Transduction of human CD34 and CD133 positive cell lines with a vector coding for EGFP.

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Introduction

CD133 and CD34 markers are considered as markers of young, non differentiated cells, generally named progenitor or stem cells. The unique feature of these cells is their ability to proliferate and differentiate into several cell types, depending on the growth factors present in an environment. Concentration of these cells is very low even in tissues the most rich for these cells. To work with CD133+ or CD34+ cells one have to separate them from cord blood or bone marrow before each experiment or immortalize cells of interest. In this experiment cell line KG1 and telomerase immortalized cell lines 55.1 and 55.2 were used as targets of transduction with retroviral vector produced by TE FLY GA EGFP line, (coding for EGFP –enhanced green fluorescent protein). EGFP protein is very useful cell marker, especially when cytofluorymetry is to be used for cell detection in mixtures of cells in vitro or ex vivo.

Materials and Methods

Materials

Metafecten PRO transfection agent was obtained from Bionex Laboratories GmbH (Munich, Germany), Polybrene transfection agent was obtained from Sigma.
TE FLY GA EGFP cells, producing retroviral vector were prepared in our laboratory.

Cells

Human KG1 - Human myelomonocytic CD34 positive cell line was obtained from American Type Culture Collection.
55.1 and 55.2 cell lines were obtained in our laboratory from human cord blood cells and immortalized by telomerase transfection. Both expressed CD133 marker.
All lines were growing in OptiMEM medium supplemented with 5% of fetal calf serum, penicilin (100U/ml) and streptomycin (100 micro/ml).
Cells TE FLY GA EGFP producing retrovirus coding for EGFP were also growing in in OptiMEM medium supplemented with 5% of fetal calf serum, penicilin (100U/ml) and streptomycin (100 µl/ml) to

a confluence. Then medium was changed for a medium without antibiotics, with 2.5% of serum. After 24 h culture supernatant was collected, centrifugated twice, and used as a source of infectious virus.

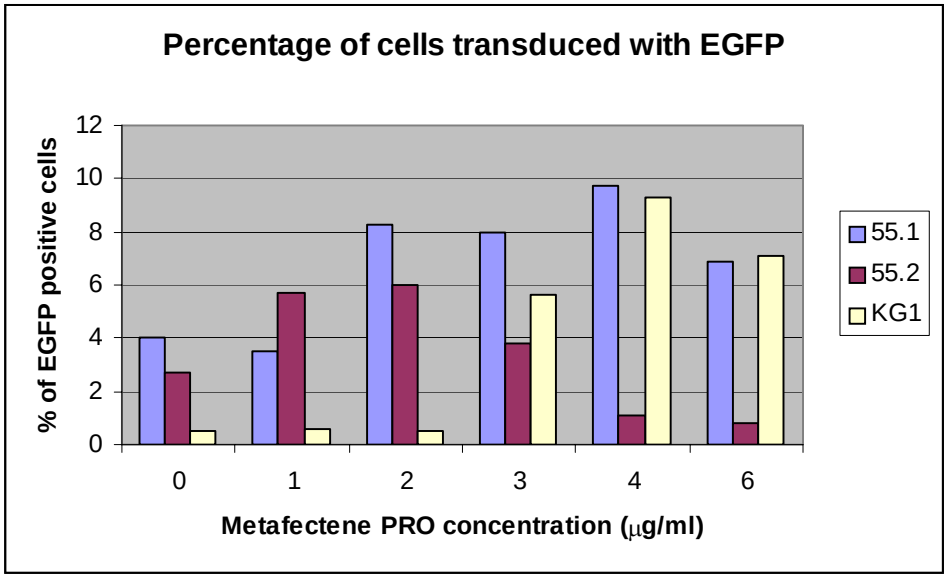
Transduction protocol

55.1 and 55.2 cells were seeded 24h before transduction in a concentration $5x10^4$ /well in 24 well plate in 500 ml of OptiMEM medium supplemented with 5% of FCS.
KG1 cells were seeded 1h before transduction in a concentration $7.5x10^4$ /well in 24 well plate, in 500 ml in OptiMEM medium without FCS.
Supernatant of TE FLY GA EGFP cells, containing viral particles was prepared by centrifugation at 800 g and then mixed with increasing doses (1, 2, 3, 4, 6 µl/ml) of Metafectene PRO or with polybren at a dose of 10 µl/ml of supernatant. After 10 min of incubation one volume of supernatant was mixed with the same volume of cells, which were to be transduced by EGFP. After 24h of incubation medium was changed for a fresh one with FCS and antibiotics. 48h after transduction percentage of EGFP positive cells was evaluated by using FACSCalibur (BD) to enumerate FL1 positive cells. (Selection of EGFP positive cells with geneticin was started on a 4^{th} day after transduction to obtain stably transduced, EGFP positive cells.)

Results

Metafectene PRO was complexed with the supernatant containig retroviral particles in concentrations 1 µl, 2 µl, 3 µl, 4 µl and 6 µl/ml and used for cell transduction. Results of one typical experiment, done with the same viral supernatant are presented in Fig.1.

Fig.1.



In every cell line tested application of Metafecten PRO augmented percentage of transduced cells. The highest percentages of transduced cells were obtained when 2-4 µg/ml of Metafecten PRO was used as an enhancing agent. For three lines tested; 55.1, 55,2 and KG1 9.7, 6.0 and 9.3% of transduced cells was detected, respectively.

When polybrene, usually used in our laboratory transfection and transduction enhancing agent was used in the same experiment as presented above, 7.7, 6.3 and 8.0% transduced cells was detected.

Conclusion

Metafecten PRO is the transfection enhancing agent which may be also efficiently used for the transduction of human cells with viral constructs.