performed without addition of the K2® multiplier because previous experiments demonstrated no beneficial effects of the multiplier reagent. Non-specific effects were determined and controlled for by parallel transfection with an unrelated negative control siRNA.

Materials and Methods

Cell culture

The stable luciferase-expressing cell line SKOV-3-Luc was cultivated under 5 % CO_2 at 37 °C in a humid atmosphere in Iscove's Modified Dulbecco's Medium containing 10 % FCS without penicillin/streptomycin.

For transfection experiments, 15,000 cells / well were seeded in 48-well plates. One day later, the transfections were carried out in 0.25 mL Iscove's Modified Dulbecco's Medium containing 10 % FCS without antibiotics as detailed below.

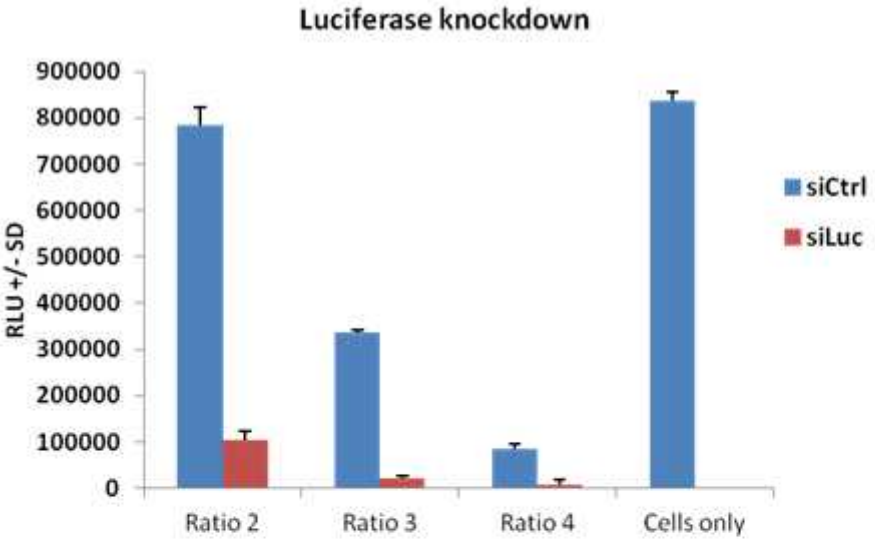
Cell transfection

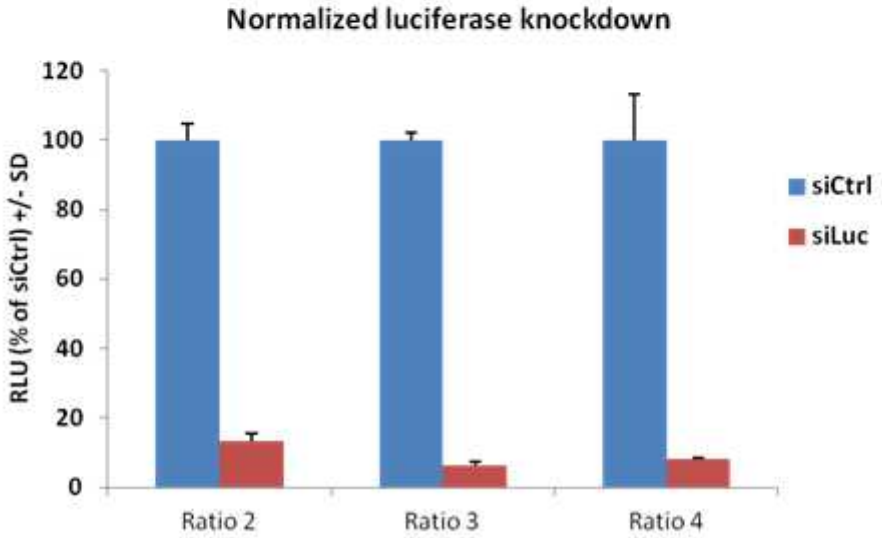
The lipoplexes were prepared following the protocol provided in the manual. Briefly, an appropriate amount of siRNA (0.2 µg or 15 pmol per well, specific for luciferase (siLuc) or non-specific negative control, siCtrl.) was mixed with serum-free IMDM. The K2® Transfection Reagent was diluted separately in IMDM at different ratios (µg siRNA/µL Transfection Reagent) as detailed in the figure. The siRNA solution was added to the diluted Transfection Reagent and mixed by gentle pipetting up and down. After 15 minutes incubation at room temperature, 30 µL of the lipoplex solution containing 0.2 µg (15 pmol) siRNA were added to the wells, followed by a gentle shaking of the well plates. The medium was changed the next day.

The determination of luciferase activity was performed 72 h after transfection using the Beetle-Juice kit from PJK (Kleinblittersdorf, Germany). Briefly, the medium was aspirated and the cells were lysed in 50 µL lysis buffer (Promega). In an appropriate tube, 25 µL luciferin substrate was mixed with 10 µL lysate and chemiluminescence was immediately determined in a luminometer (Berthold, Bad Wildbad, Germany).

Results and Conclusions

The stably luciferase-expressing cell line SKOV-3-Luc (ovarian carcinoma) was used to determine siRNA-mediated luciferase knockdown efficacies when using the K2® Transfection System. For the experimental setup, three different transfection reagent ratios were tested.
Overall, the luciferase activity was reduced to 85-95 % compared to the control siRNA "siCtrl".





Ratio 2: 1:2 [µg RNA]/[µl K2 Transfection Reagent]
Ratio 3: 1:3 [µg RNA]/[µl K2 Transfection Reagent]
Ratio 4: 1:4 [µg RNA]/[µl K2 Transfection Reagent]

Non-specific luciferase knockdown effects were higher with increasing transfection reagent ratios (blue bars in the left panel). Thus, best results in terms of strong knockdown in the absence of non-specific effects were observed at a transfection reagent ratio of 2.

Likewise, cytotoxic effects were strongly increased with higher transfection reagent ratios as indicated by decreasing luciferase activities in the control group. This was confirmed by light microscopy where a lower number of viable cells was observed (not shown). Additionally, a larger siRNA amount (30 pmol) per well was tested and found to display very high cytotoxicity already at transfection reagent ratio 2.

We conclude that the K2® Transfection System has shown very profound knockdown efficacies of luciferase reporter gene activity upon siRNA transfection. In easy to transfect cells, it worked best at lowest ratios and without the K2® Multiplier, thus avoiding non-specific effects of the transfection.