Transfection of human HeLa cells, and murine SCCVII and NIH-3T3 cells with METAFECTENE PRO

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Current treatments of head and neck squamous cell carcinoma (HNSCC) are largely unsatisfactory, and the five-year survival rate has not improved over the last two decades. The genetic approach to the treatment of HNSCC is based on the hypothesis that expression of therapeutic genes in target cells will cause a cytotoxic effect and/or mediate apoptosis. We have focused on establishing a highly efficient and tumor-specific non-viral gene delivery system for the treatment of oral SCC tumors in immunocompetent C3H/HeJ mice. This syngeneic orthotopic murine model for HNSCC, induced by the injection of SCCVII murine squamous cell carcinoma cells in the floor of the mouth, was developed by O'Malley et al. (Cancer Res.1996, 56: 1737-1741; Arch. Otolaryngol. Head Neck Surg.1997, 123: 20-24). SCCVII is an aggressive murine squamous cell carcinoma cell line that was established from the squamous cell carcinoma that developed spontaneously in C3H/HeJ mice and has been propagated subsequently in vitro. As a primer to investigating the use of non-viral vectors in an animal model of HNSCC, we examined the activity of luciferase expressed from the plasmid encoding the enzyme under the control of the cytomegalovirus promoter (pCMV.Luc) using the novel transfection reagent Metafectene PRO, in human HeLa cells, and in SCCVII cells and non-tumor murine fibroblasts, NIH 3T3 cells, derived from a NIH Swiss mouse embryo.

MATERIALS AND METHODS

Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). Alamar Blue dye (alamarBlue™) was purchased from Biosource International, Inc. (Camarillo, CA). The penicillin, streptomycin and L-glutamine solutions were obtained from Irvine Scientific (Santa Ana, CA).
The plasmid, pCMV.Luc (VR-1216; a gift of Dr. P. Felgner (Vical, San Diego, CA)) encoding luciferase was used for evaluating transfection efficiency.

Cells

The human cervical epithelial cancer cell line HeLa (ATCC, Rockville, MD), NIH-3T3 cells (UCSF Cell Culture Facilities) and SCCVII cells, kindly provided by Drs. D. Li and B. O'Malley (University of Pennsylvania, Philadelphia, PA), were cultured in Dulbecco's modified Eagle's MEM (DMEM) (Irvine Scientific) supplemented with 10% FBS (Sigma,

St. Louis, MO), penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine (4 mM) (DMEM/10).

Transfection protocol

For transfection, HeLa (1.5 x 10^5 cells/well), SCCVII (1.0 x 10^5 cells/well) and NIH-3T3 (2.5 x 10^5 cells/well) were seeded in 1 ml of DMEM/10 in 48-well culture plates one day before transfection, and used at approximately 60-80% confluence. Cells were pre-washed with serum-free DMEM medium and then covered with 0.4 ml of the same medium. Metafectene PRO was complexed with the pCMV.Luc plasmid at reagent:DNA ratios of 1 µl:0.5 µg, 2 µl:0.5 µg, 2 µl:1 µg or 4 µl:1 µg DNA with the pCMV.Luc plasmid. Complexes were prepared by mixing Metafectene PRO with 100 µl of serum-free DMEM medium, followed by the addition of plasmid DNA. The mixture was incubated for 15 min at room temperature after the addition of the transfection reagent, and another 15 min after addition of DNA. Metafectene PRO complexes with DNA were added in a volume of 0.1 ml per well, the cells were incubated for 4 h at 37°C, and then 0.5 ml of serum-containing medium (DMEM/20) was added. It was not possible to determine a +/- (cationic component/DNA) charge ratio for Metafectene PRO, since the chemical structures, the concentration of the cationic component and the molecular charges have not been disclosed by the manufacturer. Transfection efficacy was evaluated after 48 hours by measuring luciferase activity, using the Luciferase Assay System obtained from Promega and a Turner Designs TD-20/20 luminometer. The data were expressed as relative light unites (RLU) per ml of cell lysate or per mg of protein (determined by the Bio-Rad BCA assay).

Cell viability assay

Cell morphology was evaluated by inverted phase contrast microscopy at 25x magnification. The number of viable cells used for the experiments was determined by Trypan Blue exclusion. Cell viability was quantified by a modified Alamar Blue assay (Konopka et al. Biochim. Biophys. Acta 1996, 1312: 186-196). Cell viability (as a percentage of mock-treated control cells) was calculated according to the formula [(A_{570} - A_{600}) of test cells] x100 / [(A_{570} - A_{600}) of control cells].

RESULTS

Metafectene PRO was complexed with the pCMV.Luc plasmid at reagent:DNA ratios of 1 µl:0.5 µg, 2 µl:0.5 µg, 2 µl:1 µg or 4 µl:1 µg DNA with the pCMV.Luc plasmid. The highest expression of luciferase was obtained with 2 µl Metafectene PRO:1 µg DNA. All three cell lines displayed significant luciferase activity when transfected with pCMV.Luc (Fig. 1).

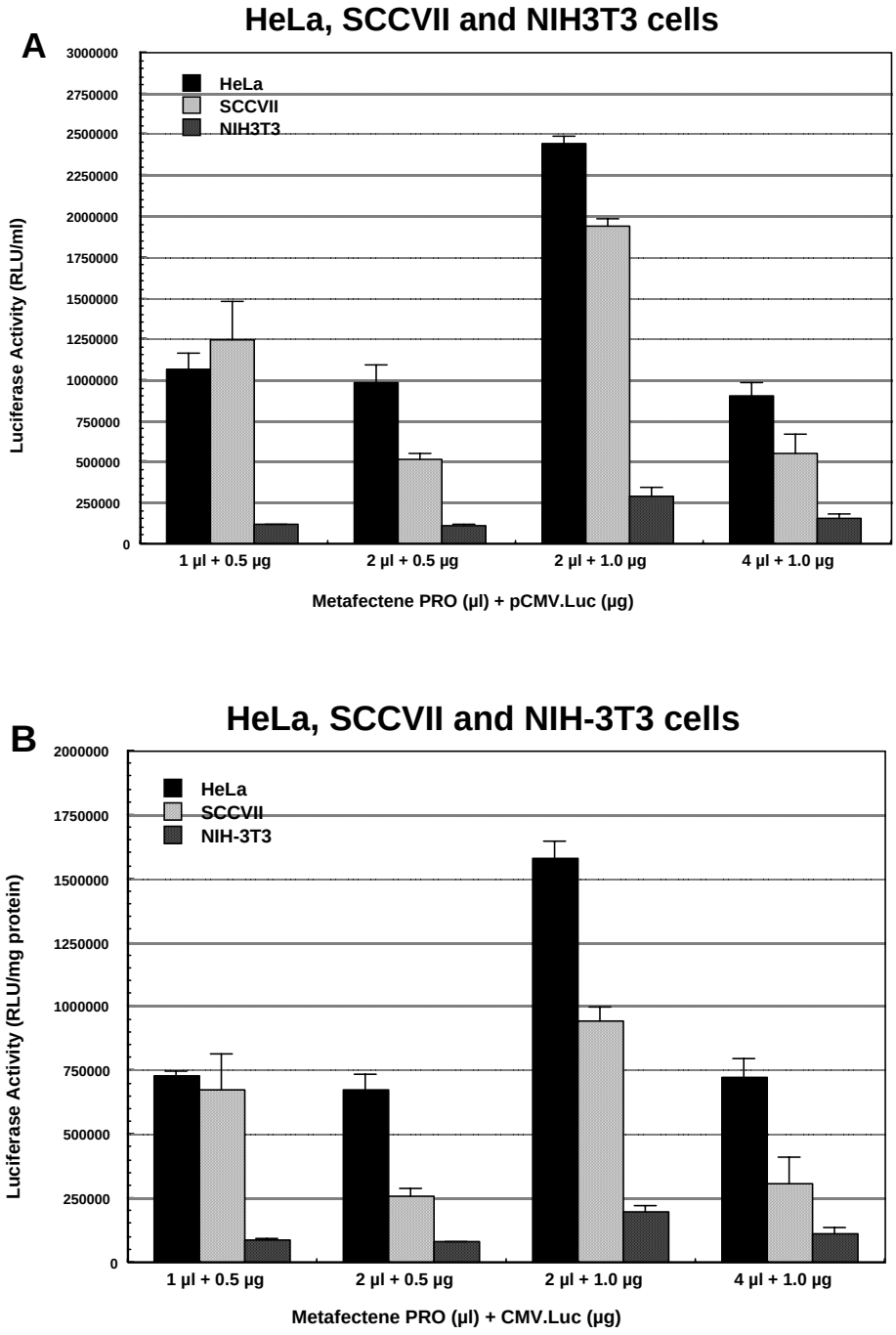

Figure 1. Optimization of transfection efficiency in HeLa, SCCVII and NIH-3T3 cells. The cells were transfected with the indicated amounts of Metafectene PRO and the pCMV.Luc plasmid. Results are expressed as relative light units (RLU) per ml **(A)** or RLU/mg protein **(B)** of cell lysate. Data represent the mean ± S.D. obtained from triplicate wells, and are representative of two independent experiments.

When compared to mock-transfected controls, Metafectene PRO-mediated transfection with pCMV.Luc plasmid did not result in the reduction of total amounts of extractable cellular protein in lysates of HeLa, SCCVII and NIH-3T3 cells (data not shown). In HeLa cells, the cytotoxic effect of the Metafectene PRO-mediated transfection determined by the Alamar Blue assay was very low (Fig. 2). We have not determined cytotoxicity using the Alamar Blue assay in SCCVII and NIH-3T3 cells, because of the difficulties with replacement of DMEM/10 with the medium/Alamar Blue mixture. These fast growing cells have tendency to detach during these procedures. We were unable to solve this problem by plating a lower number of cells and performing transfection experiments using less confluent cells.

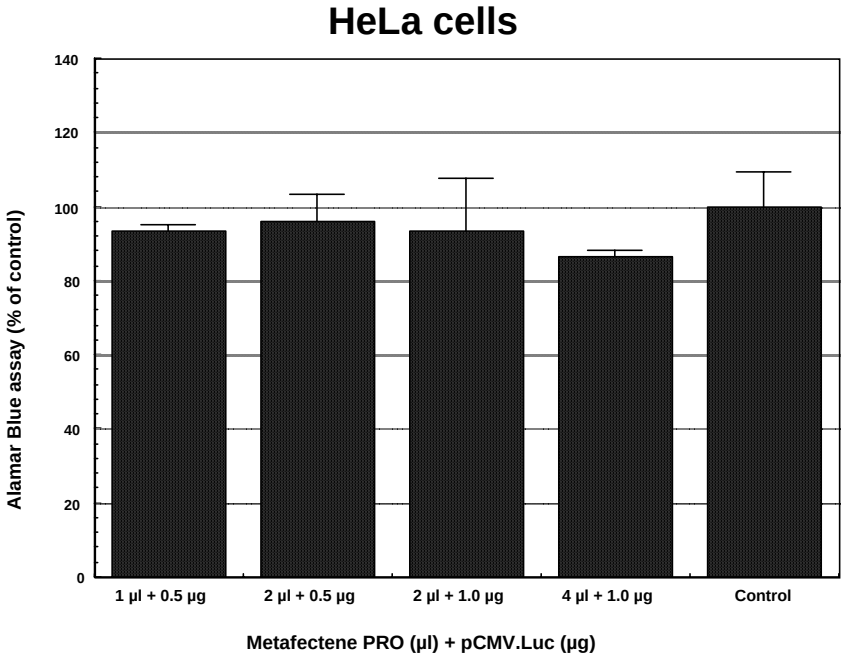

Figure 2. Effect of Metafectene PRO-mediated transfection with the pCMV.Luc plasmid on the viability of HeLa cells was measured by the Alamar Blue assay (1 hour 15 minutes incubation at 37°C). Results are expressed as a percentage of a mock-transfected control. Data represent the mean ± S.D. obtained from triplicate wells.

CONCLUSIONS

Metafectene PRO was highly effective in transfecting human HeLa cells, and murine SCCVII and NIH-3T3 cells. Toxicity of Metafectene PRO was lower, when compared to Metafectene.