Suicidal gene therapy of leiomyoma via delivery of herpes thymidine kinase gene by means of αvβ3 integrin-targeted peptide-based carriers and magnetic nanoparticles.

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Introduction

Uterine leiomyoma (UL) is the most common benign tumor of the female reproductive tract. Precise ultrasound localization of tumors and their availability by means of different endoscopic techniques makes UL a perfect target for suicidal gene therapy *in situ* using e.g. herpes thymidine kinase (HSV1-TK) gene transfer. Development of specific and efficient carriers for targeted delivery of genetic constructs to cells is a current problem of gene therapy. We developed novel RGD-targeted peptide-based carriers for plasmid DNA delivery into the αvβ3-expressing cells, including primary UL cells. For successful penetration into the inner layers of the UL, magnetic nanoparticles (MNPs) were non-covalently bound to DNA-peptide complexes.

Materials and methods

The efficiency of DNA condensation in the polyplexes with MNPs was evaluated by the quenching of ethidium bromide. DNA stability in the polyplexes with MNPs was studied using a DNAase I protection assay. The specificity of the cell penetration and DNA delivery have been demonstrated by ligand-competitive DNA uptake and transfection experiments performed on PANC-1 cells. Suicidal gene therapy with HSV1-TK gene encoding plasmid pPTK-1 and subsequent ganciclovir treatment was held for primary leiomyoma cells at early passages.

Results

Non-viral carriers composed of RGD ligand-conjugated arginine-rich peptides and ligand-free peptides were synthesized. Physicochemical properties of DNA-complexes with MNPs have been studied.

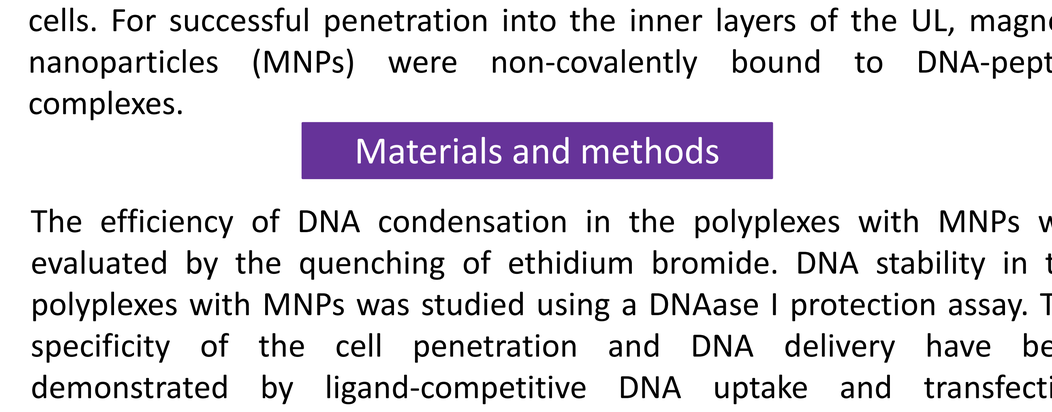

Fig. 1 - Study of DNA/cRGD-R6 complexes stability with increasing weight ratios of DNA-complexes and magnetic nanoparticles. The results are presented as mean and S.D.

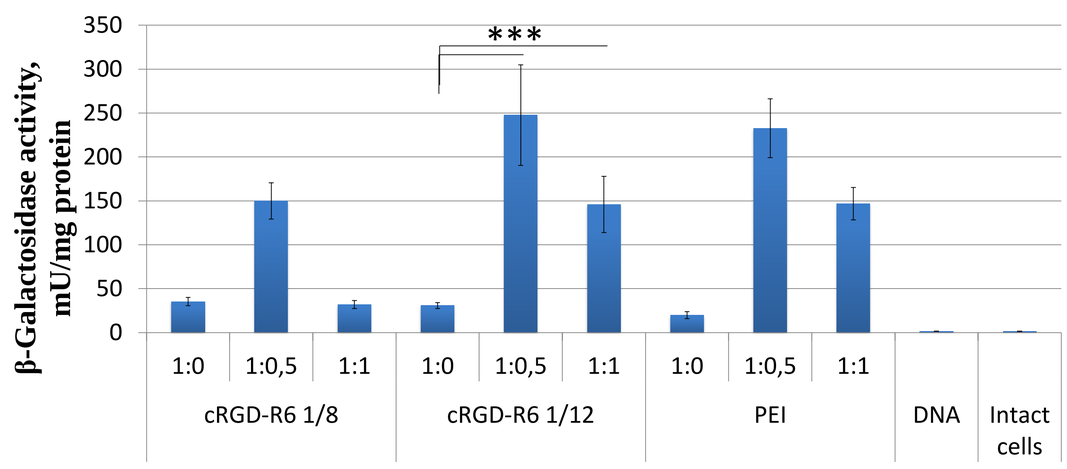

Fig. 2 - Activity of β-galactosidase in PANC-1 cells after magnetofection with pCMV-LacZ complexes with cRGD-R6 carrier and MNPs . The results are presented as mean and S.E.M. (*** - p-value < 0.005).

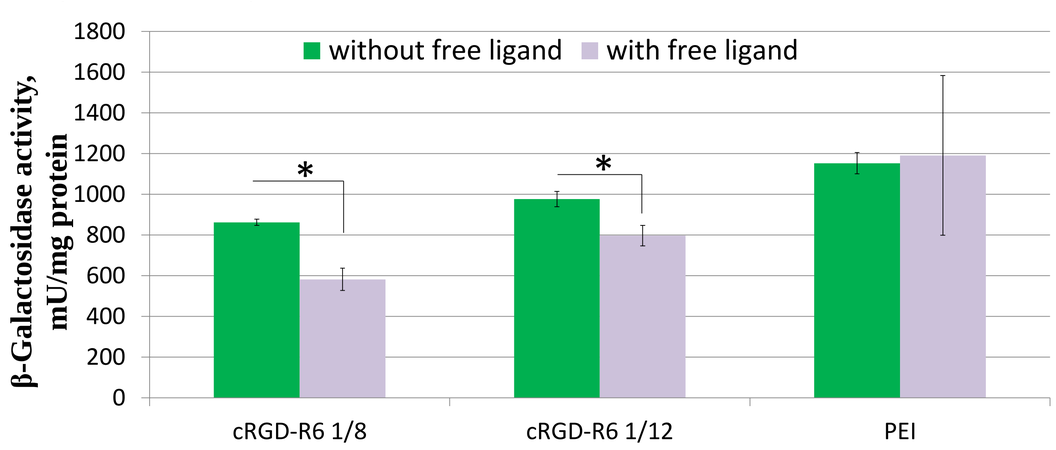

Fig. 3 - Activity of β-galactosidase after magnetofection of PANC-1 cells with nucleopeptide complexes with MNPs with and without the addition of a free ligand (* - p.value < 0.05).

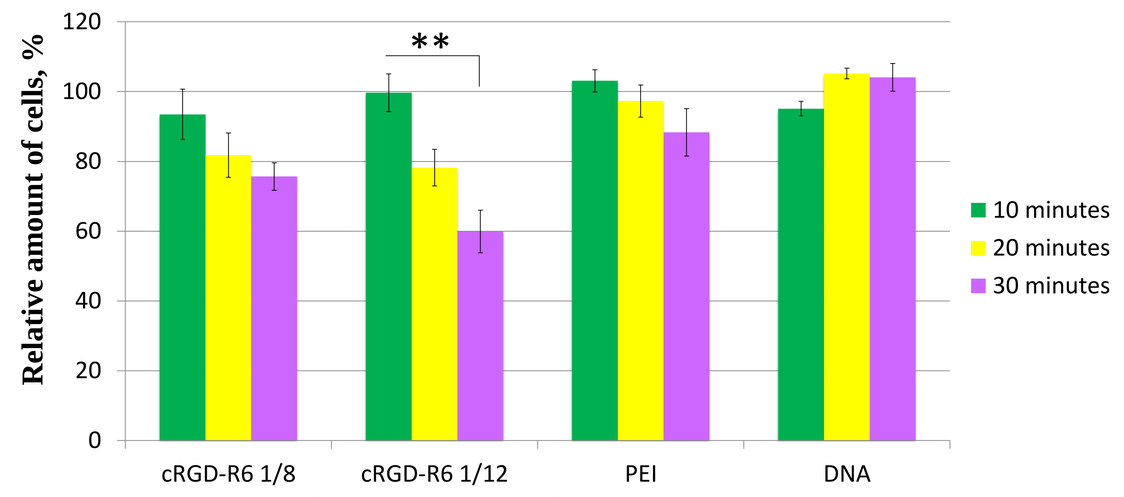

Fig. 4 - Relative number of PANC-1 cells (%) after magnetofection with complexes with DNA / cRGD-R6 and MNPs. DNA:MNP weight ratio is 1:0.5 and different incubation times of complexes with cells (10, 20, and 30 minutes) p-value < 0.01).

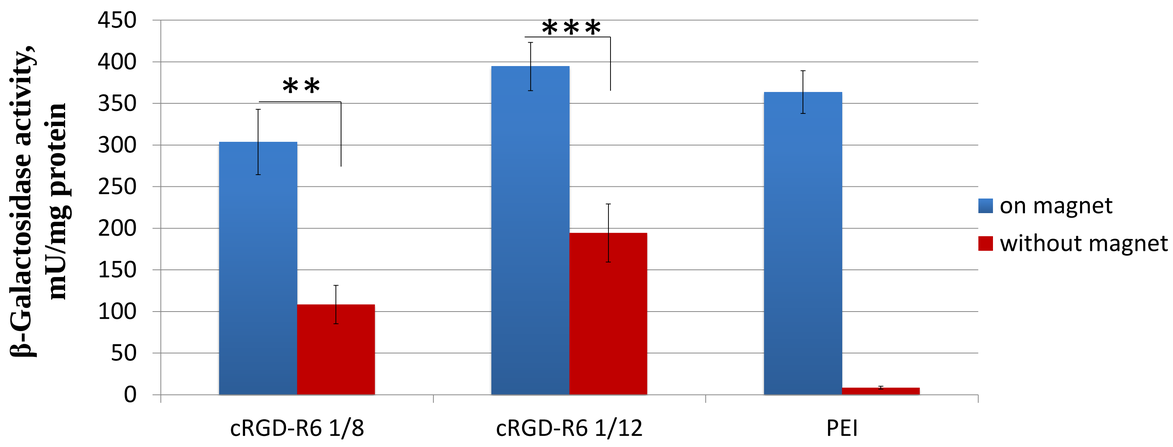

Fig. 5 - Activity of β-galactosidase after transfection of PANC-1 cells with nucleopeptide complexes with MNPs in the presence and absence of a magnet (** - p.value < 0,01, *** - p.value < 0.005).

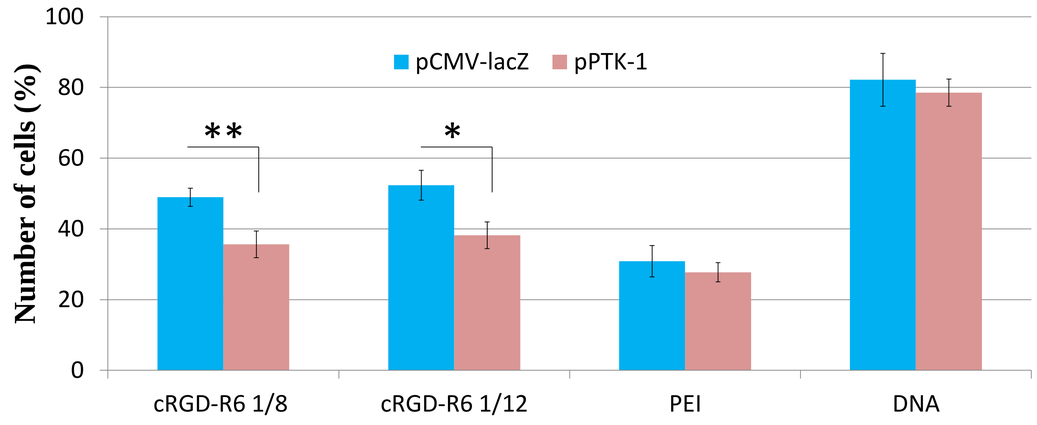

Fig. 6 - Results of Alamar blue assay after transfection of primary uterine leiomyoma cells after transfection with DNA-complexes with MNPs (DNA:MNP weight ratio is 1:0.5) (* - p-value < 0.05, ** - p-value < 0.01).

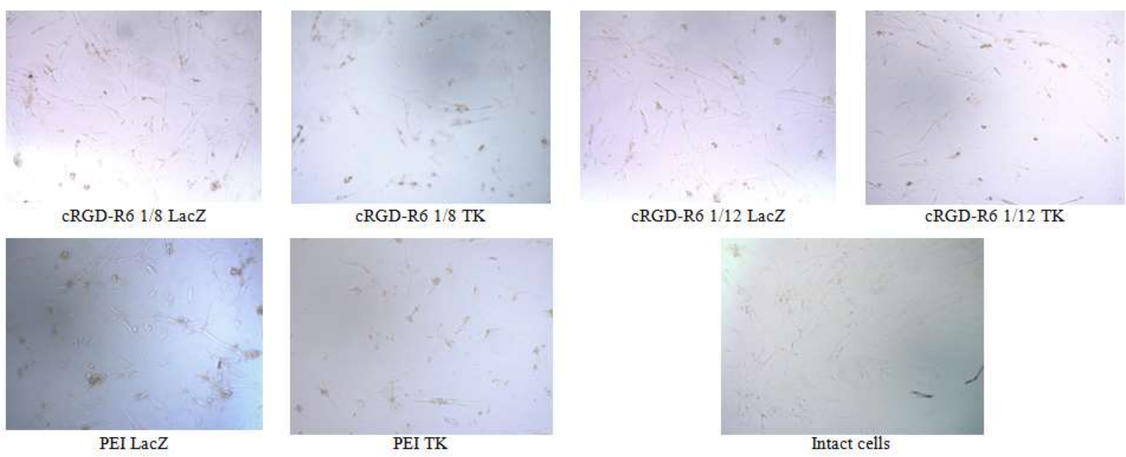

Fig. 7 - Uterine leiomyoma cells after magnetofection with DNA-peptide polyplexes with MNPs (DNA:MNP weight ratio 1:0.5) carrying the LacZ gene and with the HSV1-TK gene.

Conclusion

The developed DNA-complexes with MNP demonstrated high specificity and transfection efficiency of leiomyoma cells with subsequent successful suicide gene therapy, which makes them promising for the development of UL gene therapy.

Acknowledgements

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