Gene transfer efficiency of Metafectene in human colon carcinoma cell lines

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Human colon carcinoma represents a malignancy with high incidence in developed industrial countries and ranks second in incidence to lung cancer in men and breast cancer in women. Therefore, analysis and treatment of this type of malignanacy is of great importance, which demands experimental model systems that provide the potential for the testing of novel therapeutic approaches to treat colorectal tumors. In this context, cell lines derived from human colon carcinomas represent such an in vitro model for research studies.

To test the gene transfer efficiency of Metafectene in colon carcinome cell lines, in a first experimenal setting the parameters for effective gene transfer of the ß-glactosidase(LacZ) reporter gene were tested in the human colon carcinoma line HCT116 using the pCMVß plasmid (Clontech). The experimental conditions were as follows: 2.5 µg plasmid/5 µl Metafectene; 5 µg plasmid/10 µl Metafectene; 2.5 µg plasmid/10µl Metafectene; 5 µg plasmid/20 µl Metafectene. For transfection 1.5 x 10^5 HCT116 cells per well were used in 6-well plates. The procedure of Metafectene-transfection was as recommended by the manufacturer.

Expression of the LacZ reporter gene was detected in formalin (3%) fixed cells using the X-gal staining (see Fig. 1). This experiment revealed that in HCT116 cells 2.5 µg plasmid/5 µl Metafectene (Fig 1A) was optimal for gene transfer and was therefore used for the following experiments in the different human colon carcinoma cell lines.

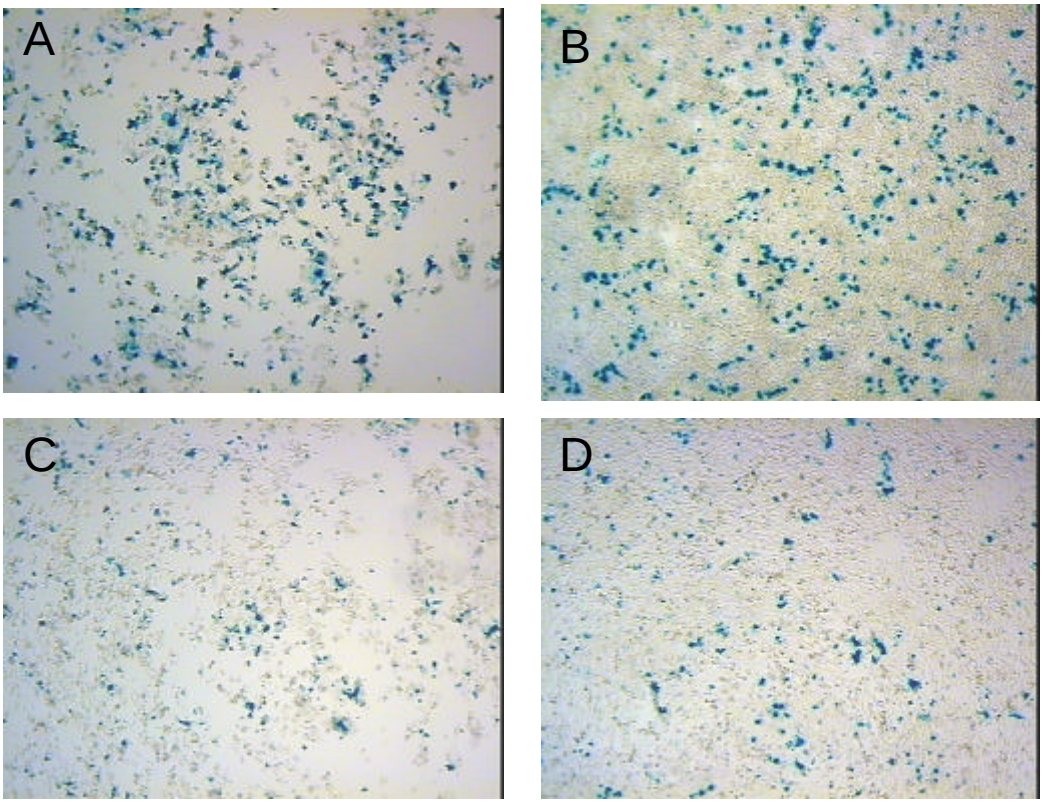

Fig. 1: ß-galactosidase expression in Metafectene-transduced HCT116 cells. The cells were formalin-fixed and stained using the X-gal procedure.
A: 2.5 µg plasmid/5 µl Metafectene;

B: 5 µg plasmid/10 µl Metafectene;
C: 2.5 µg plasmid/10 µl Metafectene;
D: 5 µg Plasmid/20 µl Metafectene

Metafectene was then used to transduce the green fluorescence protein (GFP)-expressing reporter plasmid pEGFP-N1 (Clontech) into 6 different human colon carcinoma cell lines: SW480, LoVo, Colo205, LS174T, HCT116, HCT15). For this, 2.5 µg plasmid/ 5 µl Metafectene was used to transduce 1.5 x 10^5 cells of each cell ine. After 48 hours gene transfer efficiency was analyzed in fluorescence microscopy (see Fig. 2) and quantified by FACscan-analysis (see Fig. 3)

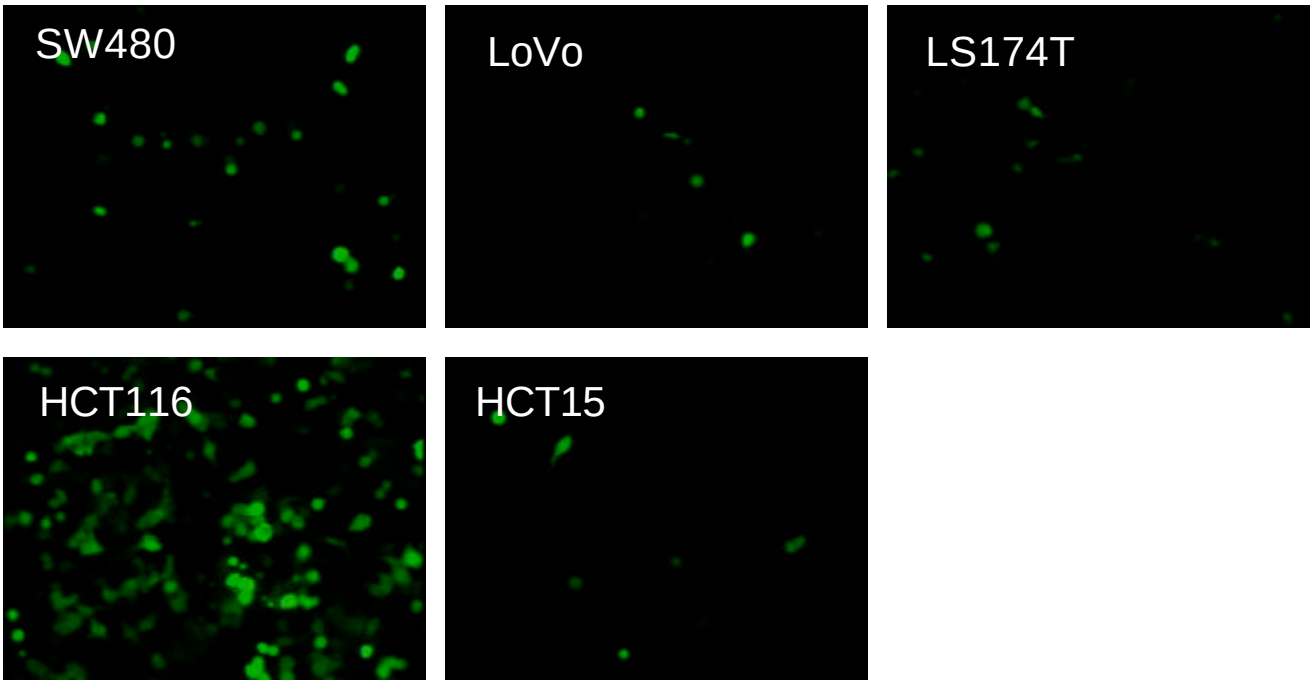

Fig. 2. GFP gene transfer in human colon carcinoma cell lines using Metafectene. GFP expression was analyzed 48h after gene transfer by fluorescence microscopy.

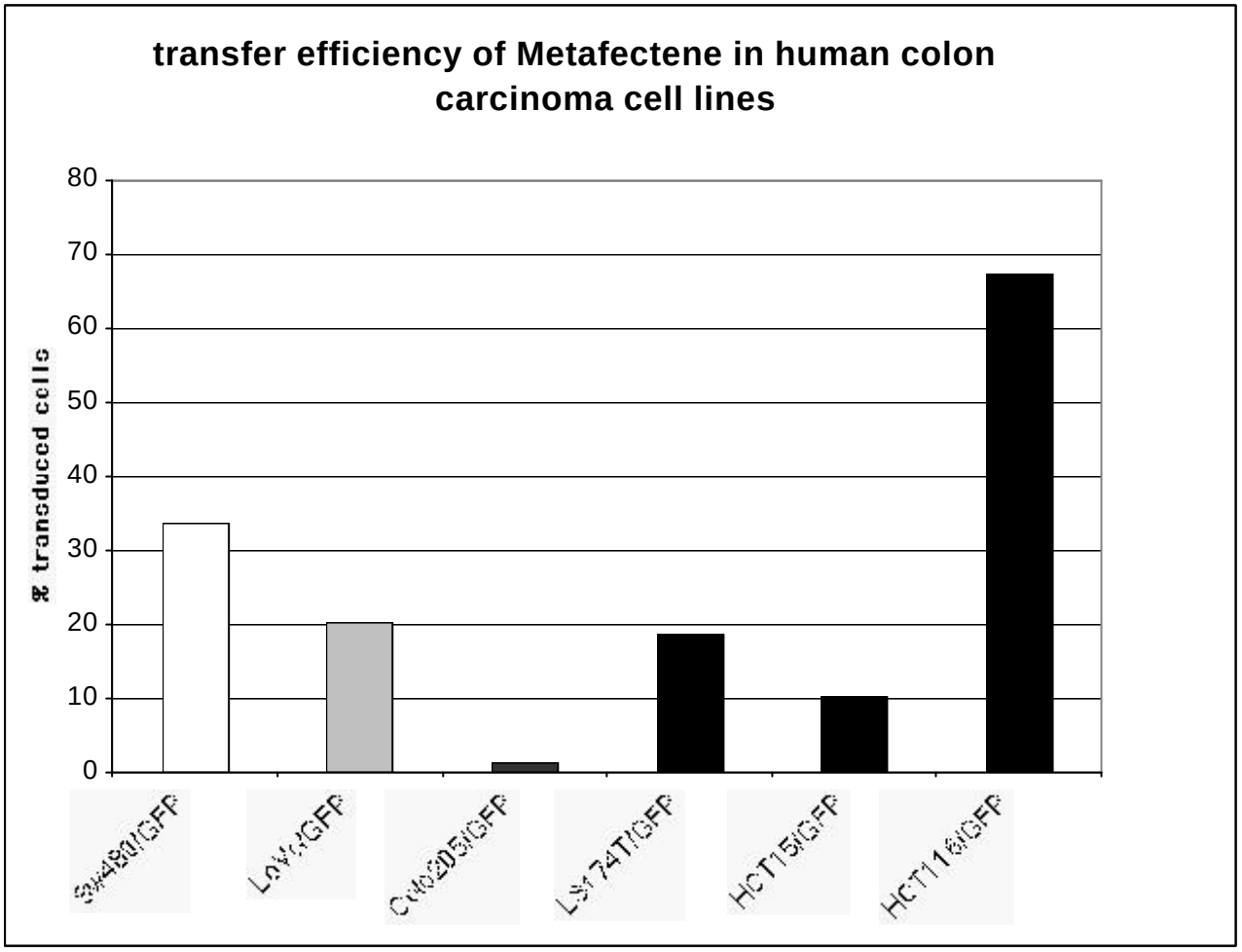

Fig. 3: Quantitation of GFP expression in Metafectene transduced human colon carcinoma cells by FACscan analysis. The transfer efficiency is given as percent of GFP-expressing (transduced cells) cells out of 10.000 cells counted for each cell line.

Conclusion:

In HCT116 cells proportion of 2.5 µg plasmid/ 5 µl Metafectene was effective for gene transfer. This proportion of plasmid DNA and Metafectene was also efficent for the colon carcinoma cell lines SW480, LoVo, LS174T and HCT15, although further optimization might be required to increase the percentage of transduced cells by Metafectene. These brief experiments show, that Metafectene can be used for the in vitro gene transfer in human colon carcinoma cell lines.

Acknowledgement

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