VALIDATION OF TRANSFECTION PROTOCOL CONCERNING HUMAN CANCER CELL LINES

dott. Luca Bruni

Department of Genetic, Biology of Microorganisms, Anthropology and Evolution, University of Parma; Laboratory of cancer biology and stem cells.

Group leader: Prof. Roberto Perris

e-mail kaneluca.77@hotmail.com

Adress: Viale degli Usberti 11/A 43100 Parma Italy.

Introduction

The scientific focus of our research group, is the studies of membrane associated eparan sulphate proteoglicans (HSPSs). These glycoproteins seem involved on metastasis formation and our efforts are employed to characterize two families of HSPSs: the glypicans and the syndecans in different human cancer cell line of sarcomas and melanomas. This chose is linked to both scientific interests of our laboratory and the reduced knowledge about metastasis formation HSPSs dipendent. This project is divided in different steps:

- to characterize both constitutive gene expression and membrane protein expression in different human sarcoma and melanoma cell lines;

- to create an *in vitro* stable cellular model and to modify the constitutive gene expression pattern (to overexpress not much express gene by gene transfer and to silence the express ones by siRNA tools);

- to carry out *in vitro* assay to get know about the invasive potential of the new model cell; if there are significative differences between not manipulated cells and get modified gene expression cells, we will testing *in vivo* cancer potential by xenograft transplant in nude mice.

To complete the first step of our sperimental project, we are transfecting the different human sarcoma and melanoma cancer cell lines choose with designated genes and the transfectant reagent; so we need an optimal ratio DNA:transfectant reagent and a transfectant reagent less toxic.

To standardize a transfection protocol means to find an excellent ratio DNA/transfectant then to use it to transfect different human sarcoma cell lines and melanoma one. The transfectant reagent is the METAFECTEN PRO. The DNA employed for these transfection tries is GFP (green fluorescent protein), clonated in pN1, a commercial vector. GFP is very common reporter gene, broadly used in biological research. 24h after transfection the cell are looked at fluorescent microscope.

The transfection will performed correctly if the cell will be green because GFP excites at the correct frequency give out green light; according to the cells have GFP correctly folded. So it is easy and fast to detect the transfected cells and define the transfection index.

In order to our scientific project, to find an optimal transfection reagent it is essential because after preliminary transfection tries, we will ovexpress the gene not much express; the aim is to select the

cell clones stably transfected. This selection is based on antibiotic resistance, conferred to cell from pEGFPN1 because this genetic vector had cloned inside the geneticin gene witch codify a protein that confer the antibiotic resistance.

So we have to start informed about both the optimal ratio DNA:transfectant reagent and the toxic effect of transfectant reagent. In this way we avoid the high toxicity relative to incorrect ratio DNA:metafecten pro and the low transfection indexes come from a poor quantity of metafecten pro.

Materials and methods

CELL LINE: A375(melanoma);143B(osteosarcoma); RD(rabdomiosarcoma); SW982(sarcoma sinoviale); SAOS-2(osteosarcoma); A204(rabdomiosarcoma); SK-UT-1(leiomiosarcoma); HT1080(fibrosarcoma); MG-63(osteosarcoma). Cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). GLUTAMINE PENICILIN/STREPTOMICIN obtained Cambrex. DMEM (Dulbecco's modify, medium) with 1g/l glucose obtain by Lonza. FBS (fetal serum bovine) obtained by GIBCO. PBS obtained by Cambrex. TRIPSIN obtained by GIBCO. METAFECTEN PRO was gently offered by BIONTEX LABORATORIES. pEGFPN1 was obtained by Clontech.

The cell line were grew on incubator with 5%CO_2 at 37°C. Were maintained in DMEM supplemented in 1% glutamine, 1% penicillin/streptomicin and 10% FBS. When cells were at confluence, was washed by PBS, followed by tripsinization. After that add DMEM without pen/strep because are tranfection interference and seeded following the transfection experimental protocol.

Experimental procedures / transfection protocol

The cells must be mycoplasma free at the transfection time, because the contamination impairs the transfection results.

I worked according to example protocol during my tries;

1. To seed $1x10^5$/$1,2x10^5$ cell/ml in a tissue culture plate 12 well 16 hours before transfection. The cells are plate in DMEM without pen/strep, because the antibiotics interference during transfection. The range of sowing amount depend on cell type and size. The cell must be at 80%-85% confluence at the transfection start point.
2. Incubate the multiwell in incubator at 5%CO_2 at 37°C.
3. METAFECTEN PRO and pEGFPN1 must use at room temperature an both gently mixed before use.
4. The following solution made in a tissue culture plate (96 well) with the concave base.
5. To pipette 50 ml of DMEM pen/strep and serum free and add the following DNA amount:

   0.5mg DNA in A1-A4; 1.0mg DNA B1-B4; 1.5mg DNA C1-C4; mix the solution pipetting one time. The capital letters are referred to the well reaction.
6. To pipette 50 ml of DMEM pen/strep and serum free and add the following METAFECTEN PRO amount:

   1, 2, 4, 6 ml METAFECTENE PRO in D1-D4; 2, 4, 8, 12ml of METAFECTENE PRO in E1-E4; 4, 8, 12, 16 ml METAFECTEN PRO in F1-F4; mix the solution pipetting one time.
7. To make the following well combinations: A1+D1, A2+D2; B1+E1; C1+F1; without any mix.

   Wait 20 minutes; cover the 96well tissue plate because the GFP is photo sensible.
8. Add to any cell seeded well, the transfection solution obtained at the point 7.
9. After 4 hours post transfection change medium.
10. 24 hours post transfection, to remove the transfected cells by add tripsin; after that to count at the fluorescent microscope the total cells and the transfected cells. Follow it, to calculate the transfection index: GREEN CELLS∶TOTAL CELLS

Results and discussion

The kind of cell lines is involved on transfection index determination, because there is different sensitivity to transfection elements (DNA transfeced and transfectant reagent). The experimental condition are uniform and equal in the matter of cell treated at time transfection: confluence about 80%, mycoplasma free and an optimal cell viability. The cell viability was easy to detect it because the cells grew adherent to cell plate and the cell dead was in suspension. 24 hours after transfection, I have choose the wells where the cells did not show high toxicity; than I made cell count by fluorescent microscope to determine the number of transfected cells. A good transfection index is a compromise between cell toxicity and efficiency of transfection. I considered both the number of cell effectively transfected and the sum of total cell at the time of chose the best index. If the cells counted after transfection were more than initial cells number, it meant that transfection experiment is carried out in correct way because even if the transfection was going on, the cells was dividing. A colleague of mine, tried to transfect some human cancer cell line by lipofectamine. I report these information because I would underline the different characteristics in order to both reagents. The lipofectamine is an useful transfectant reagent. It carry out a good transfection index but it show an high toxicity. Following I explain some examples very noteworthy.

143B (human osteosarcoma cell line) are easily transfectable. 143B transfected by high ratio lipofectamine:DNA, carried out a transfection index about 80%, significantly higher than transfection index by metafectene pro. However metafectene showed appreciable low toxicity rate in order to lipofectamine, maintaining substantially high transfection index of 48%, when we use the maximum ratio DNA:METAFECTEN PRO.

MG-63 (human osteosarcoma cell line) transfected by maximum lipofectamine:DNA ratio, showed a trasfection index of 35% and an elevated toxicity rate. The same cells transfected using the maximum of metafectene pro:DNA ratio showed an index of transfection of 37.9% and less toxicity than lipofectamine try.

HT1080 (human fibrosarcoma cell line) transfected by maximum ratio lipofectamine:DNA carried out a transfection index to 80%, displayed always strong toxicity. While transfected by intermediate ratio DNA:METAFECTEN PRO put on showed a significantly low transfection index linked to an high toxicity. HT1080, are more sensitive than 143B to transfection reagent used.

Conclusion / summary

The ***Metafecten Pro*** is an optimal tranfectan reagent because it give a good transfection index and generally not strong cell toxicity. The characteristics of ***Metafecten Pro*** are differents. Extremely fast and easy procedure; in 24 hours it is know the transfection index and it is necessary the basic laboratory instrument. It is possible to transfect a broad cell line range; there is any restriction. It is possible to transfect using very low quantity DNA and to have an optimal experimental reproducibility, absolutely important in scientific contest. The ***Metafecten Pro*** get me knew about an optimal transfectant reagent because it is a good compromise between the transfection efficiency and toxicity by transfection reagent.

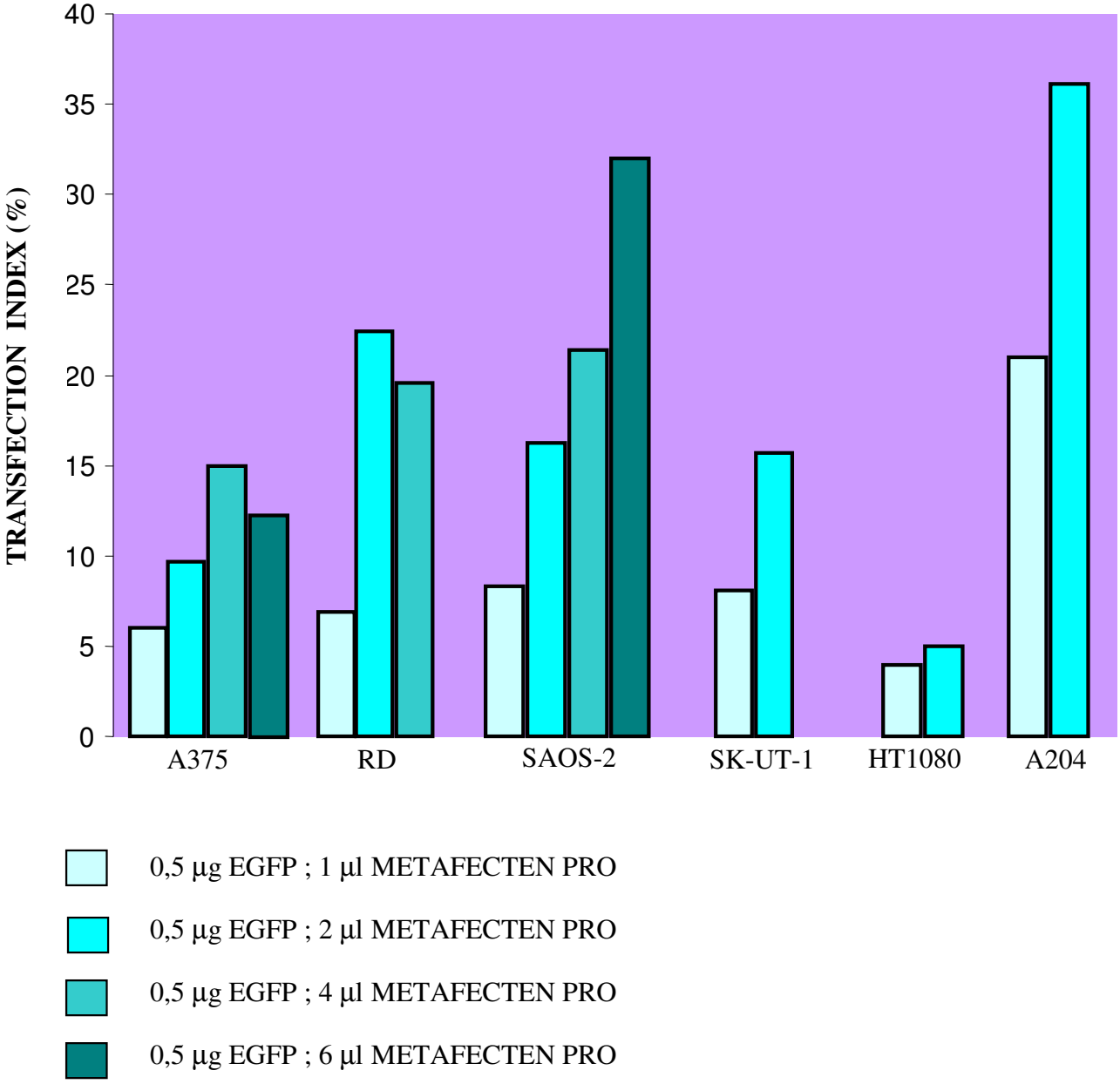
Following it can find a resume table which highlight the optimal EGFP:METAFECTEN PRO values, about every cell line tested and three graphs that draw attention to the different response between human cell line and diverse ratio EGFP:METAFECTEN PRO.

RESULTS OF TRANSFECTION STANDARDIZATION

Cell lines	*DNA (μg)*	*Transfectant reagent (μl)*	*Transfection index (%)*
143B	1.5	12	48
SW982	1.5	4	18.7
3T3*	1.75	3.5	35
HeLa*	0.5	1.5	38
A375	0.5	4	15
RD	1	4	25.6
SAOS-2	1	6	32.8
SK-UT-1	1	2	22
HT 1080	1	4	11.9
A 204	0.5	2	36.1
MG-63	1.0	12	37.9

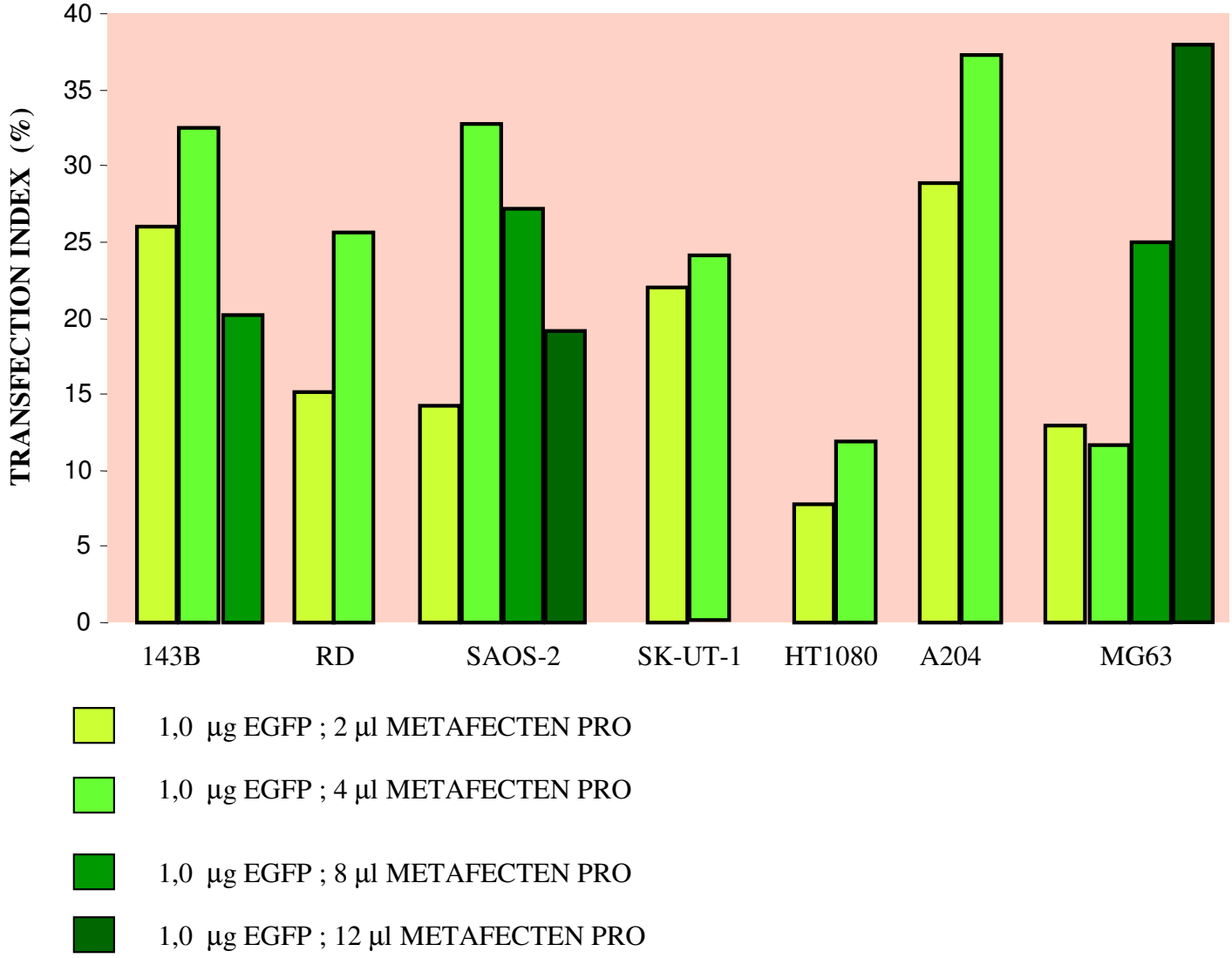
* results obtained out of transfection standardizzation tries.
DNA tested was GFP and transfectant reagent was metafecten pro. I did not report this data on the following pictures.

METAFECTEN RESULTS



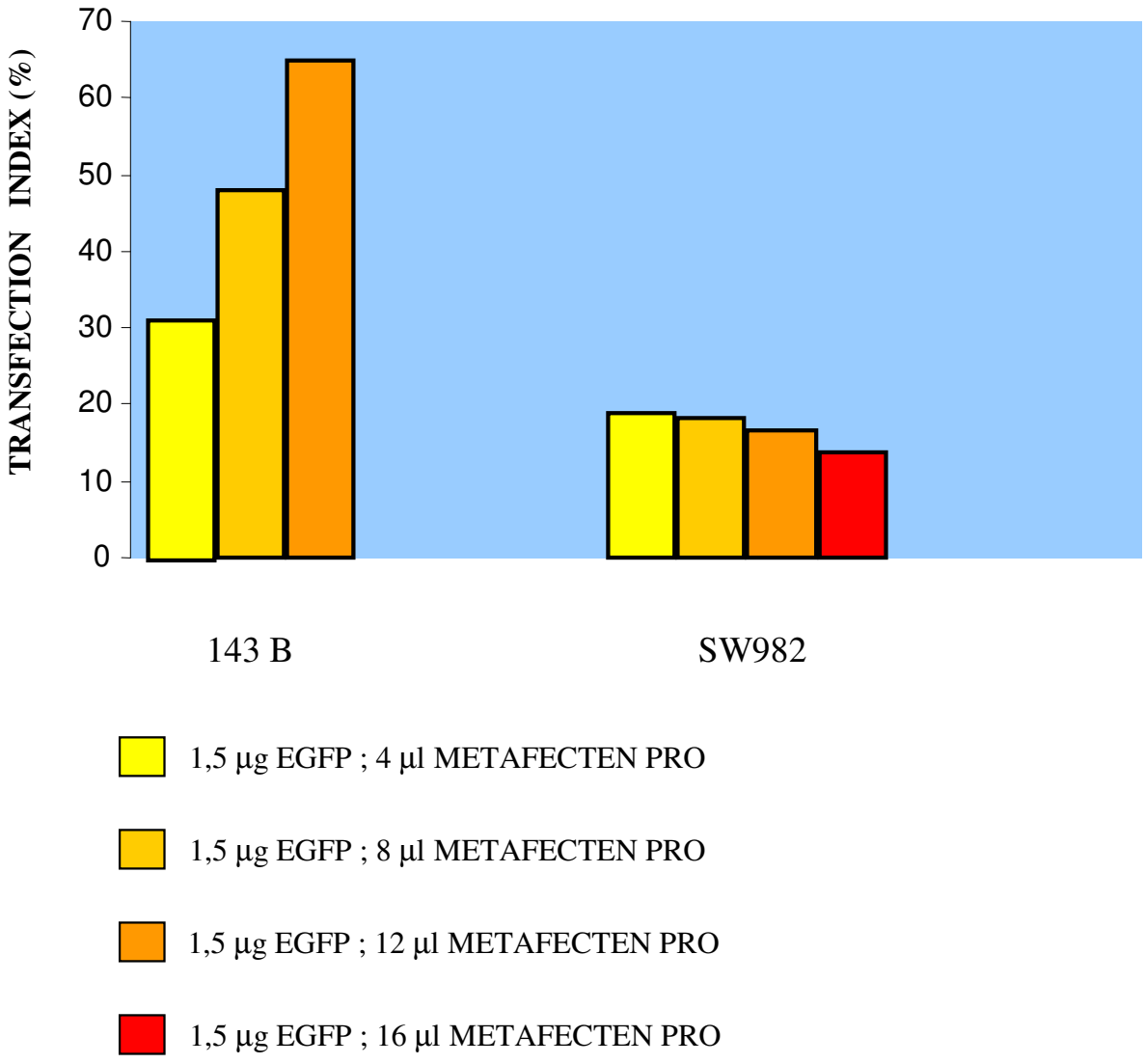
In this picture I reported the data came out to lower ratio EGFP:METAFECTEN PRO tries following experimental protocol. I do not show some values about some cell line, because not show an appreciable transfection index.

METAFECTEN RESULTS



In this picture I reported the intermediate EGFP:METAFECTEN PRO tries. At these ratio the cell lines showed firsts toxic effects; about this reason I did not report data because was not significant.

METAFECTEN RESULTS



In this picture I showed two tries at higher ratio EGFP:METAFECTEN PRO. The toxic effect from metafecten pro were so highs that I decided to leave this ratio and carried on the transfection tries by lows and intermediates ratio EGFP:METAFECTEN PRO.