Dynamic Magnetic Field Increases Transduction Efficacy of GFP-modTAT into Prostate Carcinoma PC3 Cells

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Introduction. Delivery of sufficient amount of drugs to tumour cells with minimal damage to normal cells is one of the main requirements for successful cancer treatment. In the previous study we concluded that rapid concentration of lipoplexes on the cell surface achieved by the use of a dynamic magnetic field enhances gene delivery into human prostate carcinoma PC3 and hepatocarcinoma HepG2 cell lines. Cell-penetrating peptides (protein transduction domain) bear great potential as vehicles for the delivery of pharmaceuticals, due to their ability to enter various types of cells, high internalization efficiency, low cytotoxicity and flexible structural design. Human immunodeficiency virus type 1 (HIV1) Tat protein transduction domain (PTD) has been successfully used for introduction of wide range of macromolecules including proteins in cell cultures and *in vivo*. Combination of magnetic field enhanced cell surface accumulation of magnetic nanoparticles and TAT-mediated delivery have opened a new avenue for the development of high-efficacy drug delivery. However, there are only few reports where this approach has been used.

The aim. The aim of this study was to estimate the impact of dynamic magnetic field on efficacy of TAT-mediated transduction of green fluorescent protein into the cells.

Materials and methods. The plasmid GFP-modTAT-6xHis was generated by fusion of green fluorescent protein sequence to slightly modified canonical HIV 1 Tat PTD sequence using standard fusion PCR technique. The fusion protein was expressed in *E. coli* and purified by nickel Sepharose affinity chromatography. As a model system human prostate PC3 cells were used. GFP-modTAT-6xHis was non-covalently binded with CombiMag supermagnetic nanoparticles and magnetotransduction performed under dynamic magnetic field on the device "DynaFECTOR" as well as using static magnetic field. Various magnets' rotation frequency and different concentration of fusion have been used. The transduction efficacy of fusion was estimated by fluorescence microscopy and immunoblot analysis.

Results. The transduction efficacy of GFP-modTAT-6xHis under dynamic magnetic field was higher compared to that under static magnetic field and transduction itself. The highest transduction efficacy was detected using magnets' rotation frequency 50 rpm/min. The magnetotransduction efficacy under dynamic magnetic field was dose dependent and lead to strengthening of fluorescence signal intensity using 4-times less concentration of fusion. The non-covalent binding of 10 μg of fusion with superparamagnetic nanoparticles resulted in 10 fold increase of quantity of internalized GFP compare to amount of GFP detected in PC3 cells following transduction.

Conclusion. The significantly increased amount of internalized GFP demonstrated in PC3 cells following magnetotranduction under dynamic magnetic field gives evidence that TAT-mediated transport under dynamic magnetic field allows to achieve high-efficient delivery of protein into the cells.