Transfection of human primary glioma cells, human and murine glioma cell lines as well as other eukaryotic cell lines with METAFECTENE PRO

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INTRODUCTION

The most common malignant brain tumor, glioblastoma, is rather resistant to current approaches of therapy. Standard therapy includes cytoreductive neurosurgery, radiotherapy and adjuvant chemotherapy and prolongs the median survival from about 3-4 months without therapy to 12-15 months. Glioblastomas kill by local destructive growth, but do not metastasize outside the brain. Therefore, glioblastoma is a major target for novel strategies of somatic gene therapy. Somatic gene therapy is based on the expression of therapy genes which will cause cytotoxic effects, induce apoptosis or stimulate the immune response against the cancer cell. On the other hand, shRNA-based knockdown of cancer-relevant genes in tumor cells could be used a therapy strategy. At least for *in vitro* studies, a highly efficient delivery system is needed to generate stably or transiently genetically engineered cells. We here focused to establish a powerful transfection system for primary and established glioma cells *in vitro,* allowing effective delivery of plasmids and oligonucleotides without major cytotoxicity . For the transfection of human primary glioma cultures, established human and murine glioma cell lines and for a variety of other cell lines of different origin, we evaluated the transfection efficiency achieved by Metafectene PRO in comparison to some other liposomal transfection reagents. As a reporter gene to investigate the efficiency of plasmid transfection, we used a vector expressing the enhanced green fluorescent protein (EGFP) under the control of the constitutive active CMV-promoter (pCMV-EGFP). In a second approach, the transfection efficiency of siRNA was evaluated in a human glioma cell line.

MATERIALS AND METHODS

Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). The si*CONTROL* Tox Transfection Control was obtained from Dharmacon (Lafayette, CO), cell culture medium, fetal calf serum (FCS), penicillin and streptomycin were purchased from Cambrex (Verviers, Belgium). The plasmid, pCMV-EGFP, a gift of B. Vogelstein (The John Hopkins Cancer Center, Baltimore, MD), was used for the evaluation of transfection efficieny using fluorescent based cell sorting analysis (FACS).

Cells

Human primary glioma cells were prepared as described (Rieger, 2003). The human glioma cell line T98G was obtained from ATCC (Rockville, MD), LN-308, U87MG, A172 and LNT-229 glioma cells were kindly provided by N. de Tribolet (Lausanne, Switzerland), the mouse glioma cell line SMA-560 was from D. D. Bigner (Durham, NC). Human N-Tera2 teratocarcinoma cells were a kindly gift of S. DiGiovanni (Tübingen, Germany), the mink epithelial lung cell line (MLEC32) was established by D.B.

Rifkin (NYU Medical Center, New York, NY), human microvascular endothelial cells were from H. Eltzschig (Tübingen, Germany). All cells except HMEC which grow in MCDB-131 supplemented with EGF and hydrocortisone, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, penicillin (100 U/ml) and streptomycin (100 µg/ml).

Transfection protocol

For transfection, the cells (2 x 10^4 cells/well, approximately 60% confluence) were seeded in 48-well culture plates and allowed to attach. The cells were pre-washed with serum-containing DMEM and then covered with 250 µl of the same medium. Metafectene PRO was complexed with pCMV-EGFP plasmid at reagent : DNA ratios of 0.4µl : 0.35µg, 1µl : 0.35µg, 2µl : 0.35µg, 5µl : 0.35µg, 7µl : 0.35µg or 10µl : 0.35µg DNA. As a control, lipid X was used in parallel according to the manufactures instructions in reagent : DNA ratios of 0.4µl : 0.18µg, 0.8µl : 0.36µg, 1.3µl : 0.36µg, 1.6µl : 0.36µg, 1.6µl : 0.18µg and 2.7µl : 0.36µg. Complexes were prepared by mixing the DNA with 20 µl of DMEM containing no antibiotics and serum, followed by the addition of transfection reagent. The mixture was incubated for 20 min at room temperature. Transfection complexes were added to the cells in a total of 250 µl serum-containing DMEM for 4 h at 37°C. To avoid toxicity, the cells were washed with 1 ml DMEM followed by the addition of 0.5 ml DMEM. Transfection efficiency was evaluated after 48 hours using FACS analysis on a DakoCytomation CyAn device. Data were expressed as percentages of EGFP-expressing cells. For primary glioma cells, the transfection efficiency was detected by visually counting EGFP-positive cells using a fluorescence microscope.

To assess the transfection efficiency of siRNA oligonucleotides, LN-308 glioma cells were seeded (2.5 x 10^4 cells/well) in 96-well culture plates and allowed to attach. The si*CONTROL* Tox Transfection Control was used according to the manufactures instructions using Metafectene PRO or siRNA transfection reagents from three other providers. Cell density was assessed by crystal violett staining.

Cell viability assay

For primary cells, cell morphology was evaluated by inverted phase contrast microscopy at 40x magnification. For cell lines, the number of viable cells was determined by propidium iodide (PI) exclusion using FACS analysis in parallel to the detection of EGFP-expression. Data were evaluated as percentages of PI-positive cells in comparison to non-transfected cells.

RESULTS

Metafectene PRO/pCMV-EGFP complexes were generated using ratios of reagent : DNA from 1.1 up to 28 for Metafectene PRO and from 2.2 to 7.5 for a second lipofection reagent (lipid X), according to the manufacture´s instructions. Dependent on the cell line, up to 60 % EGFP-positive cells were detected 48 h after transfection with Metafectene PRO (Fig. 1). For most cell lines, Metafectene PRO was even the best transfection reagent tested so far (Fig. 1). For primary glioma cells from patient biopsies, transfection rates were very low, but, in contrast to lipid X, EGFP-positive cells could be detected when using Metafectene PRO (Fig. 1B). Therefore the establishment of stably transfected primary cells from these patient biopsies is feasible. For transfection conditions using lipid amounts of less than 8 µl Metafectene PRO, cytotoxicity was low (< 5% of transfected cells, data not shown). When adding more than 7 µl of the transfection reagent, cyotoxicity was dramatically enhanced in glioma cell lines.

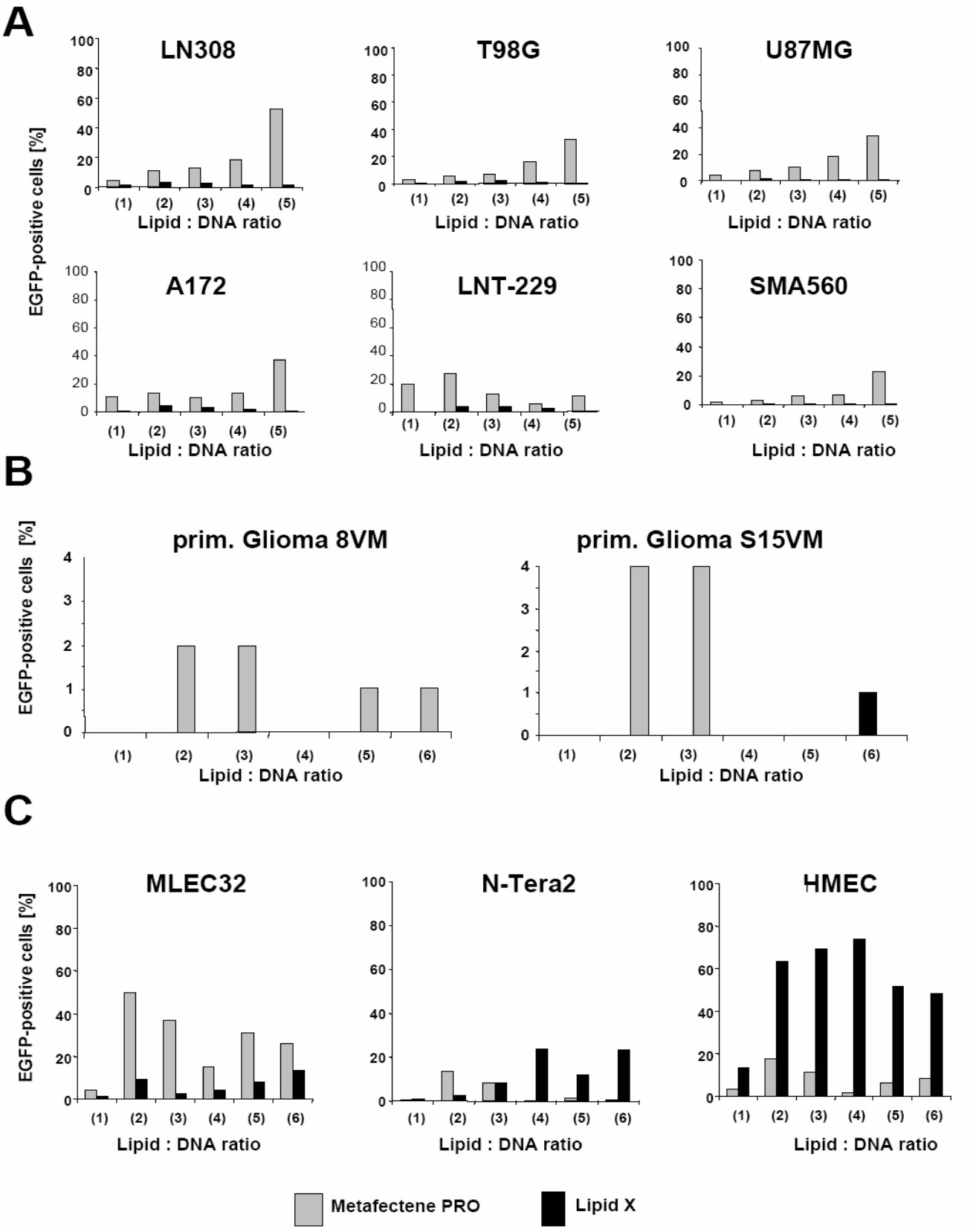

Fig.1 Transfection of primary cells and cell lines with Metafectene PRO or lipid X. Reagent : DNA ratio: Metafectene PRO: (1) 0.4µl:0.35µg, (2) 1µl:0.35µg, (3) 2µl:0.35µg, (4) 5µl:0.35µg, (5) 7µl:0.35µg or (6) 10µl : 0.35µg. Lipid X: (1) 0.4µl:0.18µg, (2) 0.8µl:0.35µg, (3) 1.3µl:0.35µg, (4) 1.6µl:0.35µg, (5) 1.6µl:0.18µg and (6) 2.7µl:0.35µg A: established glioma cell lines, B: primary glioma cells, C: non-glioma cell lines

Using the siTOX Assay, we tested the efficiency or Metafectene PRO as a transfection reagent for siRNA oligonucleotids in the human glioma cell line LN-308. Cells successfully transfected with the si*CONTROL* TOX Transfection Control undergo apoptosis and cell death within 24-48 hours, the degree of cell death can then be correlated to transfection efficiency. As shown in Fig. 2, Metafectene PRO showed best transfection results (> 80% of siTox transfected cells, black bars) at low concentrations when compared to reagents of other providers. And, in contrast to siRNA transfection reagents from other providers, toxicity of Metafectene PRO was low or not detectable (white bars).

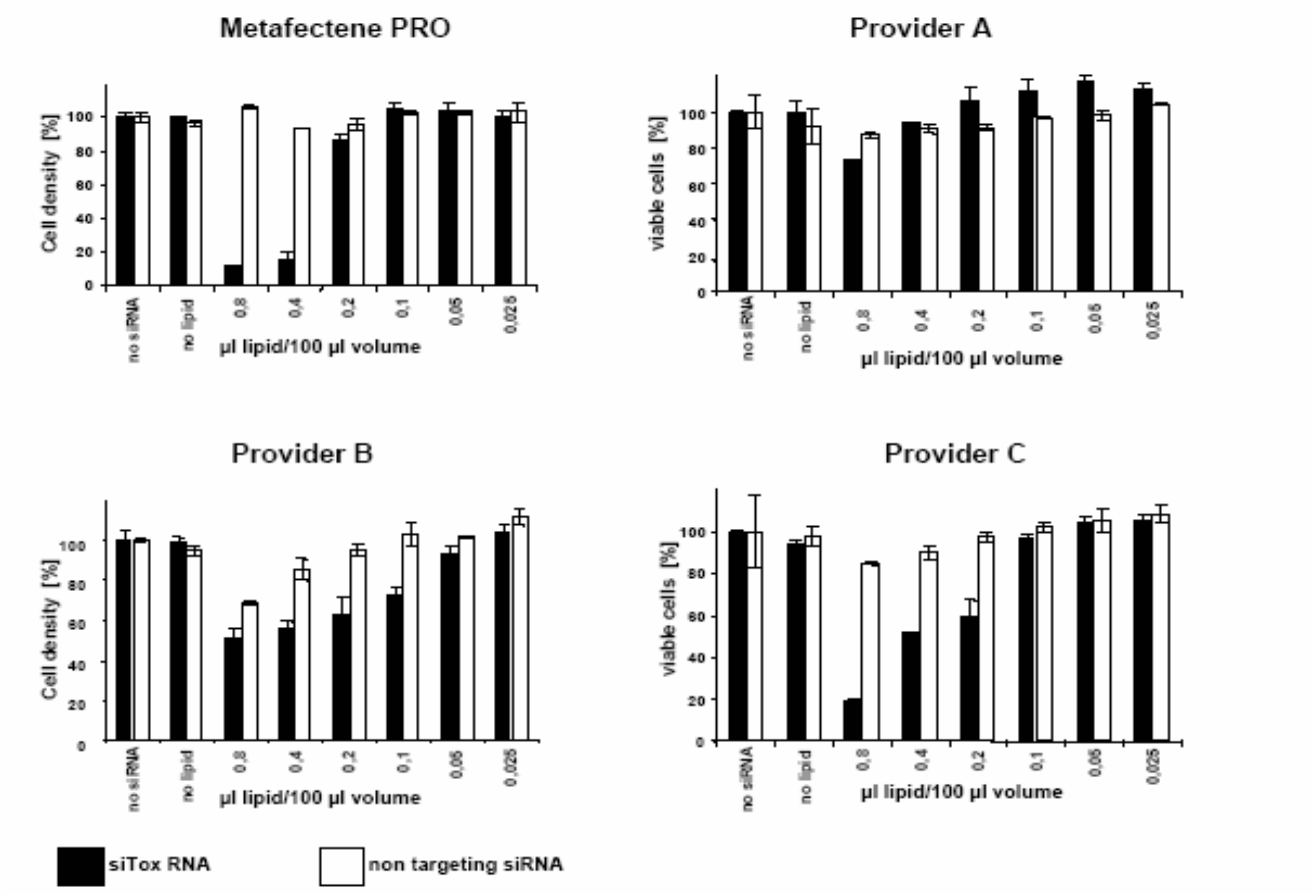

Fig. 2: Transfection of siRNA by Metafectene PRO or lipids of other providers in the glioma cell line LN-308.

CONCLUSIONS

Metafectene PRO was highly effective in the transfection of a variety of human and murine cell lines as well as primary glioma cell from patient biopsies. For plasmid transfection, cytotoxicity was low when using less than 8 µl of Metafectene PRO in a total volume of 250 µl cell culture medium. Remarkably, in siRNA transfection experiments, we did not observe any cytotoxic effect of Metafectene PRO reagent.

LITERATURE

Rieger, J., Wick, W., Weller, M. (2003) Human malignant glioma cells express semaphorins and their receptors, neuropilins and plexins. Glia 42:379-389