GRP78 siRNA transfection of A2780/A2780cis ovarian cancer cells with K2 reagent

Maximilian Kullmann, Institute of Pharmacy, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

Materials and Methods

Cell culture

The ovarian carcinoma cell line A2780 and the cisplatin-resistant variant A2780cis (European Collection of Cell Cultures, United Kingdom) were cultivated as monolayers in RPMI-1640® medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 0.1 mg/mL streptomycin (37 °C, 5% CO2). Prior to transfection cells were seeded in 6-well plates at 0.5 x 10^6 cells per well in 2 mL RPMI 1640 without antibiotics and incubated for 24 h. After that, cells were grown in RPMI1640 with 10% FCS and antibiotics for an additional 48 h to evaluate protein knockdown.

Cell transfection

(Please find accurate reagent amounts in the table below)

Cells were treated with K2® Multiplier, 2 hours before siRNA transfection. 30 µL multiplier was added carefully to each well. By gently swaying the plates the multiplier was mixed with the medium. The siRNA was diluted in RPMI 1640 medium without FCS and antibiotics. This dilution was added to the dilution of K2 transfection reagent in RPMI1640 medium, mixed by pipetting once and incubated for 15 minutes. Each well was supplemented with 250 µL of the mixture. Again, plates were gently swayed to assure a uniform distribution of transfection reagent. After 24 h the medium was replaced with full medium. Efficiency of knockdown was assessed by Western Blot.

For 2 wells		
RPMI 1640 w/o FCS and AB	250	µL
K2 reagent	27	µL
RPMI 1640 w/o FCS and AB	250	µL
siRNA (10 µM)	10	µL
Diluted siRNA	250	µL
Diluted K2	250	µL
Amount siRNA used per well	50	pmol
Concentration of siRNA	22,2	nM

Results

The relative expression of GRP78 with respect to untreated cells after knockdown was determined by Western Blot. Figure 1 shows the expression of GRP78 in the cells after

transfection with GRP78 siRNA or with negative control siRNA and in untreated cells. GAPDH was used as a loading control.

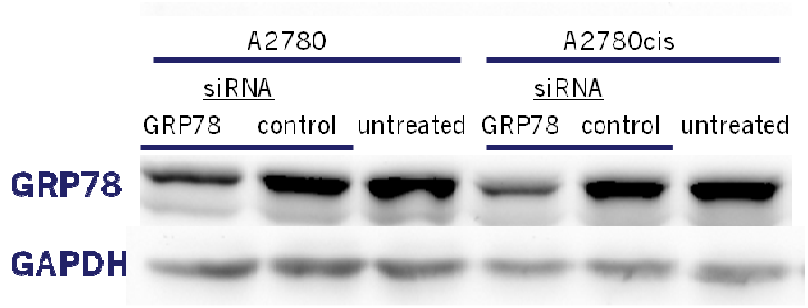

Figure 1.

In Figure 2 the relative expression of GRP78 in the cells transfected with GRP78 siRNA and with negative control siRNA with respect to untreated cells is presented.

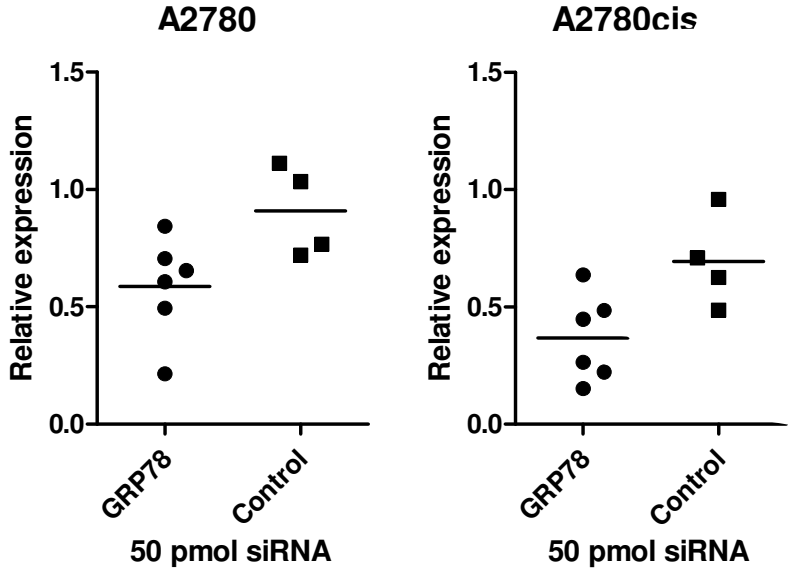

Figure 2.

In A2780 cells expression of GRP78 could be reduced to 59 ± 9 % and in A2780cis cells to 37 ± 8 % (n= 4-6). Cells transfected with negative control siRNA showed no significant change in GRP78 expression compared to untreated cells.

Conclusions

The K2 transfection system was successfully applied for transfecting the A2780/A2780cis cell line pair with siRNA for the specific knockdown of GRP78. Cells are slightly influenced by the knockdown procedure but still proliferate after the knockdown. However, the proliferation rate is reduced compared to untreated control cells.