CAPZA siRNA transfection of A2780/A2780cis cancer cells with K4 Transfection System

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Materials and Methods

Cell culture

The human ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780cis 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% CO_2).

Prior to transfection, cells were seeded in 6-well plates at the density 5 x 10^5 cells per well in 1 mL RPMI® 1640 with 10% FCS without antibiotics and incubated for 24 h.

After transfection, cells were grown in RPMI® 1640 with 10% FCS and antibiotics for additional 48 h and subsequently the efficiency of protein knockdown was evaluated.

Cell transfection

Cells were treated with K4® Multiplier two hours before siRNA transfection. For this purpose, 10 µL multiplier were 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 K4® Transfection Reagent in RPMI® 1640 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
K4® Reagent	27 µL
RPMI® 1640 w/o FCS and AB	250 µL
siRNA (20 µM)	10 µL
Diluted siRNA	260 µL
Diluted K4® Reagent	260 µL
Amount siRNA used per well	100 pmol
Concentration of siRNA	78,7 nM

Results

The relative expression of CAPZA1 after knockdown was determined by Western Blot. Figure 1 shows a representative experiment to determine the expression of this protein in the cells after transfection with CAPZA1 siRNA or with negative control siRNA and in untreated cells. GAPDH was used as a loading control.

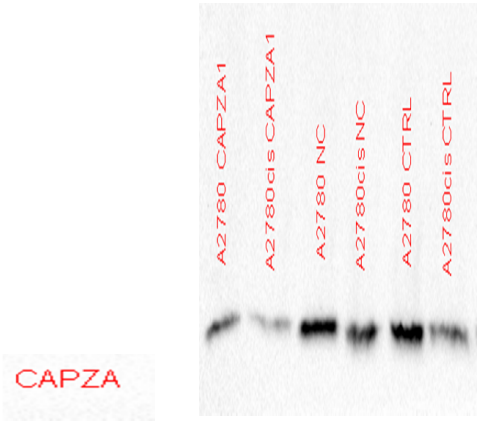

Figure 1. Representative Western Blot of CAPZA1 expression in A2780 and A2780cis cells after transfection with the negative control siRNA (NC), CAPZA1 siRNA (CAPZA1) and in untreated cells (CTRL).

In Figure 2 and 3, the quantification of the relative expression of CAPZA1 in the cells transfected with CAPZA1 siRNA or with negative control siRNA and in untreated cells is presented.

Figure 2. Relative expression of CAPZA1 in A2780 cells after transfection with the negative control siRNA (NC), CAPZA1 siRNA (CAPZA1) and in untreated cells (CTRL).

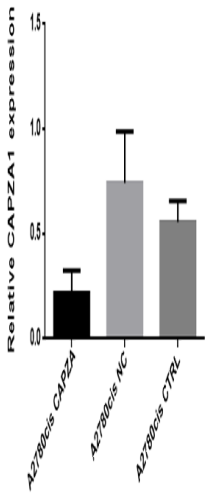

Figure 3. Relative expression of CAPZA1 in A2780cis cells after transfection with the negative control siRNA (NC), CAPZA1 siRNA (CAPZA1) and in untreated cells (CTRL).

In A2780 cells, the level of CAPZA1 was significantly reduced to 36.2 ± 12.6% (mean ± SD, n = 4). The expression of the protein was diminished to 34.0 ± 19.7% in A2780cis cells (mean ± SD, n = 4).

Conclusions

The K4® Transfection System was successfully applied in A2780/A2780cis cell line pair with siRNA for the specific knockdown of CAPZA1.