Transfection of human neuroblastoma SK-N-BE cells with Metafectene Pro reagent

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Methods

Cell culture

Human neuroblastoma SK-N-BE cells (American Type Culture Collection, ATCC, LGC-Promochem Teddington, Middlesex, U.K.) were grown in monolayer in RPMI 1640 medium (Sigma Chemical Co, St. Louis, MO, USA) supplemented with 10% Foetal Bovine Serum (FBS, Sigma Chemical Co, St. Louis, MO, USA), 2 mM L-glutamine, 50 μg/ml streptomicin and 50 IU/ml penicillin; the cells were kept in a 5% CO_2 and 95% air atmosphere at 37°C.

Metafectene transfection protocol

$5x10^5$ SK-N-BE cells were plated in 60mm Petri dishes in 3 ml of complete medium and incubated for 18h before transfection. At the time of transfection cells reached 60-70% confluency. The specified amounts of DNA were mixed in a 1.5ml Eppendorf tube with 50μl of serum- and antibiotic-free medium. Empy vector DNA was used to bring the amount of DNA transfected to 2.5 ug for all samples. (Sol. A) In another test tube the specified amounts of Metafectene Pro transfection reagent were mixed with 50μl of serum- and antibiotic-free medium. (Sol. B)

Then, Sol. A was added to Sol. B and the mixture was incubated at room temperature for 30min.

After 4h of incubation at 37°C, medium was replaced and cells were harvested for Western blotting analysis and β-gal activity assay after 24h as described below.

Lipofectamine transfection protocol

SK-N-BE were plated in 60mm Petri dishes as described above and incubated for 18h before transfection.

Sol. A: The specified amounts of DNA were added to 200μl serum- and antibiotic-free medium in Eppendorf tube. Two micrograms of empy vector DNA were used in the control sample transfected with β-gal plasmid alone.

Sol. B: 12 μl of lipofectamine (Life Technologies) were added to 200μl serum- and antibiotic-free medium in Eppendorf tube.

Sol. A and Sol. B were mixed, kept at room temperature for 45min and then added to the cells in 2ml of serum- and antibiotics-free medium . After 4h the medium was replaced with complete medium and cells were harvested for Western blotting analysis and β-gal activity assay after 24h as described below.

Plasmids

β-gal and p67^{phox} plasmids were all inserted in PC DNA3 plasmid under the control of CMV promoter. The empty vector CMV PCDNA3 plasmid was used as control.

Western blotting analysis

Cells were harvested by scraping them into RIPA buffer (50mM Tris-HCl, pH 7.5, NaCl 150mM, 1%NP40, 0.5% deoxycholate, 0.1% SDS) containing 2.5mM Na-pyrophosphate, 1mM β-glycerophosphate, 1mM Na_3VO_4, 1mM NaF, 0.5mM PMSF and a cocktail of protease inhibitors (Boheringer – Mannheim Co., Mannheim, Germany). Next, cell lysates were centrifuged for 10 min at 11600xg and the pellets were discarded. 40 micrograms of protein in Laemmli sample buffer (Laemmli 1970) was boiled for 5 min, resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. The membranes were then probed either with anti p67phox antibody (from Upstate), at 1:1000 dilution following the manifacturer's instructions. To normalize for sample loading and protein transfer the membranes were then stripped and reprobed with antibodies at 1:2000 dilution against monoclonal α-tubulin (from Sigma). Protein bands were revealed by enhanced chemiluminescence (ECL), (Amersham Pharmacia Biotech, Milano, Italia) and quantified by densitometry.

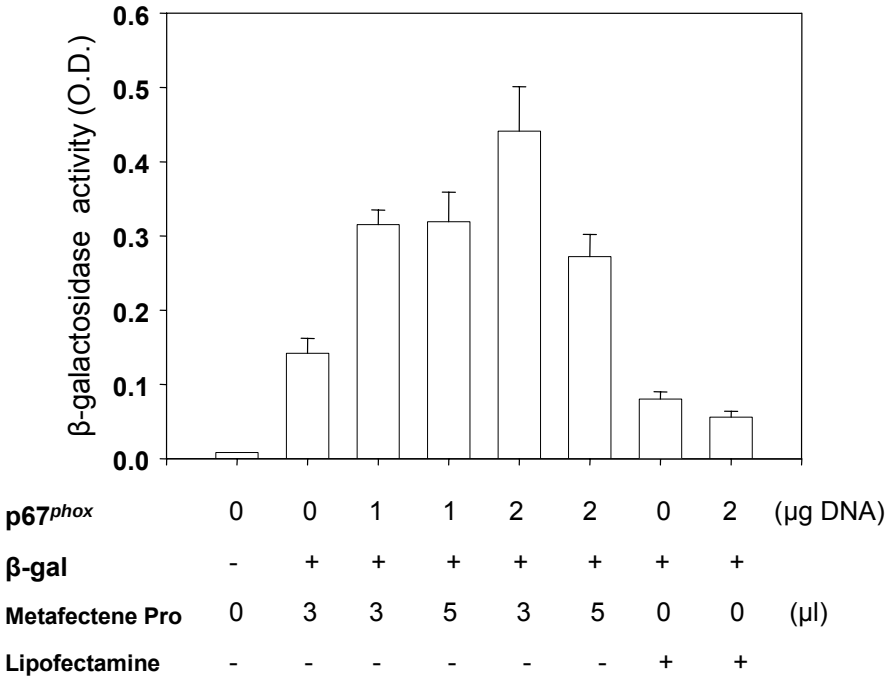

Fig. 1 Beta-galactosidase activity assay of cells transfected either with Metafectene Pro or Lipofectamine reagent solution as described in Methods. 0.5μg of β-gal DNA were used.

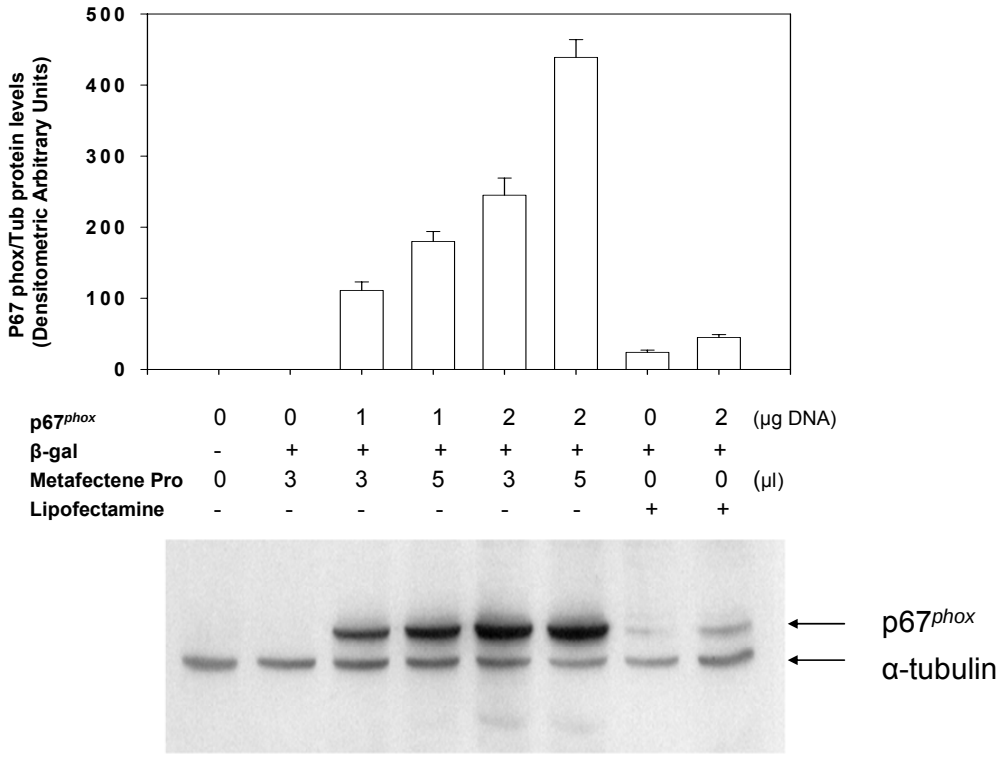

Fig. 2 Western blotting analisys of cells transfected with p67^{phox} and β-gal plasmids with Metafectene Pro or Lipofectamine reagent solution as described in methods. 0.5 ug of β-gal DNA were used. The hystograms shows the densitometric values (means +/- SE) of three different experiments. A representative experiment is shown in the lower part of the figure.

Conclusions

Metafectene Pro transfection reagent has been used for transient transfection of neuroblastoma SK-N-BE cells. These cells are very difficult or impossibile to transiently transfect using Lipofectamine or other transfection reagents (i.e. Fugene; data not shown). Stable transfection works fine with these reagents.

The best results were obtained using 5µl of Metafectene and 2.5µg of DNA. In these conditions, the efficiency of transfection was higher than 10 fold compared to the Lipofectamine reagent.

With other cells, such as Hela, we did not notice greater differences. We should point out that the number of experiments was lower than those reported above.