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Efficient Delivery of Genetic Constructs to Uterine Fibroid Cells Using Peptide Nanocomplexes

Alina Roman[1], Sofia Shtykalova [1], Anna Egorova[1], Anton Kiselev [1]

Laboratory of Molecular Genetics and Gene Therapy, Research Institute of Obstetrics, Gynecology & Reproductology named after D.O. Ott, Saint-Petersburg, 199034, Russia

INTRODUCTION & AIM

INTRODUCTION

Gene therapy holds great promise for treating genetic disorders, but its success relies on efficient and safe delivery of nucleic acids into target cells. Non-viral vectors, particularly peptide-based carriers, offer a promising alternative to viral systems due to their improved safety profile and modularity. However, their efficacy often requires optimization. This study focuses on modifying cationic and anionic peptide carriers and fine-tuning nanocomplex formation parameters to maximize delivery efficiency while minimizing injection volumes.

AIM

To develop novel ternary polyelectrolyte nanocomplexes, functionalized with an αvβ3 integrin-specific ligand, for the targeted and efficient delivery of genetic constructs into uterine fibroid cells.

METHODS

1. **Ethidium bromide intercalating dye displacement assay** – assesses the ability of carriers to effectively bind DNA.
2. **Fluorescence intensity measurement** to determine the degree of DNA release from complexes under the influence of dextran sulfate.
3. **Resazurin assay** to analyze the metabolic activity of PANC-1 cells after transfection with DNA/carrier/anion complexes.
4. **Flow cytometry** – to determine the efficiency of *in vitro* transfection of PANC-1 cells with DNA/carrier/anion complexes.
5. ***Ex vivo* transfection** of myomatous nodules followed by visualization of the number of GFP-positive cells using fluorescence microscopy.
6. **Dynamic light scattering** to measure the size of DNA/carrier/anion complexes.
7. **Electrophoretic light scattering** to measure the zeta potentials of DNA/carrier/anion complexes.

Name of the carrier	Description
R6p	Arginine-rich polycondensed peptide carrier
R6pH	Arginine-rich polycondensed peptide carrier with increased histidine content
E6p	Glutamate-rich polycondensed peptide carrier
cRGD-E6p	Glutamate-rich polycondensed peptide carrier with a ligand to αvβ3 integrins

RESULTS & DISCUSSION

Arginine-rich peptides and glutamate-rich peptides, modified with a cyclic RGD ligand, were synthesized and used to form ternary nanocomplexes with *GFP* and *lacZ*-encoding plasmids. These complexes were subsequently analyzed for their physicochemical characteristics and cytotoxicity.

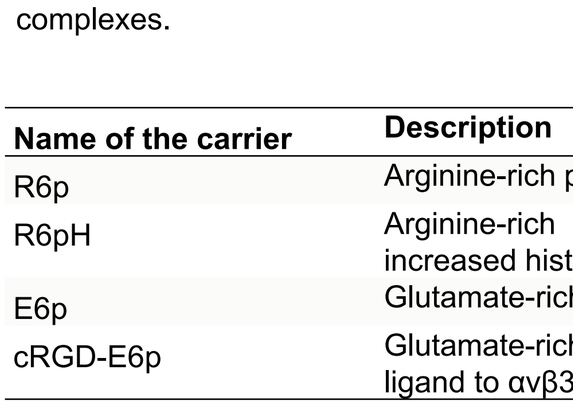

Fig. 1. Relative fluorescence level of ethidium bromide with increasing content of the anionic component in complexes with carriers for a formation volume of 1 μg DNA in 8 μl. The degree of DNA condensation in ternary complexes with a reduced formation volume was assessed.

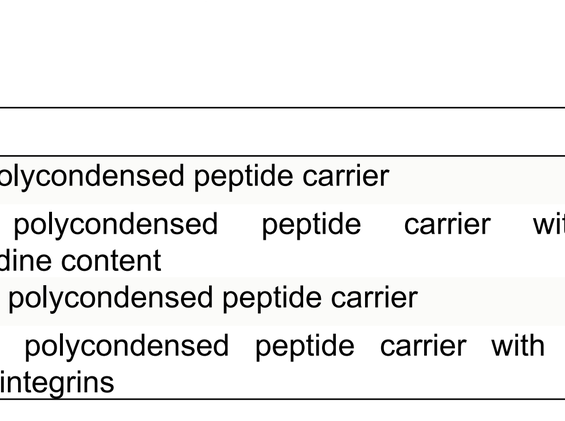

Fig. 2. The viability of PANC-1 cells was assessed after transfection with DNA/carrier/anion complexes formed in small volumes.
No statistically significant differences ($p > 0.05$) in PANC-1 cell viability were found between the groups treated with the various DNA/carrier/anion complexes and the control.

Experiments were performed on transfection of PANC-1 cells with complexes containing the pEXPR-IBA5-eGFP plasmid, which confirmed the efficiency of complexes formed in a small volume. A high percentage of GFP-positive cells was observed when using R6pH/E6p complexes with charge ratios of 1/12/12 and 1/12/24. The carrier that demonstrated the highest transfection efficiency, R6pH/E6p, was selected for modification with ligand.

An experiment examining the viability of PANC-1 cells after transfection with ligand-modified R6pH/cRGD-E6p complexes showed that all tested complexes had little effect on cell viability. Furthermore, extended incubation with the polyanion indicated that these complexes maintained stable DNA binding while gradually relaxing over time.

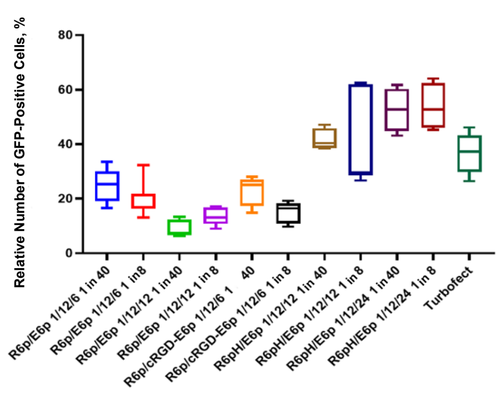

Fig. 3. Relative abundance of GFP-positive PANC-1 cells after transfection with DNA/carrier/anion complexes formed in a small volume. Pairwise comparisons between formation volumes of 1 μg DNA in 8 μl and 1 μg in 40 μl for each carrier showed no statistically significant differences (Kruskall-Wallis test with Dunn's correction), indicating the efficiency of both types of packaging.

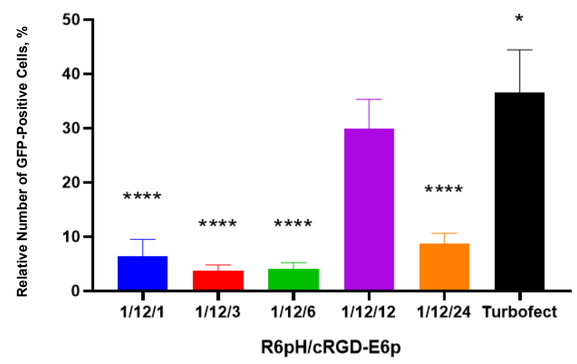

Fig. 4. Relative abundance of GFP-positive PANC-1 cells after transfection with ligand-modified complexes. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons ($\alpha = 0.05$) with the 1/12/12 group showed statistically significant differences ($p < 0.0001$) for the 1/12/1, 1/12/3, 1/12/6, 1/12/24, and Turbofect groups (marked with * in the graph).

Preincubation of cells with a tenfold excess of ligand before addition of R6pH/cRGD-E6p complexes resulted in a significant decrease in gene transfer rate ($p = 0.00752$). This suggests that the modified complexes enter cells primarily by binding to αvβ3 receptors, and that excess free ligand inhibits this process. For unmodified R6pH/E6p complexes, the addition of ligand did not affect gene transfer efficiency ($p = 0.981$).
Results from transfection of PANC-1 cells with R6pH/cRGD-E6p complexes at a reduced formation volume demonstrate that reducing the complex formation volume from 40 μl to 8 μl leads to increased gene transfer efficiency.

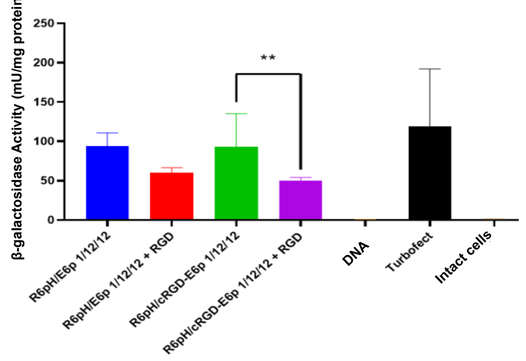

Fig. 5. β-galactosidase activity after transfection of PANC1 cells with complexes containing the lacZ marker gene with the addition of a 10-fold excess of free ligand. A statistically significant difference ** - $p < 0.01$ for R6pH/cRGD-E6p complexes indicates that their uptake by cells depends on interaction with αvβ3 receptors.

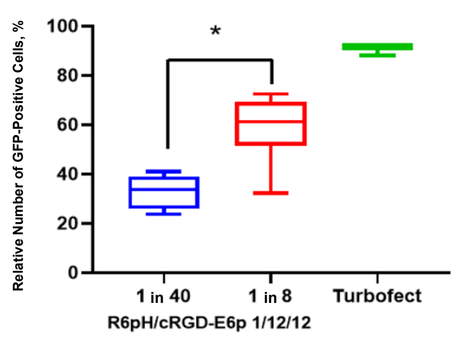

Fig. 6. The number of GFP-positive PANC1 cells after transfection with complexes with reduced formation volume. The results of comparison using the Mann-Whitney test showed statistically significant differences between the groups ($p = 0.0120$), with a higher median for the 1 μg in 8 μl group

Based on the experimental results, R6pH/cRGD-E6p and R6pH/E6p carriers with charge ratios of 1/12/12 and 1/12/12, 1/12/24, respectively, were selected and used to deliver plasmid DNA with the GFP gene *ex vivo* to myomatous nodes.

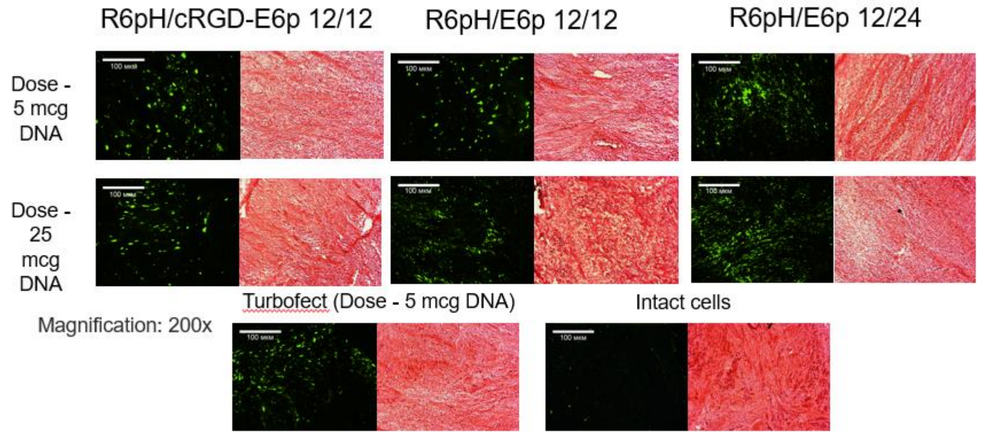

Fig. 7. Microphotographs of sections of myomatous nodes after delivery of plasmid DNA with the GFP gene using peptide carriers.

CONCLUSION

The results demonstrate that reducing the formation volume of the nucleopeptide complexes does not compromise their stability, and the administration of these compact complexes shows no significant cytotoxicity in PANC-1 cells. Importantly, the reduced-volume formulation maintains high transfection efficiency. Furthermore, we successfully demonstrated efficient *ex vivo* transfection of myomatous nodules using the developed ternary nucleopeptide complexes prepared in the minimized volume, thereby highlighting their significant potential for *in situ* gene therapy of uterine fibroids..