



# Polyethylenimine as a gene delivery tool in triple-negative breast cancer cell line and breast cancer stem cell model

MIHAELA MATOVINA<sup>1</sup>  
STEFFI LEMMENS<sup>2</sup>  
MARIJETA KRALJ<sup>2</sup>  
KATJA ESTER<sup>2\*</sup>

<sup>1</sup>Laboratory for Protein Biochemistry and Molecular Modelling, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia

<sup>2</sup>Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

**\*Correspondence:**

Katja Ester

E-mail address: kester@irb.hr

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**Abbreviations:**

PEI, polyethylenimine

pDNA, plasmid DNA

GFP, green fluorescent protein

TNBC, triple-negative breast cancer

CSC, cancer stem cell

EMT, epithelial-to-mesenchymal transition

## Abstract:

**Background and purpose:** Polyethylenimine is a cationic polymer able to neutralise negative DNA charges and to condense large genes which makes it suitable for gene delivery in human cells. Because of its low cost, simplicity of use and moderate toxicity, there is still room for broader usage and experimental adjustments, especially in cell lines that are difficult to transfect. In the presented research, we used polyethylenimine for the delivery of plasmid DNA into triple-negative breast cancer cell line SUM159 and breast cancer stem cell model HMLE-Twist.

**Material and methods:** Cultured cells were transfected with GFP-expressing plasmid using both polyethylenimine and Lipofectamine. Transfection efficiency was determined by flow cytometry measurements of the intensity of the green fluorescence, while viability was determined by measuring intensity of the red fluorescence after propidium iodide staining.

**Results:** In SUM159 and HMLE-Twist cells we obtained transfection efficiency between 30-40% using polyethylenimine, while cytotoxicity was generally low to moderate. Polyethylenimine caused 10% of cell death in SUM159 and 20% in HMLE-Twist. Transfection efficiency of polyethylenimine was comparable and even higher than the efficiency of the Lipofectamine in both SUM159 and HMLE-Twist, but not significantly different. In mammary epithelia (control HMLE) we obtained only 20% transfection efficiency using both carriers.

**Conclusions:** We demonstrated for the first time that polyethylenimine represents a suitable nanocarrier for gene delivery into breast cancer stem cell model. We successfully transfected both breast cancer stem cell model HMLE-Twist and triple-negative breast cancer line SUM159. Since polyethylenimine is inexpensive and easy to use, we recommend it for further exploitations of these cell lines in triple-negative breast cancer research.

## INTRODUCTION

Gene manipulations and delivery into human cells have been used and improved over decades. Gene therapy based on delivery of genes or gene-drug combinations that will target and destroy specific type of cancer represents promising alternative to the classical chemotherapy (1). For successful gene delivery, it was critical to develop safe and efficient carrier. Nowadays, the utilisation of non-viral DNA carriers is increasing, especially chemical systems such as liposomes and polymers (2).

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Polyethylenimine (PEI) is a cationic polymer used for DNA delivery since 1995 (3). It is widely used since it is easy to synthesize, efficient and stable. PEI efficiently binds anionic DNA and RNA within the physiological pH range, thereby making it efficient as both pDNA and siRNA carrier (4, 5). Recent modifications of PEI include functionalisations that reduce cytotoxicity and enhance biodegradation (6). PEI can be successfully used as a gene/drug co-delivery tool, since tailor-made co-delivery represents a novel and promising anticancer strategy, especially for tumours with poor prognosis such as triple-negative breast cancer (TNBC), defined by the lack of estrogen, progesterone and human epidermal growth factor receptor (1, 7). Despite its low cost, simplicity of use and low toxicity, there is still a room for broader use and further experimental adjustments, especially in cell lines that are difficult to transfect.

Breast cancer is the second most common cause of death from cancer in woman. One of the most hazardous types of breast cancer is TNBC, a heterogeneous disease, characterised by poor prognosis and lack of efficient therapy (8). Several subtypes of TNBC that feature high metastatic potential, recurrence ability and high percentage of chemotherapy resistant cells are more aggressive than any other types of breast cancer. TNBC cell lines such as SUM159 and MDA-MB231 represent useful tools and are widely used for research (9).

There is a high percentage of cancer stem cells (CSCs) among TNBC, which are the key contributors to its chemoresistance (10, 11). CSCs, also named tumour initiating cells, share some characteristic of healthy stem cells as pluripotency and self-renewal, while they could give rise to new tumour and often survive chemo and radiotherapy (12). CSCs subpopulation within a tumour overlaps with a subpopulation of cells that have undergone epithelial-to-mesenchymal transition (EMT), developmental program activated during cancer invasion and metastasis (13). Since there is a growing need for studying breast CSCs, model cell lines that share all of the CSCs characteristics, but are easy to propagate in the cell culture, were designed. Cell line HMLE-Twist represents breast CSCs model where immortalised breast primary epithelial cells HMLE are forced to pass EMT by overexpression of transcription factor Twist, which was sufficient for the acquirement of CSC-like phenotype (14).

In this study, we used PEI for the delivery of plasmid DNA into triple-negative breast tumour cell line SUM159, breast cancer stem-like cells HMLE-Twist and control HMLE that represent mammary epithelia. Although there are reports on PEI-based transfection in TNBC cell lines as SUM159 and MDA231 (15, 16), there is no data on PEI in the breast cancer stem-like cells to our knowledge. We compared PEI transfection efficiency and cytotoxicity in all of the tested cell lines. Using the same experimental conditions, we compared the efficiency of PEI with a market dominant, but more expensive lipid based complex Lipofectamine.

## MATERIAL AND METHODS

### Cells and Reagents

We used linear PEI (Mr=25000) (Polysciences Europe GmbH, Germany) and Lipofectamine 2000 (Invitrogen, California, USA). HumeC Media with supplements and Opti-MEM Media were from Gibco (NY, USA). Flow-cytometry reagents were from BD Bio sciences (San Jose, California, USA). All the remaining reagents were purchased from Sigma (Sigma Aldrich, MO, USA) or Kemika (Zagreb, Croatia). HMLE cells transduced with lenti-Twist (HMLE-Twist) and HMLE cells transduced with an empty control vector (control HMLE) were kindly provided by Dr. Robert A. Weinberg and Tamer T. Onder and maintained in HumeC media with supplements, as previously described (17). SUM 159 cells were kindly provided by Dr. Robert A. Weinberg and were maintained in Ham's F-12 media with L-Glutamine and Pen/Strep, with 5 % FBS, 5 µg/ml Insulin and 1 µg/ml Hydrocortisone.

### Plasmid preparations

We transformed electrocompetent Top10 *E. coli* by electroporation with plasmid pEGFP-C1 (Clontech, Laboratories Inc., Mountain View CA, USA). Plasmid DNA was purified using Qiagen maxiprep kit (Qiagen, Hilden, Germany), visualised by agarose gel electrophoresis and concentration was determined by Biospec-nano (Shimadzu, Japan).

### Transient transfection using PEI and Lipofectamine 2000

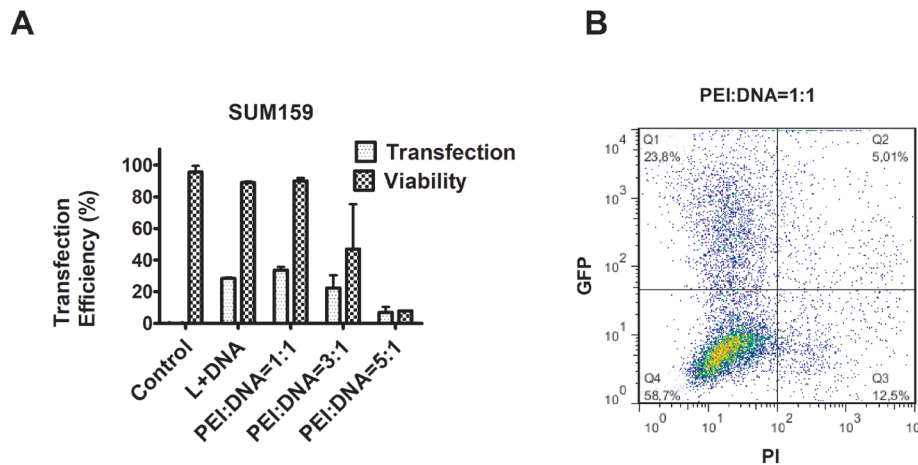
Cells were seeded in 24-well plate at  $5 \times 10^4$ /well as duplicates and grown overnight. PEI was diluted in ultra-pure water, pH=7.2. For the transfection, 500 ng of pDNA/well was mixed with diluted PEI in PEI to DNA ratios 1:1, 3:1 and 5:1 (w/v). Mix was incubated in cell media without FBS at RT for 15 min prior to the addition to the cells. For Lipofectamine transfection, Lipofectamine reagent was mixed with 500 ng pDNA/well in v/v 1:1 in Opti-MEM Media, incubated for 15 min and added to the cells. Transfection efficiency was evaluated 48 hours after the transfection using flow cytometer.

### Transfection efficiency and cytotoxicity analysis

Transfected cells were represented as GFP positive cells on a BD FACSCalibur flow cytometer with excitation at 488 nm while the emitted fluorescence was collected through a 530 nm bandpass filter. Dead cells were represented as Propidium iodide positive cells on BD FACSCalibur with excitation at 488 nm and emission at 585 nm. Data analysis was performed using FlowJo software.

### Statistics

Results are represented as mean values from at least three separate experiments. All graphics with error bars are pre-



**Figure 1. Transient transfection using PEI in SUM159 cells.** Cells were seeded in 24-well plate at  $5 \times 10^4$ /well as duplicates and grown overnight. 500 ng of pEGFP-C1 DNA/well was mixed with PEI in PEI to DNA ratios 1:1, 3:1 and 5:1 (w/v), or with Lipofectamine. After 48 hr, transfection efficiency was determined using flow cytometer by the percentage of GFP positive cells and viability by the percentage of PI positive cells. **A** Graphical representation with bars where each bar represents a mean value  $\pm$  s.d. of at least three individual experiments. **B** Representative dot plot with percentages of transfected (Q1 and Q2; GFP positive) and dead (Q2 and Q3; PI positive) cell populations.

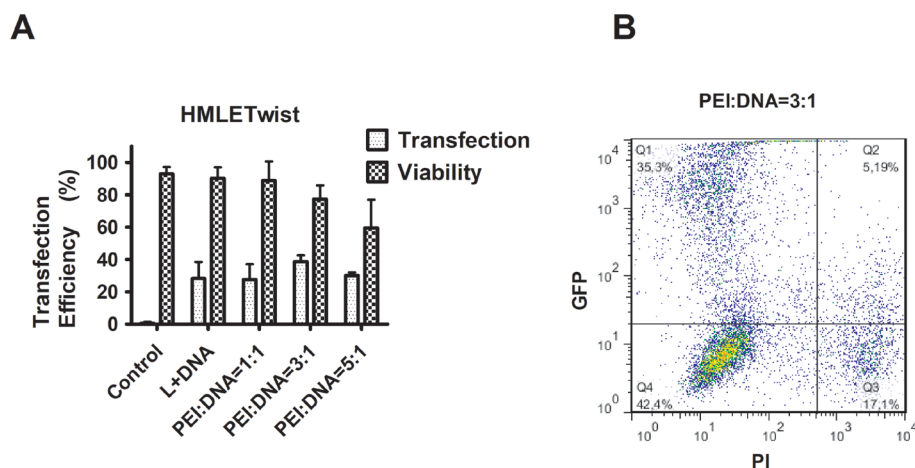
sented as mean  $\pm$  s.d. and were generated in GraphPad Prism 5 software. To determine statistical significance between samples, one-way ANOVA with post-hoc Tukey's multiple comparison test was performed in GraphPad Prism (NS – non-significant; \* –  $p < 0.05$ , \*\* –  $p < 0.01$ ).

## RESULTS

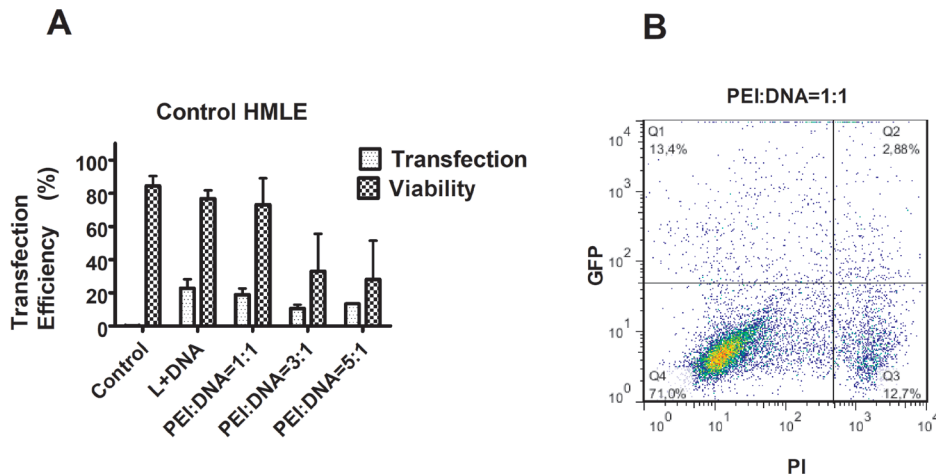
In the presented research, we used PEI for the delivery of plasmid DNA into TNBC cell line SUM159, breast CSC model HMLE-Twist, and mammary epithelial cells control HMLE. At first, we had to adjust all of the ex-

perimental conditions using PEI to obtain sufficient transfection efficiency, while keeping cytotoxicity at minimum. Experimental conditions for Lipofectamine were previously adjusted to obtain maximal transfection efficiency using protocol recommended by manufacturer.

In SUM159 cells, transfection efficiency was highest using PEI to DNA ratio 1:1 (w/v) (Figure 1). When PEI was used at higher concentrations, transfection efficiency was not improved, and PEI became cytotoxic. Transfection efficiency of the Lipofectamine was to some extent lower, but not significantly different (Figure 4) while toxicity was similar to PEI.



**Figure 2. Transient transfection using PEI in HMLE-Twist.** Cells were seeded in 24-well plate at  $5 \times 10^4$ /well as duplicates and grown overnight. 500 ng of pEGFP-C1 DNA/well was mixed with PEI in PEI to DNA ratios 1:1, 3:1 and 5:1 (w/v), or with Lipofectamine. After 48 hr, transfection efficiency was determined using flow cytometer by the percentage of GFP positive cells and viability by the percentage of PI positive cells. **A** Graphical representation with bars where each bar represents a mean value  $\pm$  s.d. of at least three individual experiments. **B** Representative dot plot with percentages of transfected (Q1 and Q2; GFP positive) and dead (Q2 and Q3; PI positive) cell populations.



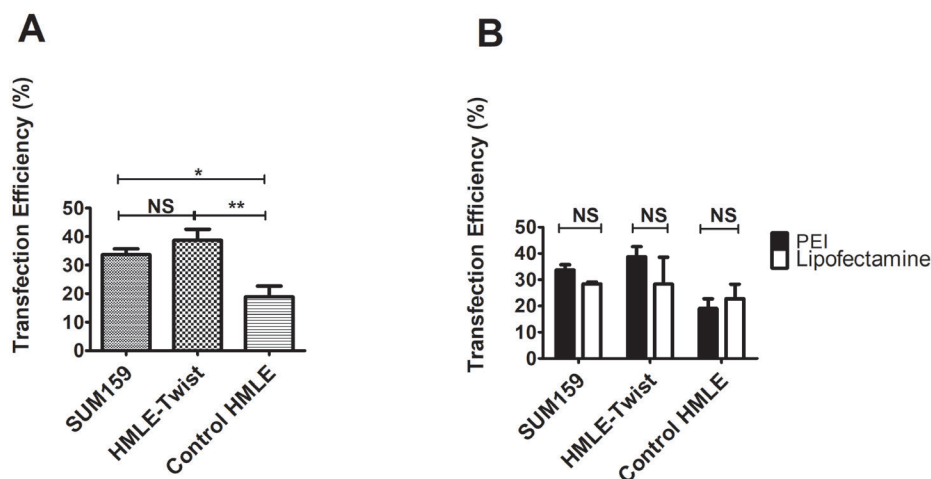
**Figure 3. Transient transfection using PEI in Control HMLE.** Cells were seeded in 24-well plate at  $5 \times 10^4$ /well as duplicates and grown overnight. 500 ng of pEGFP-C1 DNA/well was mixed with PEI in PEI to DNA ratios 1:1, 3:1 and 5:1 (w/v), or with Lipofectamine. After 48 hr, transfection efficiency was determined using flow cytometer by the percentage of GFP positive cells and viability by the percentage of PI positive cells. **A** Graphical representation with bars where each bar represents a mean value  $\pm$  s.d. of at least three individual experiments. **B** Representative dot plot with percentages of transfected (Q1 and Q2; GFP positive) and dead (Q2 and Q3; PI positive) cell populations.

In HMLE-Twist, transfection efficiency was the highest using PEI to DNA ratio 3:1 (w/v) where PEI was slightly more efficient than Lipofectamine, but still not too toxic (Figure 2). Higher concentration of PEI did not enhance the efficiency, but increased cytotoxicity.

Control HMLE cells were the most difficult to transfect (Figure 3). The highest transfection efficiency, but not significantly different, was reached using Lipofectamine (Figure 4). Regarding PEI, transfection efficiency was highest using PEI to DNA ratio 1:1 (w/v).

Cytotoxicity of PEI was generally low to moderate in our hands. PEI caused only 10% of cell death in SUM159 (Figure 1), and 20% in HMLE-Twist (Figure 2). Viability in HMLE-Twist was lower because we used PEI to DNA ratio 3:1 (w/v). In control HMLE, PEI to DNA ratio 1:1 (w/v) caused more than 20% of cell death (Figure 3).

In Figure 4, we summarised data from Figures 1-3 and compared maximal transfection efficiency of PEI among cell lines (Figure 4). Transfection efficiency of PEI was similar (30-40%) in SUM159 and HMLE-Twist. Inter-



**Figure 4. A** Comparison of transfection efficiency among cell lines using PEI. Each bar represents a mean value  $\pm$  s.d. of at least three individual experiments. One-way ANOVA with Tukey's post-hoc test was used for statistical analysis (\* -  $p < 0.05$ , \*\* -  $p < 0.01$ ). **B** Transfection efficiency of PEI vs Lipofectamine. Each bar represents a mean value  $\pm$  s.d. of at least three individual experiments. One-way ANOVA with Tukey's post-hoc test was used for statistical analysis (NS - not significant).

estingly, it was significantly lower in control HMLE, where it did not reach 20% (Figure 4).

Transfection efficiency of PEI was comparable and even higher than the efficiency of the lipid complex Lipofectamine in both SUM159 and HMLE-Twist, but not significantly different (Figure 4), while it was lower in control HMLE.

## DISCUSSION

TNBC and the cell lines derived are a heterogeneous group of tumours which complicates the investigation of molecular basis of disease and aberrations in signal transduction pathways. In this study, we focused on cell lines that represent chemoresistant subpopulation within the tumour. Human mammary epithelial cell line HMLE bears CD24<sup>+</sup>/CD44<sup>-</sup> phenotype. On the other hand, HMLE-Twist cells, that represent breast cancer stem cell line, bear CD44<sup>+</sup>/CD24<sup>-</sup> surface phenotype and are unresponsive to chemotherapy (14). Cell line SUM159 consists of high percentage of breast CSCs among whole cell population that bear the same CD44<sup>+</sup>/CD24<sup>-</sup> phenotype. This cell line most likely derived from metaplastic cancer, a subset of breast cancer that is extremely resistant to treatment (9). In both cell lines we obtained similar results regarding PEI efficiency that additionally indicated substantial level of similarity between these lines. In parental HMLE cells, control HMLE, the transfection efficiency was lower using both carriers, leaving mammary epithelial cells as a challenging cell line regarding gene delivery. At the moment, we cannot speculate what is the basis of these cells resistance, and moreover, what is the link between EMT and improvement of genes delivery.

Branched PEI usually has greater transfection efficiency than linear PEI, but it could also be more toxic for the cells (18). Therefore, we used linear PEI 25kD that allowed us to avoid high cytotoxicity.

In our hands, gene delivery by PEI was comparable with the lipid complex Lipofectamine, which is market dominant, but expensive. Our findings correlate with study in glial cells where Lipofectamine was compared to polyethylenimine Viromer Red (19).

In conclusion, we successfully transfected triple-negative breast cancer cell line SUM159 and breast CSC model HMLE-Twist with plasmid DNA using PEI as a carrier. Since PEI is inexpensive and easy to use, we recommend it for further exploitations of these cell lines in TNBC research.

Cationic polymer PEI represents a suitable nanocarrier for gene delivery in cell lines that represent models for breast cancer stem cells and triple-negative breast cancer. These findings will contribute to the pursuit for an efficient treatment of triple-negative breast cancer.

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