Transfection of mouse melanoma cell lines S91 and B16 F10 with Metafectene Pro

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Introduction

Melanoma is an aggressive malignant type of cancer derived from melanocytes of the skin or eye. One of its characteristic, yet ominous features is extreme plasticity and diversity of cell morphology and differentiation status. This variability is responsible for frequent difficulties in development of a good transfection method for research purposes.

The oncogenic transformation is frequently attributed to deregulated signal transduction pathways within the cells. The transformation as an altered gene expression pattern is a result of hyperactivity of certain transcription factors – such situation is often the case in melanoma. The studies on transcription factor activity employ a variety of reporter vectors which create a need for improved transfection techniques, e.g. based on combination of high transfection efficiency and low toxicity. The purpose of the experiments presented here was the optimization of transfection method of two mouse melanoma cell lines with GFP expressing plasmid using Metafectene Pro lipofilic agent in comparison with two other methods: FuGene (Roche) and Amaxa Nucleofector kit R (Amaxa).

Materials and methods

Cell culture

S91 and B16 F10 mouse melanoma cell lines were maintained in RPMI medium supplemented with 10 % fetal bovine serum (FBS) and 50 U/ml penicillin, 50 ng/ml streptomycin (all cell culture reagents were purchased from Invitrogen, Grand Island, NY, USA).

Reagents

Metafectene Pro, a polycationic lipoplex transfection reagent was kindly provided by dr. Stefan Hofreiter from Biontex Laboratories GmbH (Martinsried, Germany). The GFP expression plasmid - pmaxFP-Green was from Amaxa, USA. The trypan blue (Sigma Aldrich, Steinheim, Germany) was used to evaluate cell viability.

Transfection protocol

The cells were seeded onto 24-well plate in the density of $8x10^4$ cells per well, in 0.5 ml of medium. The chosen cell density guaranteed approximately 70 % of confluency after 24 h and about 90 % after 48 h culture. The transfection was carried out within an hour after cell seeding. The amount of plasmid used was 0.5 µg per well, with the DNA / Metafectene Pro ratios (µg / µl): 1:1, 1:2, 1:4 and 1:8. For each condition DNA and a proper amount of Metafectene Pro were dissolved in 50 µl of sterile phosphate buffered saline (PBS) in separate vials, then combined (DNA solution was added into Metafectene Pro solution) and incubated in room temperature for 20 min. Next, the solution with DNA – lipid complex (100 µl) was poured dropwise into the cell culture. The transfection efficiency was evaluated 24 and 48 h

after transfection, and calculated as a percentage of green cells observed in the fluorescent microscope (TS 100, Nikon, Japan).

Results and disscussion

The highest transfection efficiency for was achieved with the 1:4 DNA / Metafectene Pro ratio, reaching almost 30 % for S91 cells and almost 18 % for B16 F10 cells after 24 h (Fig. 1). There is a decrease in transfection efficiency values of after 48 h due to cell proliferation.

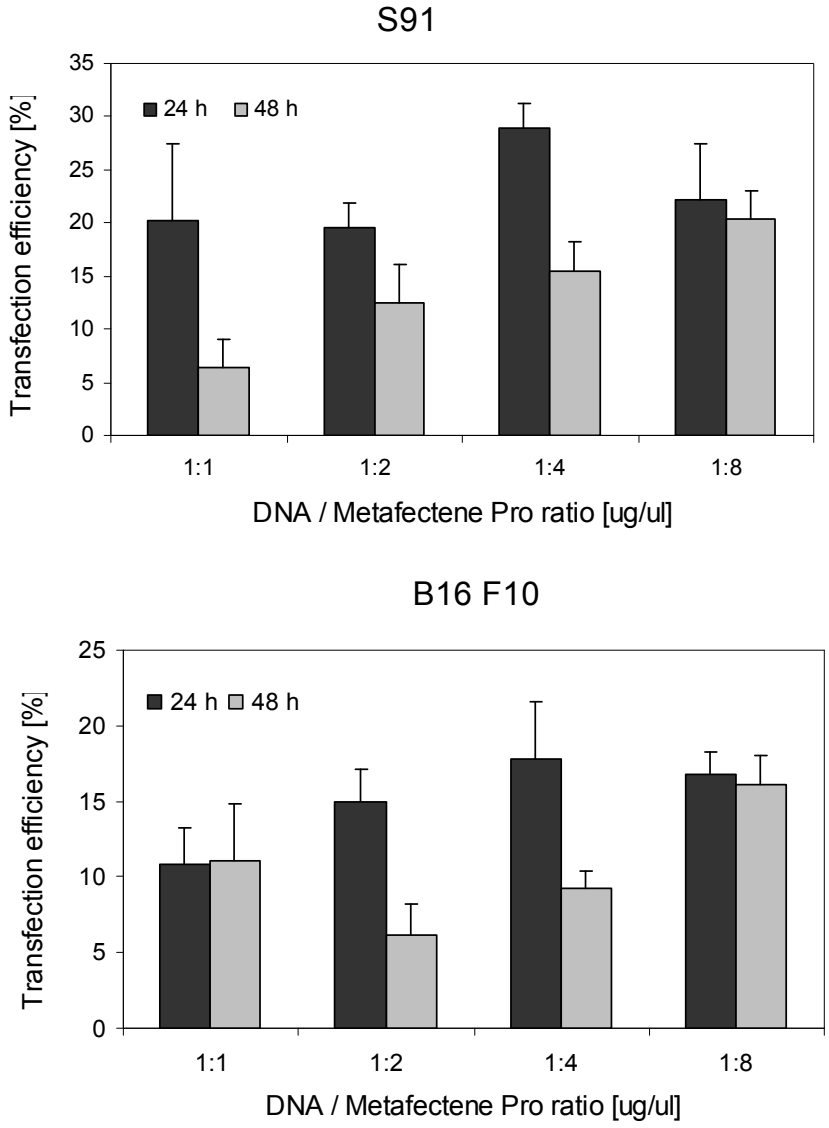

Fig. 1. The optimization of DNA / Metafectene Pro ratio used in the transfection of S91 (upper panel) and B16 F10 cells (lower panel) with the GFP expressing plasmid. The transfection efficiency values represent mean ± SD, n=3.

The viability assays showed none or very low toxicity of the lipid transfection reagent (0 – 2 % of dead cells).The cell morphology or proliferation rate was not affected by Metafectene Pro (not shown).

Table 1. summarizes the results of three transfection methods applied to S91 cells. FuGene (Roche, Mannheim, Germany) and Metafectene Pro (Biontex) are lipofilic agents with chemical properties which enable nucleic acid transfer into the cells. Cell nucleofector kit R (Amaxa, Gaithersburg, USA) employs electroporation, which is generally more efficient but simultaneously harsh method and gives a higher percentage of cytotoxicity than chemical methods. Metafectene Pro is significantly more efficient than FuGene and is not toxic. The difference in time profile of the observed activity of transfected vector (GFP expression) is worth noticing: in case of Metafectene Pro the maximal response (i.e. percentage of fluorescent cells) is seen after 24 h, as quickly as for nucleoporation. In FuGene mediated transfection the maximal response is observed after 48 h, which probably indicates slightly longer DNA uptake kinetics. Moreover, FuGene should be applied for cells culture of high confluency, plated a day before, which additionally prolong duration of the experiment. Metafectene Pro can be applied to freshly seeded cells, and therefore the experiment is as short as with the nucleofection.

Table. 1. The characteristics of S91 cells transfection with three different methods.

Transfection method	**Plasmid**	**Transfection efficiency [%] (mean ± SD)**	**Cytotoxicity [%]**	**Time after transfection [h]**
Metafectene Pro (Biontex, Germany)	pmaxFP-Green (Amaxa, USA)	28.9 ± 2.2	0 - 2	24
Cell line nucleofector kit R (Amaxa, USA)	pmaxFP-Green (Amaxa, USA)	min 38.7 ± 3.1 max 75.1 ± 12.5 (depending on the applied program)	5 - 10	24
FuGene (Roche, Germany)	pmaxFP-Green (Amaxa, USA)	4.3 ± 0.5	1 - 7	48

Conclusion

Metafectene Pro lipophilic agent was proved to be efficient in plasmid DNA transfer into mouse melanoma cell lines S91 and B16F10 and shows very low or no cytotoxicity. Therefore it can be recommended as an alternative for electroporation methods.