Transfection of Chinese hamster ovary [CHO] and human HT-1080 breast fibrocarcinoma cells with METAFECTENE PRO

Michael Skalski, Michelle Kean and Marc G. Coppolino

Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ont., Canada N1G 2W1

Materials and methods:

Materials

The plasmid, pEGFP-N1, encoding green fluorescent protein was used to assess transfection efficiency. Metefectene PRO was obtained from Biontex Laboratories GmbH, Munich, Germany. FuGene 6 Transfecton reagent was purchased from Roch Applied Science, Laval, QC.

Cell culture

Chinese hamster ovary (CHO-K1) and HT-1080 (ATCC, Rockville, MD) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone) supplemented with 10% fetal bovine serum (HyClone) under 5%CO_2 at 37 °C.

Transfection protocol

CHO-K1 cells: CHO-K1 cells were plated in 6-well dishes and allowed to grow overnight to 70% confluencey. Metafectene PRO was complexed with pEGFP-N1 plasmid at ratios 2 ul:2 ug, 4 ul:2 ug, 8 ul:2 ug and 16 ul:2 ug. Complexes were prepared following the manufacturer's protocol.

HT-1080 cells: HT-1080 cells were plated in 6-well dishes and allowed to grow overnight to 70 or 95% confluencey. Metafectene PRO was complexed with pEGFP-N1 plasmid at ratios 2 ul:2 ug, 4 ul:2 ug, 8 ul:2 ug, 10 ul:2 ug and 14ul:2ug. Complexes were prepared following the manufacturer protocol.

Cells were also transfected FuGene6 Transfection Reagent (Roche Applied Science, Laval, QC) following the manufacturer's suggested protocol and compared to Metafectene PRO.

Transfection efficiency and cell confluency was assessed 24 h post-transfection on a inverted Leica DM IRE2 microscope

Results and discussion:

CHO-K1: The transfection efficiency and confluency of CHO-K1 cells was as follows:

4ul FuGene 6:2ug- Approximately 35% of cells were expressing the GFP construct and cell confluency was >100%.

2ul Metafectene PRO:2ug- Approximately 50% of cells were expressing the GFP construct and cell confluency was 50%.

4ul Metafectene PRO:2ug- Approximately 70% of cells were expressing the GFP construct and cell confluency was 20%.

8ul Metafectene PRO:2ug- Approximately 90% of cells were expressing the GFP construct and cell confluency was 20%.

16ul Metafectene PRO:2ug- Approximately 90% of cells were expressing the GFP construct and cell confluency was 10%.

Untransfected control cell had a confluency greater than 100%.

HT-1080: The transfection efficiency of HT-1080 cells was as follows:

2ul:2ug- Approximately 30% of cells were expressing the GFP construct

4ul:2ug- Approximately 40% of cells were expressing the GFP construct

8ul:2ug- Approximately 50% of cells were expressing the GFP construct

10ul:2ug- Approximately 75% of cells were expressing the GFP construct

14ul:2ug- Approximately 75% of cells were expressing the GFP construct

There was low level of toxicity for HT-1080 cells transfected with GFP-N1.

Conclusion / summary:

In HT-1080 cell, Metafectene PRO provide good transfection efficiency with little toxicity to the cells; however, in CHO-K1 cells, while transfection efficiency was good, we observed significant cell death.