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Anionic coated peptide-based carriers as a delivery system for gene therapy of uterine leiomyoma

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Abstract:

Uterine leiomyoma (UL) is the most common benign tumor of the female reproductive tract. Despite being benign, this tumor can seriously affect reproductive function and lead to infertility. Localization makes the disease a perfect target for gene therapy. However, excessive extracellular matrix (ECM) remains challenging for gene delivery by nanoparticles. Anionic coating can improve stability of polyplexes and contribute to successful ECM crossing. We developed αvβ3-integrin-targeted peptide-based carrier and anionic peptide coating for DNA delivery into primary UL cells and UL nodes.

Arginine-histidine-rich peptide carriers modified with cycloRGD-ligand were synthesized. Physicochemical properties of anionic coated DNA-polyplexes were tested. Polyplexes stability was evaluated in transfection experiments in the presence of serum on PANC-1 cells. Suicidal gene therapy with *HSV1-TK* delivery and subsequent ganciclovir treatment was held for primary UL cells obtained after myomectomy. Efficiency of DNA delivery into UL nodes was demonstrated by GFP detection by fluorescent microscopy.

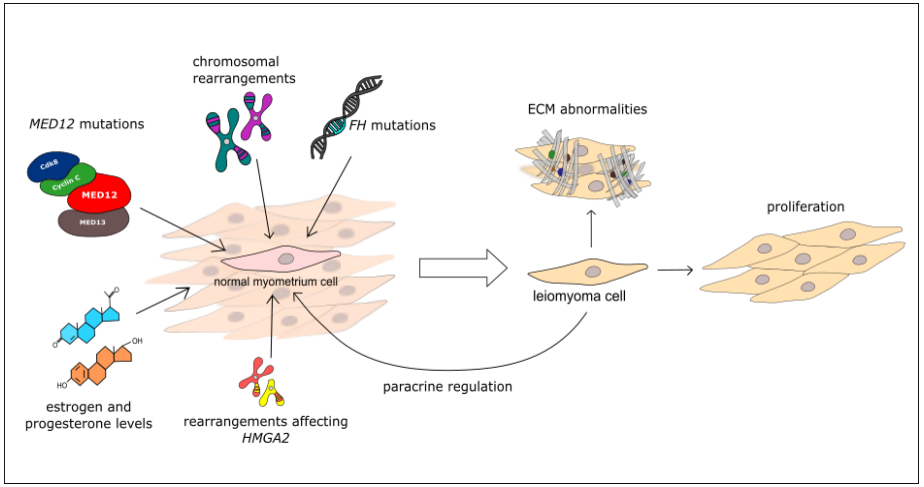
Anionic peptide coating increased stability of polyplexes and allowed successful transfection in the presence of serum. TrypanBlue staining assay showed decline of proliferative activity among primary UL cells transfected with *HSV1-TK* carrying complexes in comparison with control *lacZ* transfected cells. Fluorescence signal was detected on sections of nodes injected with GFP-polyplexes.

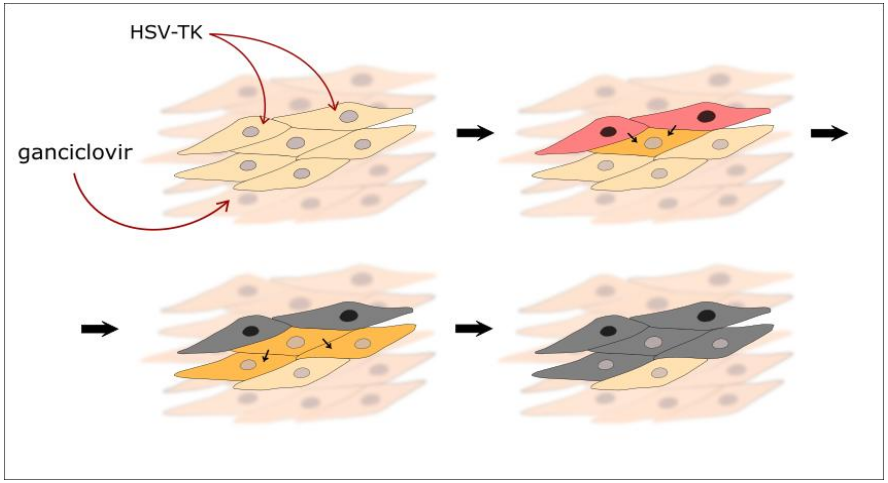
Here we showed that αvβ3-integrin-targeted peptide-based carriers with anionic peptide coating demonstrate high specificity and transfection efficacy of UL cells and successful gene delivery in UL nodes. Further development of anionic coated peptide carriers is promising for UL gene therapy.

Keywords: anionic coating; gene delivery; non-viral carriers; suicide gene therapy; uterine leiomyoma

Introduction

Uterine leiomyoma (UL) is the most common benign tumor of the female reproductive tract. Despite being benign, this tumor can seriously affect reproductive function and lead to infertility. The precise localization makes the disease a perfect target for gene therapy. However, excessive extracellular matrix (ECM) remains challenging for gene delivery by nanoparticles. Anionic coating can improve stability of polyplexes and contribute to successful ECM crossing.

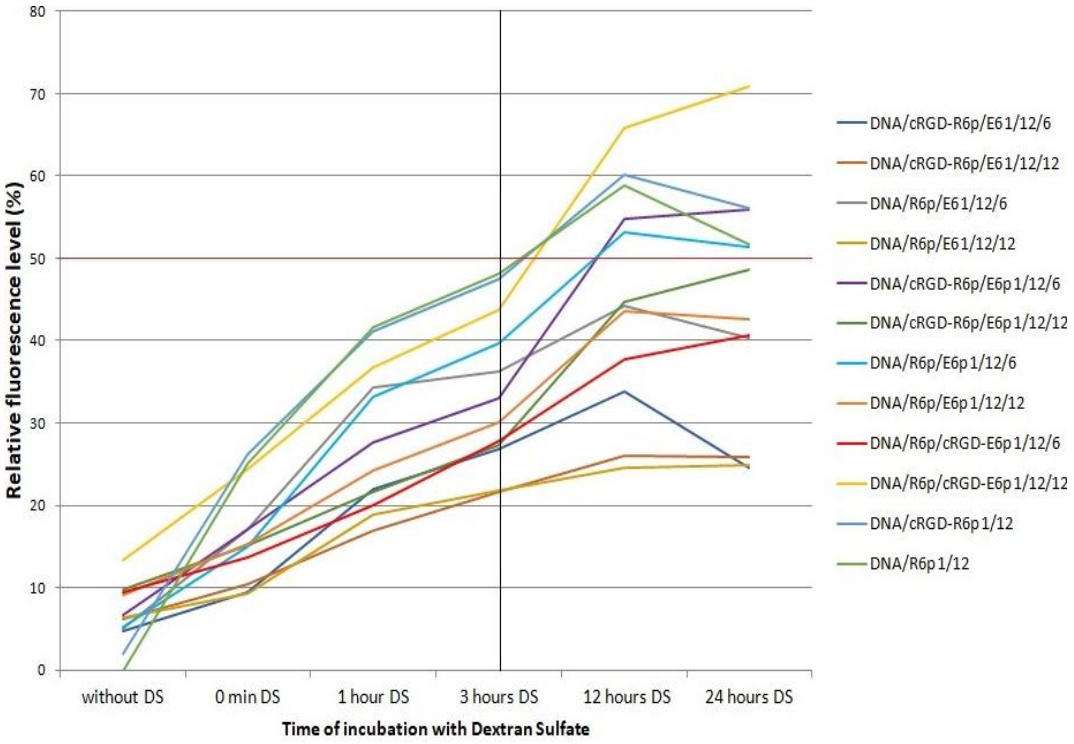


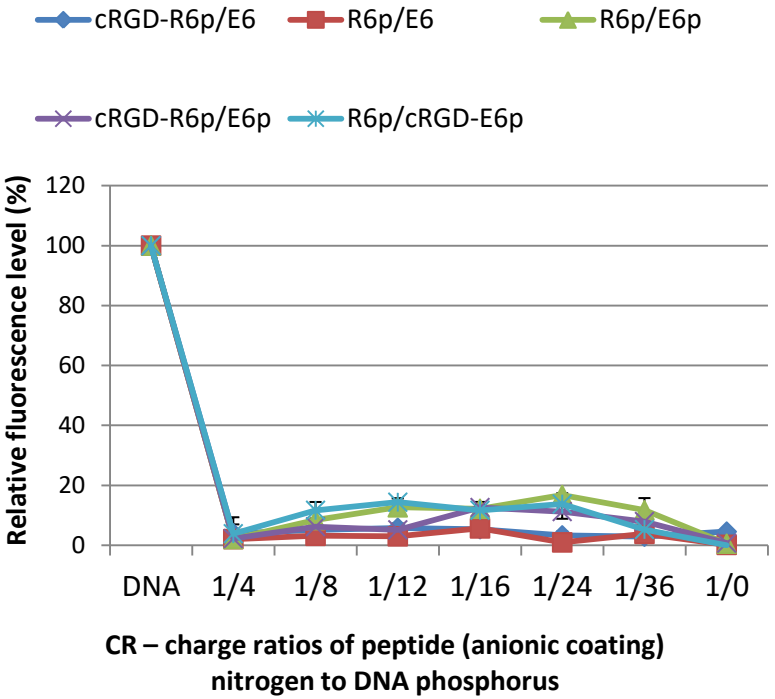


Pathogenesis of the ULs and the Bystander effect of suicide gene therapy of ULs (*Shtykalova et al., 2021).*

Shtykalova S.V., Egorova A.A., Maretina M.A., Freund S.A., Baranov V.S., Kiselev A.V. Molecular genetic basis and perspectives of uterine leiomyoma gene therapy // Russ J Genet 57, 1002–1016 (2021). doi: 10.1134/S1022795421090118

Results and discussion

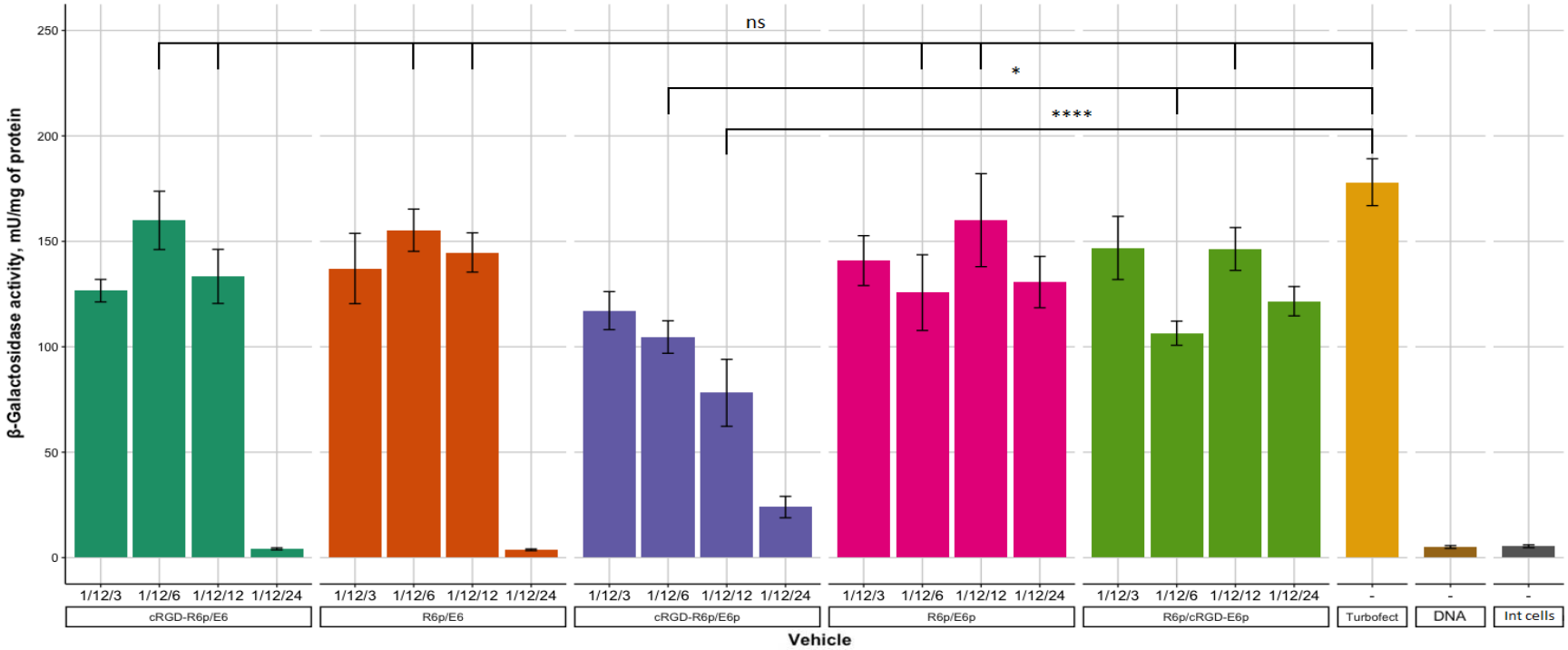




Changes in the fluorescence intensity of the ethidium bromide dye while increasing of the incubation time with Dextran Sulfate (DS).

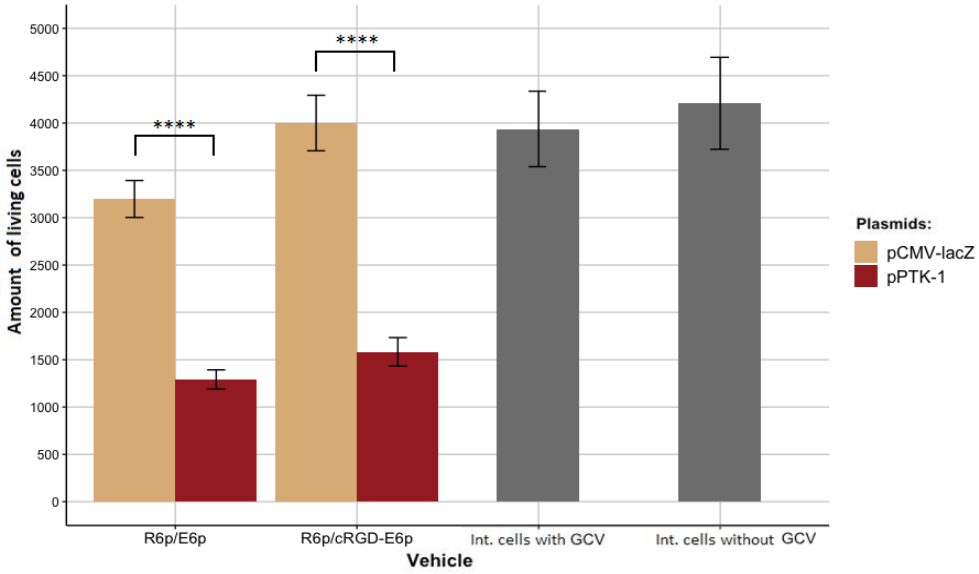
Changes in the fluorescence intensity of the ethidium bromide dye while increasing of the DNA/anionic peptide charge ratios. The results are presented as mean ± S.D.

Results and discussion



Activity of β-Galactosidase after transfection of PANC-1 cells with nucleopeptide complexes with anionic coating in presents of fetal bovine serum. The results are presented as mean ± S.E.M. Statistical significance was determined using One-way ANOVA with Sidak's multiple comparisons test (*p<0.05, ****p<0.0001).

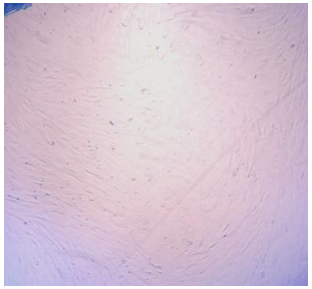
Results and discussion



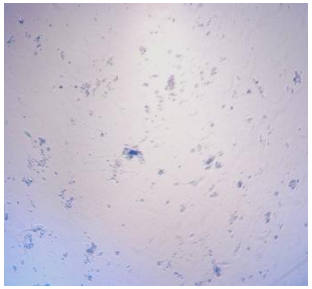
Results of Trypan blue staining after transfection of primary uterine fibroids cells.

The results are presented as mean ± S.E.M.
Statistical significance was determined using One-way ANOVA with Sidak's multiple comparisons test (****p<0.0001).

CR – charge ratios of peptide nitrogen to DNA phosphorus

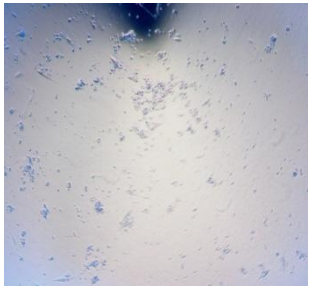


R6p/cRGD-E6p
(CR = 1/12/6)
pCMV-lacZ



R6p/cRGD-E6p
(CR = 1/12/6)
pPTK-1

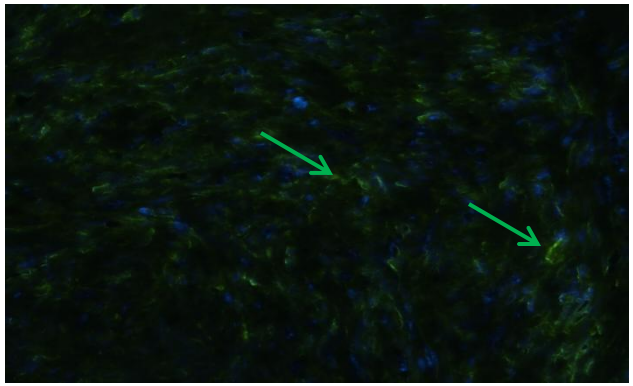
R6p/E6p
(CR = 1/12/6)
pCMV-lacZ



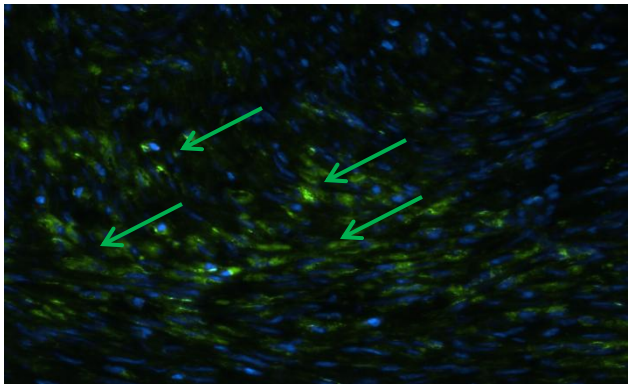
R6p/E6p
(CR = 1/12/6)
pPTK-1

UL cells after transfection with DNA-peptide polyplexes with anionic coating carrying the *lacZ* gene and the *HSV1-TK* gene.

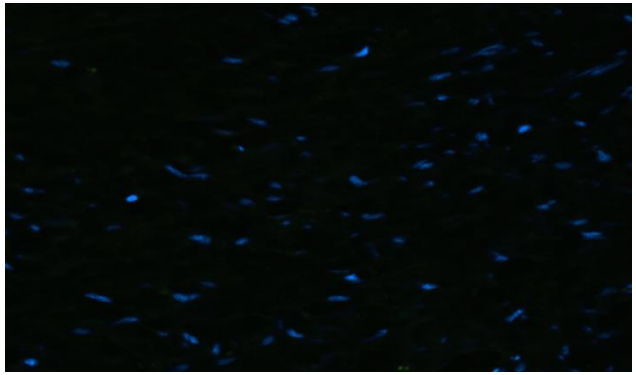
Results and discussion



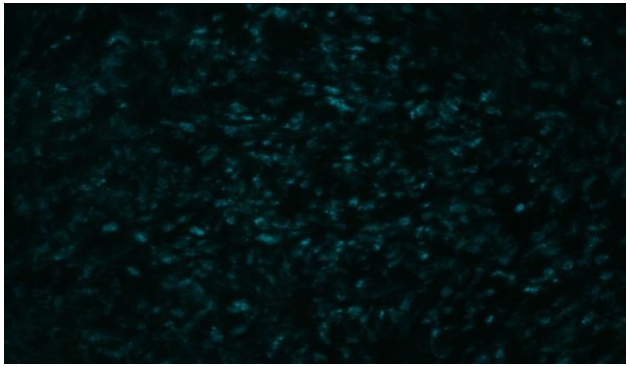
R6p/ cRGD-E6p (CR=1/12/6)



R6p/E6p (CR=1/12/12)



R6p/E6p (CR=1/12/6)



Control tissue

Sections of leiomyoma nodes stained with Hoechst 33258 dye. Blue glow marks nuclei, green glow marks accumulation of GFP protein by UL cells.

Conclusions

We showed that αvβ3-integrin-targeted peptide-based carriers with anionic peptide coating demonstrate high specificity and transfection efficacy of UL cells and successful gene delivery in UL nodes.

Further development of anionic coated peptide carriers is promising for UL gene therapy

Acknowledgments

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