Comparison of transfection efficiencies of METAFECTENE PRO and Reagent L in MDA-MB231 human breast cancer cell line using pSV-β-Galactosidase control plasmid.

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Introduction:

Our work investigates the role of ADAM (a disintegrin and metalloproteinase) family of proteins in human breast cancer. We are seeking a reagent that would give us the maximal efficiency in transfecting plamid DNA in MDA-MB231 human breast cancer cell line for usage in transient and stable transfections. The trasfection efficiency of METAFECTENE PRO, a novel reagent was compared to Reagent L which is currently used in the laboratory. The pSV-β-Galactosidase Control Vector (Promega) was used as a positive control vector for monitoring transfection efficiencies in these cells. Different volumes of the trasfection reagents and plasmid DNA were used inorder to identify the optimal transfection conditions.

Materials and methods:

MDA-MB231 human breast cancer cells was obtained from cell culture collection of Cancer Research UK. Cells were routinely cultured as monolayer cultures in DMEM with phenol red, 10 % FCS, 5mM glutamine and 1% Penicillin/streptomycin cocktail (regular growth medium). OPTIMEM serum free medium was obtained from Invitrogen. The transfection reagent METAFECTENE PRO was a gift from Dr. Stefan Hofreiter, Biontex laboratories, Germany. The trasfection reagent, Reagent L, was obtained from another supplier. Cell lysis buffer and the beta-galactosidase enzyme assay system were obatined from Promega.

Experimental procedures / transfection protocol:

Proliferating, healthy MDA-MB231 cells were seeded at a density of 3 x 10^5 cells/well in a 12 well tissue culture plate one day before transfection. On the day of transfection, cells were washed with OPTIMEM- serum free medium (Invitrogen) and then replenished with 1 ml of OPTIMEM. METAFECTENE PRO was complexed with

PSV beta gal plasmid DNA at DNA: reagent ratios 0.5µg:1µl, 0.5µg:2µl, 1.0µg:1µl, 1.0µg:2µl. Reactions were set up in a 24 well plate and in all cases diluted in a total volume of 50 µl OPTIMEM medium per reaction. Care was take to ensure that the medium was pipetted first followed by the addition of DNA as per the manufacturer's protocol. Complexes were allowed to incubate for 20 minutes after which 100 µl was added in a drop-wise manner to the cells. The medium containing the complex was substituted with regular growth medium after 5 hours. After 48 hours post-transfection, medium was removed from the cells, washed with ice-cold PBS and lysed in 80 µl 1Xcell lysis buffer (E1500, Promega). 20 µl of the cell lysates was used for estimating beta-galactosidase activity using the beta-galactosidase enzyme assay system (Promega) and data expressed as absorbance at 420nM.

Results and discussion:

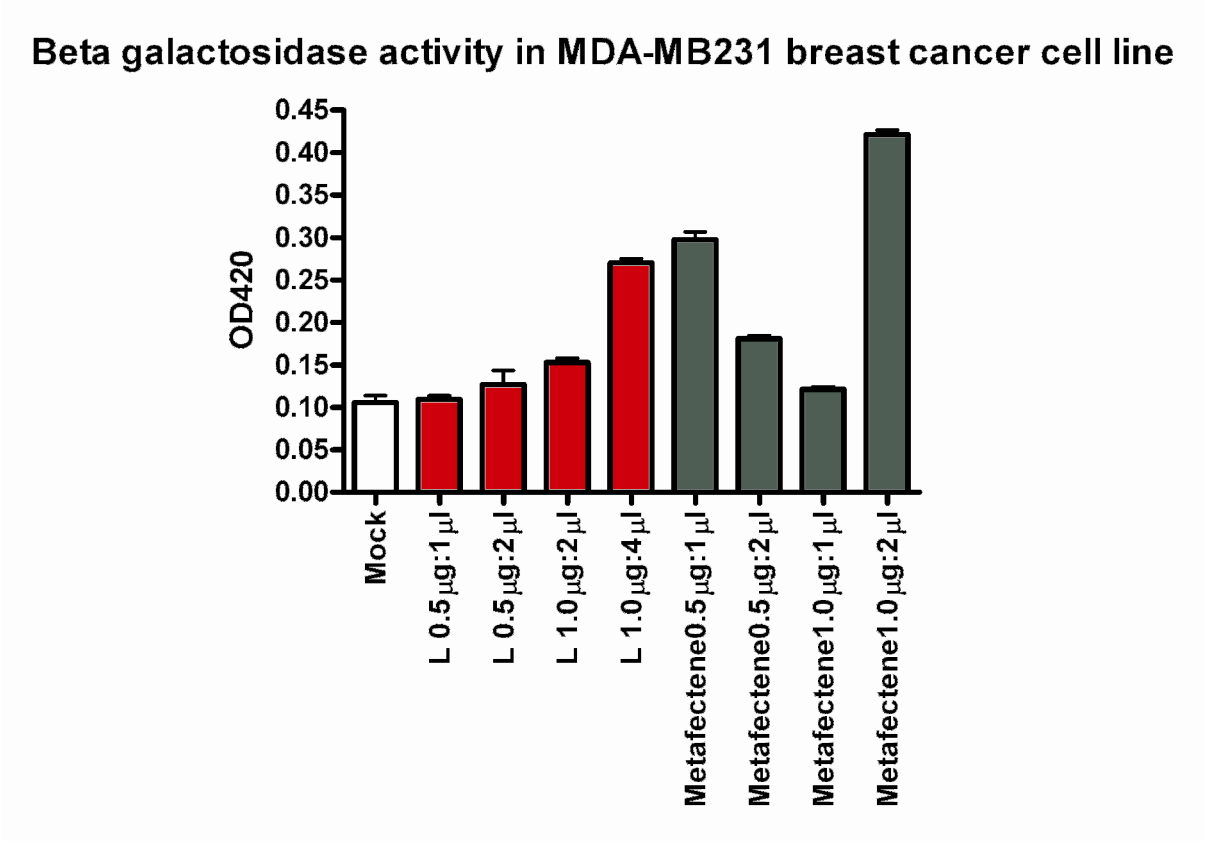
MDA-MB231 cells were transfected with METAFECTENE PRO or with Reagent L from an alternative supplier (Reagent L was used at different beta-gal plasmid and reagent ratios of 0.5µg:1µl, 0.5µg: 2µl, 1.0µg:2µl, 1.0µg:4µl) according to the instructions of the manufacturer.

Results (Figure 1) indicate that METAFECTENE PRO showed the highest beta gal activity in cells transfected with plasmid DNA reagent ratio of 1.0µg : 2µl. The values were clearly higher than that obtained with Reagent L transfected cells.

Conclusion / summary:

METAFECTENE PRO shows enhanced transfection efficiency in delivering pSV-β-Galactosidase plasmid DNA in MDA-MB231 human breast cancer cells.

Appendix: Tables and/or figures:


Beta galactosidase activity in MDA-MB231 breast cancer cell line
OD420
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Mock
L 0.5 µg:1 µl
L 0.5 µg:2 µl
L 1.0 µg:2 µl
L 1.0 µg:4 µl
Metafectene0.5 µg:1 µl
Metafectene0.5 µg:2 µl
Metafectene1.0 µg:1 µl
Metafectene1.0 µg:2 µl