Comparative analysis of METAFECTENE and METAFECTENE PRO transfection efficiencies in human tumor cell lines.

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Introduction:

Efficient and reproducible transfection rates are an essential prerequisite for many in vitro experiments. This transfection efficiency should be accomplished by use of small amounts of plasmid-DNA and associated with minimal toxicity of the transfection reagent, which is even more important if large experimental settings are envisaged. In the present study human colon carcinoma, mammary- and pancreatic carcinoma cell lines were used, since these represent important in vitro models for a variety of studies in experimental cancer research. In the study the potentially different transfection efficiency of Metafectene and Metafectene Pro was tested to evaluate, whether Metafectene Pro is advantageous over Metafectene. For this, the cell lines SW480, LS174T, Colo205, HCT116, MCF-7 und Panc-1 were used in a standardized experimental setting using the pEGFP-N1 reporter-plasmid DNA with Metafectene or Metafectene Pro respectively. The pEGFP-N1 plasmid is expressing the Green Fluorescence Protein (GFP), which is detectable by fluorescence microscopy and can be easily quantified by FACscan anlysis.

Material and Methods:

For the in vitro study the human tumor cell lines SW480, LS174T, Colo205, HCT116, MCF-7 and Panc-1 were used. These lines were transfected with the pEGFP-N1 (Clontech) reporter gene construct. In the comparative analysis Metafectene and Metafectene Pro was used, the Fugene HD transfection reagent served as control. For quantification of transfection effciency the FACScan analysis was used (Facs-Calibur, BD-Instruments).

Transfection Protocol:

24 hours prior to transfection 2 x 10^5 cells / well were seeded into a 12-well plate (Costar). For the transfection solution A and B was prepared as follows: for preparation of solution A 1.11 µL pEGFP-N1 plasmid-DNA (concentration of 1 µg DNA/µL) was added to 50 µL serum free medium and mixed; for preparation of solution B 3 µL Metafectene or Metafectene Pro were added to 50 µL serum free medium and mixed. Solution A and B were then mixed and incubated for 20 minutes at room temperature. During this incubation time, 1 mL fresh medium (+10% FCS) was added to the cells. After the 20 minute incubation the A+B mix was added to the cells and incubated over night (ON) at 37°C, 5% CO_2; then medium was changed. The transfected cells were harvested 48 hours after transfection, washed twice in PBS and GFP-fluorescence was determined by FACscan analysis. The quantification is then given as % transfected cells (GFP-positive Zellen) vs. non-transfected parental cells. The Metafectene and Metafectene Pro transfected cell were measured in parallel to ensure better comparability. The Fugene HD was used as control transfection reagent, in a ratio of 3:2 according to the recommendation of the manufacturer, using 2 µg pEGFP-plasmid DNA.

Results and Discussion:

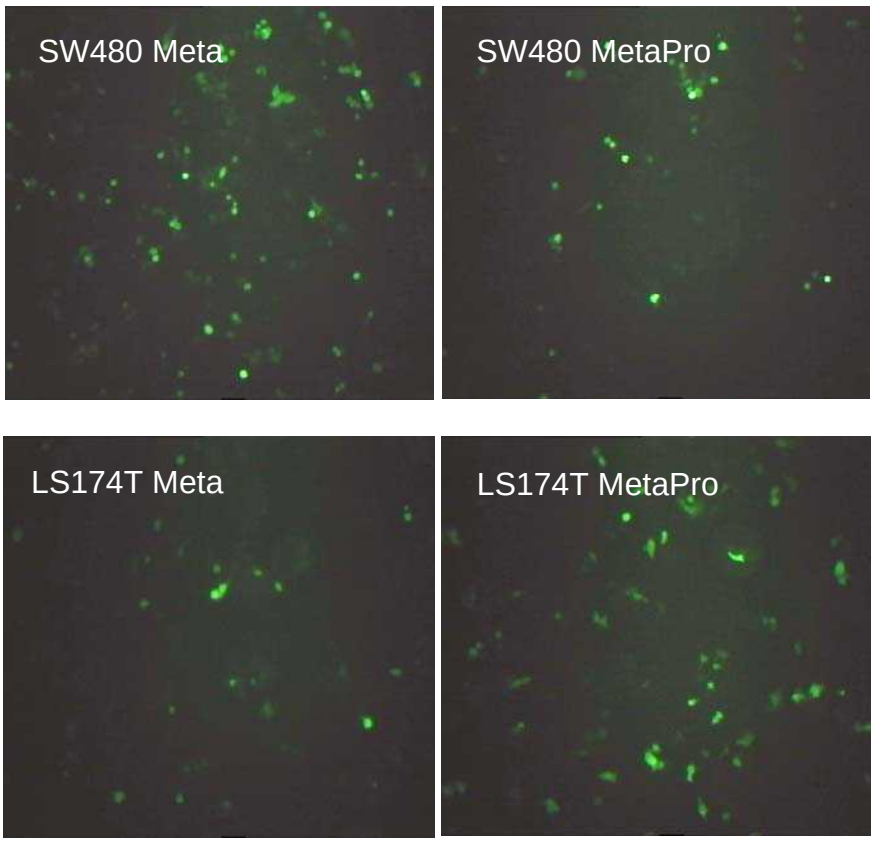
As expected, the cells react differently on the transfection reagents Metafectene, Metafectene Pro and Fugene HD (figure 1). This is also reflected by the different transfection rates, determined by FACScan analysis for the tumor cell lines (tab. 1). Since in the study similar transfection conditions were used for Metafectene and Metafectene Pro applying the same amounts of plasmid, the direct comparison of the transfection rates of these two reagents does not reveal a general/overall improvement in Metafectene Pro mediated transfections. However, for the lines LS174T (colon carcinoma), MCF-7 (breast carcinoma) und Panc-1 (pancreas carcinoma) Metafectene Pro improved the transfection efficiency (tab. 1). Moreover, the comparison of transfection rates mediated by Metafectene or Metafectene Pro are better, if compared to the control transfection reagent Fugene HD.

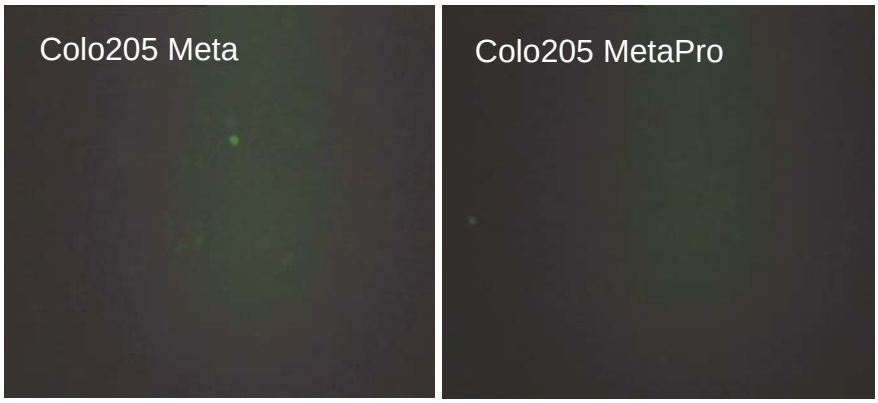
Conclusion:

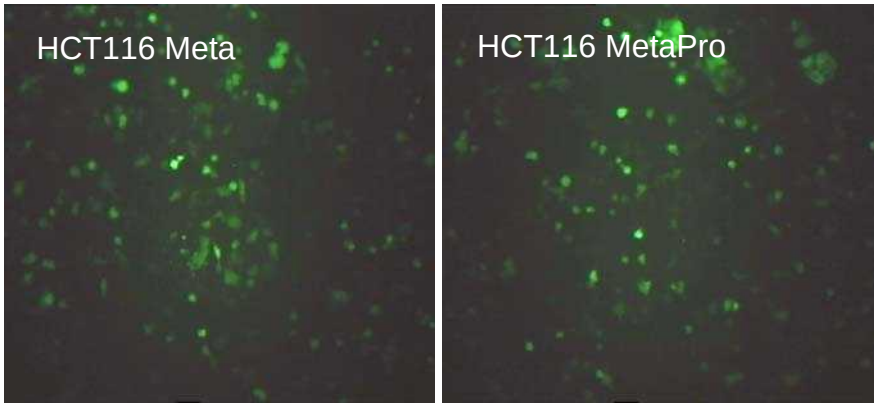
Metafectene, as well as Metafectene Pro are feasible reagents for in vitro transfection of human tumor cells of different origin. For some of these cell lines Metafectene Pro was shown to improve the transfer efficiency. However, it needs to be emphasized, that transfection conditions should be optimized for a particular cell line and application. An important advantage of Metafectene and Metafectene Pro is, that it is well tolerated by all the cell lines tested and it exerts low or no toxicity. This is not observed for other transfection reagents, but in fact, this is an important point, if large scale experiments are performed, and significant losses in cell material should be prevented (as for e.g. reporter gene experiments).

Tables and Figures:

Abb. 1: Expression of GFP in pEGFP-N1 transfected tumor cell lines. For transfection either Metafectene (Meta) or Metafectene Pro (MetaPro) was used. GFP-Expression was evaluated by fluorescence microscopy.




Colo205 Meta
Colo205 MetaPro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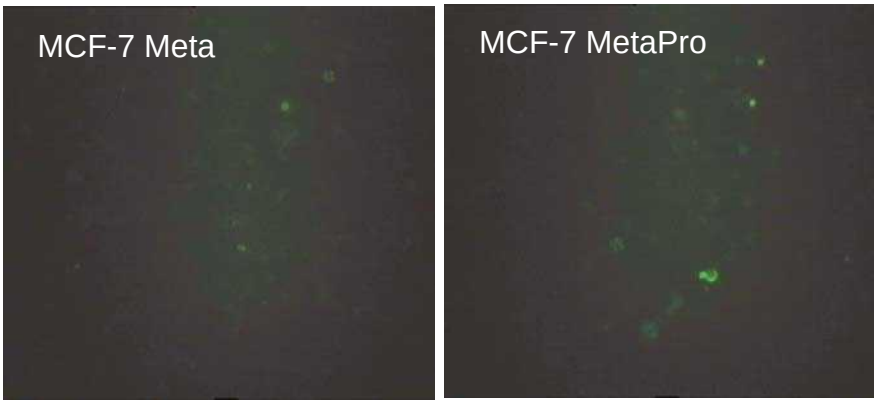
HCT116 MetaPro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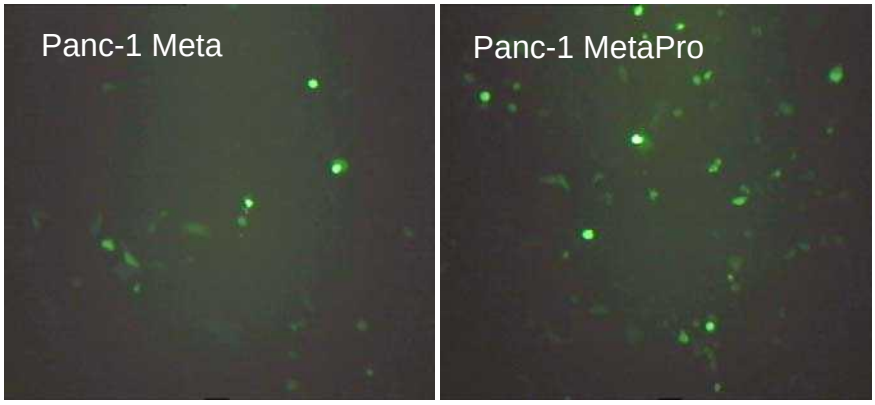
MCF-7 MetaPro


Panc-1 Meta
Panc-1 MetaPro

Tab. 1: Comparative quantitative analysis of transfection efficiency mediated by Metafectene, Metafectene Pro and Fugene HD in different pEGFP-N1 transfected human tumor cell lines. The efficiency was quantitatively determined by FACScan-analysis and is expressed as % transfected cells compared to the respective non-transfected parental cells.

Cell line	**Metafectene**	**Metafectene Pro**	**Fugene HD**
SW480	24,6 %	17 %	9,4 %
LS174T	14,8 %	28,2 %	19,3 %
Colo 205	2,7 %	2,6 %	11 %
HCT116	38,8 %	37 %	13 %
MCF-7	0,2 %	1,4 %	5 %
Panc-1	4,0 %	11,5 %	2 %