GSTP1 siRNA transfection of HCT-8/HCT-8ox ileocecal colorectal cancer cells with K4 Transfection System

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Materials and Methods

Cell culture

The human ileocecal colorectal adenocarcinoma cell line HCT-8 and its oxaliplatin-resistant variant HCT-8ox kindly provided by Dr. R. A. Hilger (University Hospital Essen, Germany) 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 2.5 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 an additional 48 h and subsequently the efficiency of protein knockdown was evaluated.

Cell transfection

(Please find accurate reagent amounts in the table below)

Cells were treated with K4® Multiplier two hours before siRNA transfection. For this purpose, 10 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 K4® Transfection Reagent in RPMI® 1640 medium, mixed by pipetting once and incubated for 15 minutes. Each well was supplemented with 260 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 48 h later.

For 2 wells		
RPMI 1640 w/o FCS and AB	250	µL
K4® Transfection Reagent	27	µL
RPMI® 1640 w/o FCS and AB	250	µL
siRNA (20 µM)	10	µL
Diluted siRNA	260	µL
Diluted K4® Transfection Reagent	260	µL
Amount siRNA used per well	100	pmol
Concentration of siRNA	78,7	nM

Results

The relative expression of GSTP1 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 GSTP1 siRNA or with negative control siRNA and in untreated cells. GAPDH was used as a loading control.

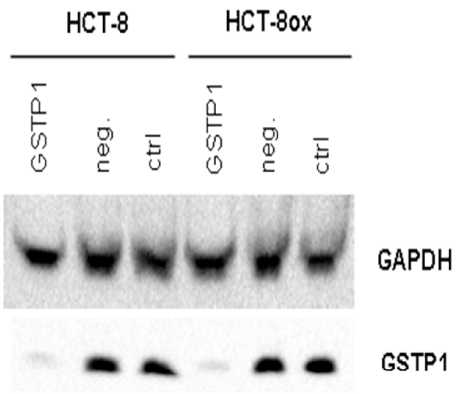

Figure 1. Representative Western Blot of the GSTP1 expression in HCT-8 and HCT-8ox cells after transfection with the negative control siRNA (neg.), GSTP1 siRNA (GSTP1) and in untreated cells (ctrl).

In Figure 2, the quantification of the relative expression of GSTP1 in the cells transfected with GSTP1 siRNA and with negative control siRNA with respect to untreated cells is presented.

Figure 2. Relative expression of GSTP1 in HCT-8 (filled symbols) and HCT-8ox (open symbols) cells after transfection with the negative control siRNA (neg.), GSTP1 siRNA (GSTP1) and in untreated cells (ctrl).

In HCT-8 cells, the level of GSTP1 could be reduced to 7.86 ± 0.16 % and in HCT-8ox cells to 7.57 ± 0.23 % (n = 3). Cells transfected with negative control siRNA showed no significant change in GSTP1 expression compared to untreated cells.

Conclusions

The K4® Transfection System was successfully applied in HCT-8/HCT-8ox cell line pair with siRNA for the specific knockdown of GSTP1. Cells were slightly influenced by the knockdown procedure but still proliferated after the knockdown. However, the proliferation rate was reduced compared to untreated control cells.