Transfection of wild type and p38α-knock-out mouse embryonic fibroblasts with METAFECTENE PRO

Alvaro Gutiérrez-Uzquiza and Almudena Porras

Departmento de Bioquímica y Biología Molecular II, Facultad de Farmacia, Universidad Complutense, Ciudad Universitaria, 28040 Madrid, Spain

Phone: 34-91-394 1627; Fax: 34-91-394 1779; e-mail: maporras@farm.ucm.es

Introduction:

Our work is focused on the analysis of the role played by p38α MAPK in the regulation of apoptosis and cell adhesion as well as the potential cross-talk with the C3G pathway. To study the potential involvement of C3G in the regulation of p38α MAPK activity and p38α-dependent apoptosis and cell adhesion, we want to characterize the effect of RNA interference for C3G in wild type and p38α MAPK knock-out mouse embryonic fibroblasts (MEFs). However, the transfection efficiency in these cells with the phosphate method is very low, so the C3G knock-down is not very efficient with this method. Thus, we have tried to improve the transfection efficiency using the Metafectene PRO reagent. We have quantified the GFP expression upon transfection of the pMAXGFP plasmid under different experimental conditions in order to set up the optimal protocol.

.

Materials and methods and Experimental procedures:

Materials

Wild type and p38α MAPK knock-out mouse embryonic fibroblasts (MEFs) were used in this study. These cells were transfected with the pMAXGFP plasmid (2mg/ml) using the Metafectene PRO, a polycationic liposomal transfection reagent, obtained from Biontex Laboratories GmbH (Munich, Germany).

Transfection protocol

MEFs were seeded 2h before transfection at approximately 60% confluence in 12 multiwell plates with 1 ml of 10% FBS-DMEM medium per well.

Transfection assays were performed using different concentrations of DNA (pMAXGFP plasmid (2mg/ml)), 1 and 1,5 µg, and different amounts of Metafectene PRO, 1, 4 and 12 µl, as indicated in the following table:

DNA/DMEM				METAFECTENE/DNA			
µl DNA (dilution 1/10)				µl METAFECTENE			
1µg	1,5 µg	1 µg	1,5 µg				
5	7,5	5	7,5	1	1	1	1
5	7,5	5	7,5	4	4	4	4
5	7,5	5	7,5	10,5	10,5	10,5	10,5

The DNA was diluted in 50 µl (final volume) of serum-free DMEM medium. In parallel, the METAFECTENE PRO was also diluted in 50 µl (final volume) of serum-free DMEM medium. The DNA solution was added slowly (drop by drop) into that of

METAFECTENE PRO and was incubated for 20 min at RT in order to get the DNA/METFECTENE complexes. Then, this mixture with the DNA/METFECTENE complexes was added to the cells (drop by drop) maintained in 5% FBS-DMEM (1ml). After 6h of incubation at 37ºC in a CO_2 incubator under these conditions, the transfection mixture was removed and replaced by the regular growing medium (10% FBS-DMEM).

Cell viability and Transfection efficiency

Cells were analyzed by fluorescence and phase contrast microscopy at different time points (0, 40, 96 and 144h). The number of attached cells in 3-4 fields was determined at the different time points. Then, cell viability was calculated as the percentage of cells maintained alive along the time as compared with the initial number of cells (cells at time 0). Similarly, the number of cells expressing GFP was quantified using fluorescence microscopy. Then, the ratio between the positive cells and the total number of cells (at time 0) was calculated to evaluate transfection efficiency.

Results and discussion

Transfection of pMAXGFP plasmid into wt and p38α-/- MEFs was very efficient as shown by the high number of GFP expressing cells. However, differences in the efficiency were found depending on the ratio DNA/METAFECTENE PRO, although a high toxicity was induced in wt cells under certain conditions. The highest number of GFP positive cells for wt cells was obtained using 1μg of DNA and 4μl or 10.5μl of METAFECTENE PRO, as well as with the ratio 1.5μg of DNA/10.5μl of METAFECTENE PRO (table 1 and figure 1). However, when the transfection was performed with 10.5μl of METAFECTENE PRO, most of the cells were dead after 40h (table 2). In contrast, this cytotoxic effect of METAFECTENE PRO was not observed in p38α-deficient cells, while the transfection efficiency was also high. Therefore, 1μg of DNA and 4μl of METAFECTENE PRO was established as the optimal condition for the transfection assays in wt and p38α-deficient MEFs. Under these conditions, the efficiency was very high and cell death was low. In fact, the efficiency obtained with the METAFECTENE PRO was around 100 fold of that obtained with the Phosphate calcium method (data not shown).

Conclusions

We show here that METAFECTENE PRO is a very efficient method for the transfection of wt and p38α-deficient MEFs. Although METAFECTENE PRO can be cytotoxic for wt cells at a higher concentration through a p38α-dependent mechanism, we found optimal conditions to overcome any problem of toxicity.

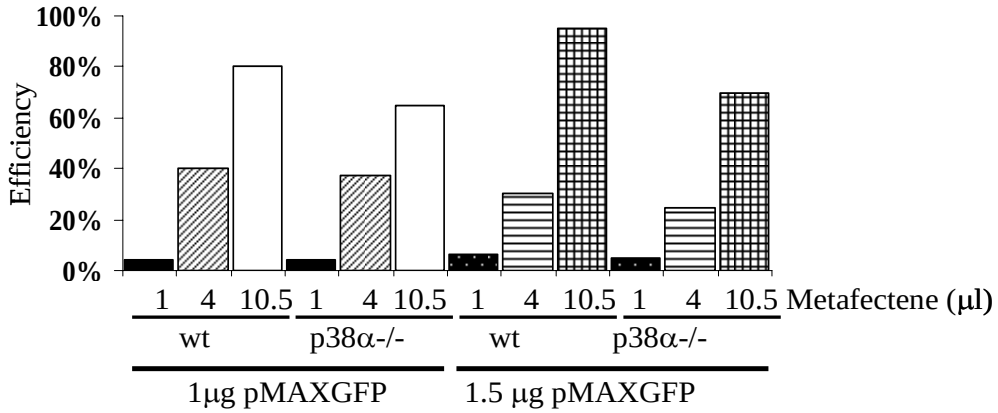

Table 1-Effect of the amount of DNA and Metafectene on transfection efficiency. MEFs (wt and p38α-/-) were transfected with the indicated amounts of Metafectene PRO and the pMAXGFP plasmid. Results are expressed as the percentage of GFP positive cells. A representative experiment is shown. Similar results were obtained in three independent experiments.

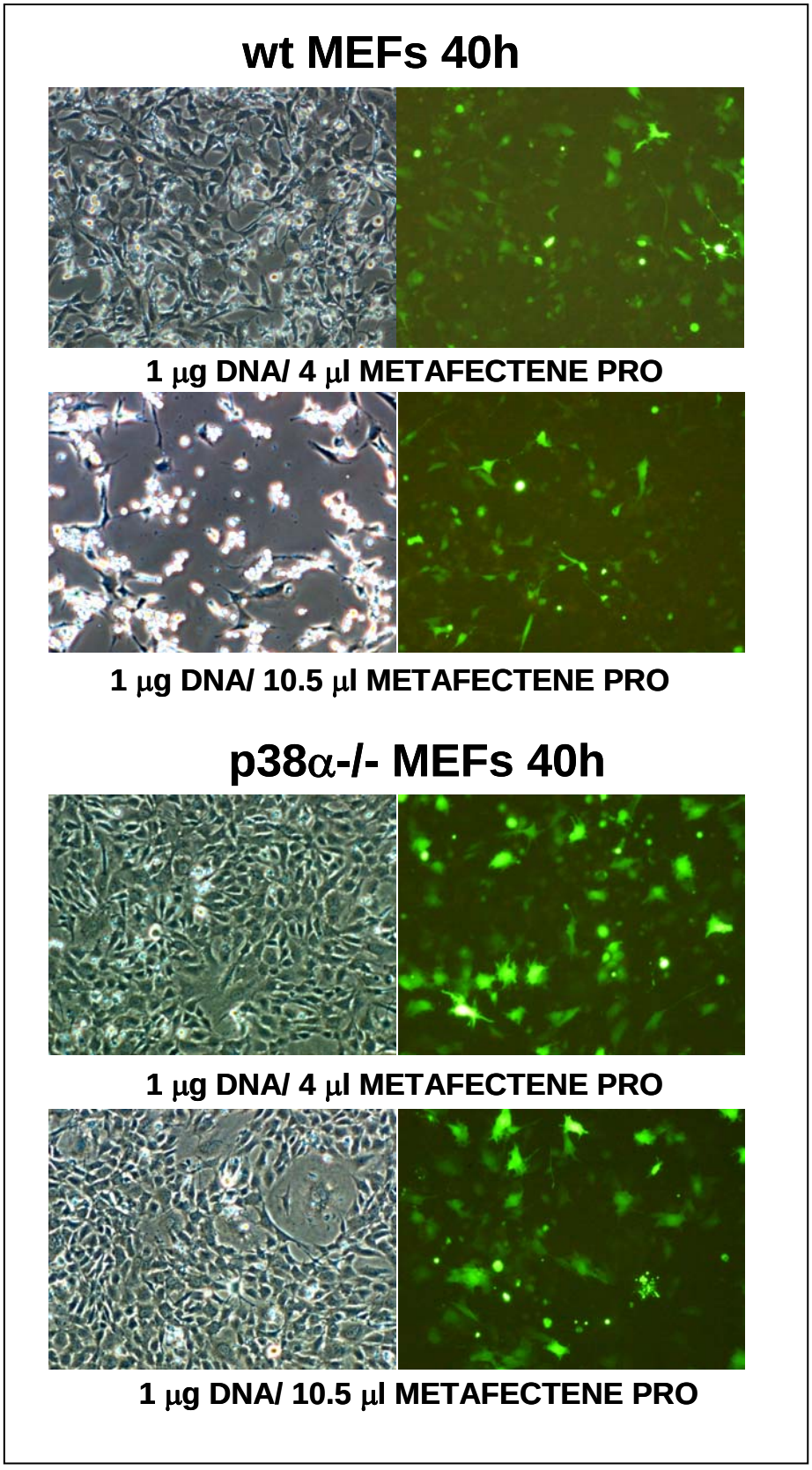

Figure 1-Fluorescence and phase contrast microscopy analysis of wt and p38α-deficient MEFs transfected with pMAXGFP using METAFECTENE PRO. Cells were transfected with 1μg of plasmid and 4 or 10.5 μl of METAFECTENE PRO, as indicated. Microscopy analysis by phase contrast (left panels) and by fluorescence (right panels) after 40h of transfection.

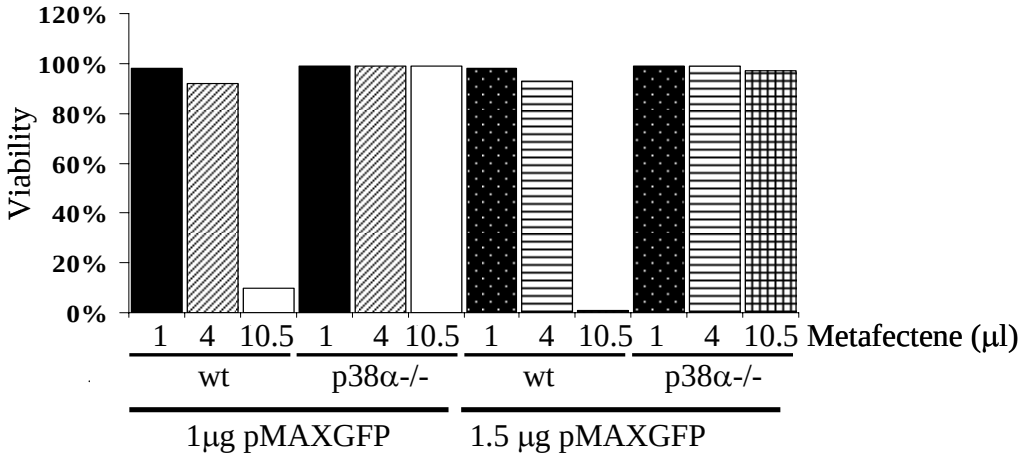

Table 2-Effect of the amount of DNA and Metafectene on cell viability. MEFs (wt and p38α-/-) were transfected with the indicated amounts of Metafectene PRO and the pMAXGFP plasmid. Viability was evaluated after 40h. Results are expressed as the percentage of cells maintained alive and attached to the plate. A representative experiment is shown. Similar results were obtained in three independent experiments.