Plasmid stable transfection of human PANC-1 (ATCC® CRL-1469™) cells using "Biontex K2® Transfection System".

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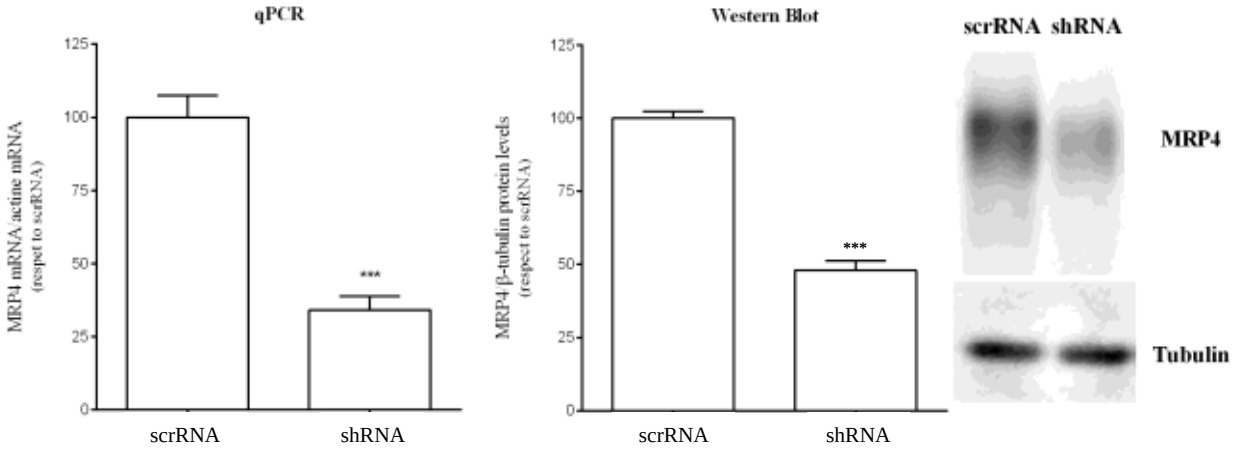
Materials and Methods

1. 500,000 PANC-1 cells (ATCC® CRL-1469™) were plate in each well of a 6-well dish in 3 ml of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum and 5 mg/ml gentamicin.
2. Cells were incubated for 24h at 37°C in a CO_2 incubator until 80-100% of confluence.
3. Cells in 1.5 mL medium were treated with 24 µl of K2® Multiplier 2 hours before adding the lipoplex. For this, K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes.
4. For each well of a 24-well dish there were prepared:
Solution A: 2 µg of plasmid coding for a shRNA specific for MRP4 or coding for a non-silencing oligonucleotide (scrRNA).
Solution B: 4 µl of K2® Transfection reagent was added to 150 µl medium without serum.
Solution A was added to the solution B (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection mix was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfected cells were incubated at 37°C and 5% CO_2 for 24 hours and then cells were selected with puromycin (antibiotic resistance encoded in the plasmid) for 48 hours and splitted into 96 wells-plate in order to obtain clonal populations. Resistant clone cells were lysed and protein and total RNA and cDNA were prepared.
Analysis of RNA was performed by quantitative RT-PCR and protein levels were measured by Western blot.

Results



Expression of MRP4 mRNA (left) and protein levels (right) after transfection. ***p<0.01 respect to scrRNA.

Conclusions

Our results show that **Biontex K2® Transfection System** reagent was successfully applied for transfecting and obtaining stable clones of PANC-1 cells with a plasmid coding for a shRNA targeting MRP4.