Transfection of C6 glioma cells with Metafectene Pro

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Introduction

Bcl-x, a member of the Bcl-2 family, plays a critical role in the control of apoptosis. Bcl-x is required for development, and Bcl-x-deficient mice die at embryonic day 13 due to extensive apoptosis of neurons and haematopoietic cells (Motoyama et al., 1995). Alternative splicing in the exon 2 of Bcl-x pre-mRNA results in the production of two isoforms with opposing activities. Bcl-xL (the large form) is anti-apoptotic, while Bcl-xS (the short form) is pro-apoptotic by antagonizing the function of Bcl-xL and Bcl-2. Bcl-x gene contains multiple promoters, which have been suggested to modulate downstream alternative splicing. Previous studies have demonstrated Bcl-x expression is regulated by several transcription factors, such as NF-κB, Ets and Stat5 in malignant cells. However, the mechanisms mediating Bcl-x expression and the transcription factors that act at multiple promoters in neural cells remain unclear. Here we cloned 2.5 kb of Bcl-x promoter region, and examined the luciferase activity under the control of this promoter using a novel transfection reagent Metafectene Pro in rat C6 glioma cells.

Materials and Methods

Materials
Metafectene Pro, a polycationic liposomal transfection reagent, was kindly provided by Dr. S. Hofreiter from Biontex Laboratories GmbH (Martinsried, Germany). The plasmids, pBcl-x.luc and pEGFP were stored at our laboratory.

Cell culture
Rat C6 glioma cell line was obtained from ATCC (Rockville, MD), and cultured in DMEM supplemented with 10% FBS (Sigma-Aldrich, St. Louis, MO) and 100 U/ml penicillin, 100 µg/ml streptomycin (Hyclone, Logan, UT).

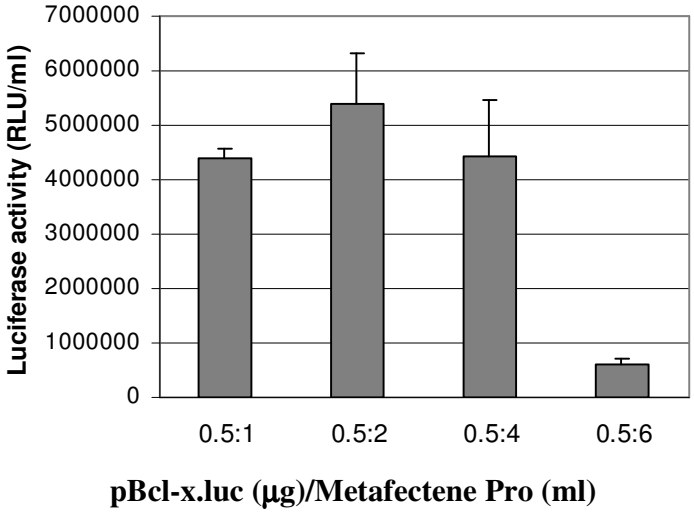
Transfection protocol

Rat C6 cells were seeded onto 12-well plate with the density of 8 x 105 cells per well, in 1 ml of medium one day before transfection, and used at 70-80% confluence. The pBcl-x.luc plasmid was complexed with Metafectene Pro at DNA:reagent ratios of 0.5 µg:1 µl, 0.5 µg:2 µl, 0.5 µg:4 µl, 0.5 µg:6 µl, 1 µg:2 µl, 1 µg:4 µl, 1 µg:8 µl, 1 µg:12 µl, 2 µg:4 µl, 2 µg:8 µl, 2 µg:12 µl and 2 µg:16 µl. Complexes were prepared by mixing Metafectene Pro with 100 µl of PBS, followed by the addition of DNA and incubated at room temperature for 20 min. The DNA-lipid complex was added dropwise into the cell culture. Six hours post transfection, medium was replaced by fresh growth medium. The transfection efficacy was evaluated after 48 hr by measuring luciferase activity, using the luciferase Assay System obtained from Promega and a luminometer (TD-20/20). The transfection efficiency was also evaluated by green signal under the fluorescent microscope (SWISS) 48 hr post transfection with pEGFP plasmid and Metafectene complex (1 µg:2 µl).

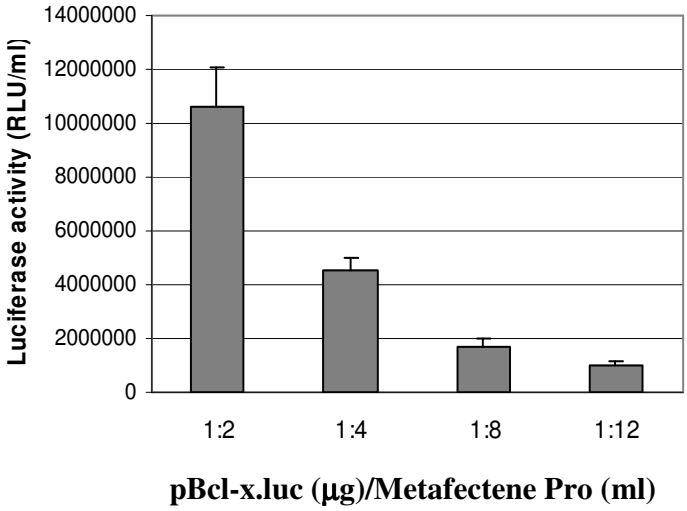
Results

The highest transfection efficiency was achieved with the 1µg:2µl DNA/Metafectene Pro ratio in C6 cells for 12-well format (Fig. 1 and 2). Cell morphology does change significantly.

A



B



C

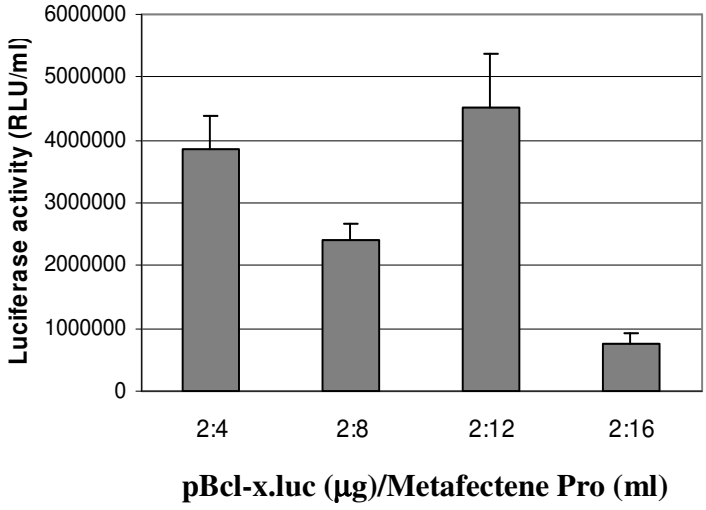


Figure 1. Optimization of transfection in C6 cells. The cells were transfected with the indicated amounts of Metafectene Pro and the pBcl-x.luc plasmid. Results are expressed as relative light units (RLU) per ml of cell lysate. Data represent the mean ± SD from three independent experiments.

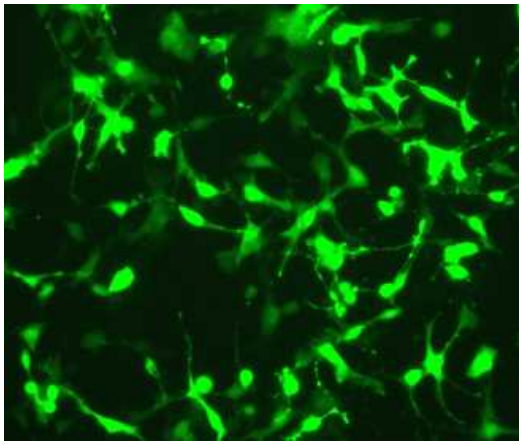


Figure 2. Transfection of C6 cells with Metafectene Pro and the pEGFP plasmid.

Conclusion

Metafectene Pro is highly effective in transferring plasmid DNA into rat C6 cells and shows very low cytotoxicity.