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The analysis of efficacy of selected gene therapy strategies – the influence of nucleus-directed maspin on breast cancer cells and preparation of carrier for genetic drug

ABSTRACT

The aim of this study was to develop a strategy for a therapy of breast cancer based on a gene therapy with a proper carrier for a genetic drug. Particular stages of study aimed at selection of the sequence of a genetic drug and analysis of its influence on breast cancer cells, the choice and preparation of the carrier for the genetic drug and evaluation of effectiveness of drug delivery to cells. The last aim was to combine those two elements of a therapy – the genetic drug and the carrier, followed by preparation and optimization of a vector, which will be ready to use in *in vitro* and *in vivo* studies.

In the first part of this study, two strategies have been chosen for a gene therapy: silencing of the oncogene and overexpression of a gene encoding a tumor suppressor protein. As an oncogene, the gene encoding a HER2 protein was selected. This protein is overexpressed in 15-25% of breast tumors, affecting the increase in the rate of tumor growth. Four silencing sequences and one control sequence have been designed. Using molecular biology techniques, these sequences were inserted under the control of the U6 promoter to the pLB plasmid. This plasmid also encodes a reporter protein EGFP under the control of the CMV promoter. Two of the designed sequences, due to the highest efficiency of silencing, were selected for further experiments. PLB plasmid is also a transfer plasmid in lentiviral system, and it has been used for the production of lentiviral vectors encoding silencing and control sequences. The cells were transduced with lentiviruses, the analysis of the functionality of the vectors was conducted and the efficiency of silencing the *erbb2* gene expression was evaluated on the control human embryonic kidney cells. However, the effective silencing was not observed in breast cancer cell lines and transduction with lentiviruses encoding silencing shRNA did not affect cell proliferation of these cells, indicating the lack of functionality of prepared vector in this model.

In the case of using a strategy related to an overproduction of tumor suppressor proteins initially the p53 protein coding sequence was used.

Mutations in this gene cause a loss of activity of this protein and its aberrant function is observed in 17% of breast tumors, leading to impaired DNA repair mechanisms and to reduce the sensitivity of cells to apoptotic signals. Transfection with a plasmid encoding this protein leads to apoptosis of transfected cells. Lentiviral vectors encoding this protein have also been prepared.

According to the literature, both of these genetic drugs ensure high effectiveness in inhibiting the tumors growth, but do not provide the specificity. When they are applied, specificity should be achieved by using a particular promoter and/or the simultaneous targeting of the carrier to specific cell surface receptors of breast cancer cells.

However, independently from previously described studies, in collaboration with the Medical University in Wroclaw, another research connected with maspin protein was conducted. It is a protein not well understood and characterized, but some studies have shown that it has the properties of the tumor suppressor to slow down or even inhibit the development of cancer, including breast cancer. Due to the lack of clear data on this protein, as well as to emerging reports on the influence of subcellular location of this protein on the proliferation of cancer cells, the aim was to verify the hypothesis that a nuclear location of maspin is responsible for the antiproliferative effect.

The first stage was the preparation of constructs encoding respectively maspin-EGFP (protein is located mainly in the cytoplasm), or maspin-NLS-EGFP (location within the nucleus), which will provide the different location of proteins in the cell. As a control two other plasmids were used: plasmid encoding EGFP (mixed location) and EGFP-NLS (nuclear location). Three breast cancer cell lines (MCF-7, MDA-MB-231, SKBR-3) and a control line from normal breast MCF10A, all have been transfected with constructs mentioned above and tested for proliferative status of the cells. It has been shown that a nuclear-localized maspin is responsible for the inhibition of cell proliferation of breast cancer, cytoplasmic maspin has no significant effect on the proliferative status of cells, as well as two control constructs. Furthermore, the location of maspin both cytoplasmic and nuclear exerted no effect on proliferation of control cells. Due to the fact that the effect of the nuclear-targeted maspin appears to be specific in reference to breast cancer cell, it was decided to change the originally planned strategy, and to use the sequence encoding maspin-NLS-EGFP as a genetic drug for use in later stages of the study.

Hence ensuring specificity of the therapy will be possible at the stage of genetic drug in contrast to two previously tested genetic drugs. Of course, other tests on cell lines derived from other tissues are necessary to determine

whether the lack of inhibitory effect on cell proliferation is observed only in normal cells from breast or it will also be observed in other tissues. In the case of obtaining the inhibitory effect on proliferation of normal cells from other tissues, it will be possible to use the strategy with an additional transcriptional targeting (specific promoter) or transductional targeting (targeting carrier for specific receptors).

Another aim of this work was to select a carrier for a genetic drug. Three types of carriers were tested: liposomal, adenoviral and lentiviral. All three of these carriers were prepared in our laboratory. Finally, it was decided to choose the adenoviral carrier due to its characteristics, efficiency of drug delivery and genetic modification of breast cancer cells.

In the last stage sequences encoding maspin-EGFP and maspin-NLS-EGFP were cloned to adenoviral transfer plasmids and all stages of production, purification and concentration of the adenoviral particles were conducted leading to obtaining the adenoviral preparations. The functionality of obtained adenoviruses and efficiency of transgene delivery *in vitro* in cell cultures and *in vivo* in a mouse animal model were evaluated. Experiments have shown the functionality of the vectors with genetic drug in a cultured cell, and the effectiveness of inhibiting the proliferation of breast cancer cells by the vector encoding the sequence of nuclear-targeted maspin. Preliminary studies *in vivo* on a mouse model demonstrated the functionality of the prepared vector providing delivery of the transgenes into the cancer cells and maintain their expression for about 30 days.

In summary, sequence encoding nuclear-targeted maspin has been chosen as a genetic drug, which may be used as a part of cancer treatment in the future studies. It has been shown that maspin located in cytoplasm inhibits proliferation of breast cancer cells, while the cytoplasmic location of the maspin does not have a significant effect on cell proliferation of breast cancer. Furthermore, overproduction of maspin, neither cytoplasmic nor nuclear, has no effect on the proliferation of control cells of the breast. Moreover, the preparation of adenoviral vectors was implemented and optimized, and the adenoviral vector encoding maspin was also prepared. It has been demonstrated that the obtained adenoviral vector with a genetic drug is functional *in vitro* and *in vivo*. Prepared adenoviral vector carrying a sequence encoding maspin-NLS-EGFP must be subjected to further optimizations and evaluation of its efficiency.