Effective Splicing Correction of *SMN2* Gene in SMA Cells after Delivery of RNA Interpolyelectrolyte Complexes

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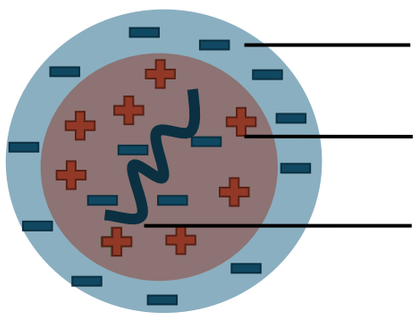
INTRODUCTION & AIM

- Splicing correction of the *SMN2* gene is a key treatment for spinal muscular atrophy.
- Intrathecal administration of antisense RNA oligonucleotides (ASOs) can cause side effects and requires repeated injections.
- The use of non-viral methods for the delivery of antisense oligonucleotides has the potential to facilitate the transition from intrathecal administration to intravenous infusion of these drugs.
- Ternary oligonucleotide--peptide complexes coated by anionic polypeptide are designed to overcome the impossibility of systemic delivery antisense RNA oligonucleotide.

METHOD

Ternary oligonucleotide—peptide complexes

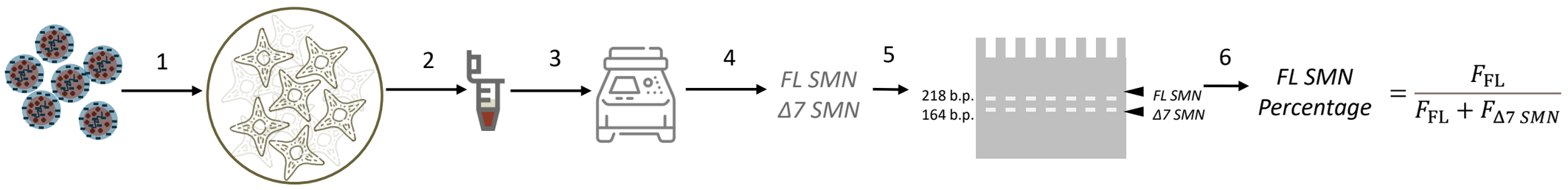


Previously, we developed a **cationic peptide carrier R6p**, and an **anionic peptide coating E6p** and **cRGD-E6p** (ligand-modified to αvβ3 integrins) [1] to deliver ASO 3UP8 [2] to a cellular model of SMA in the presence of serum, which corresponds to physiological conditions.

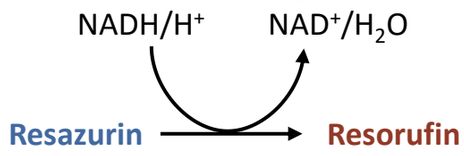
The **RNA/anionic carrier** charge ratios tested in this work:

1/4, 1/8, 1/16, 1/24.

The study involved transfecting SMA fibroblast cell culture with the serum-stable oligonucleotide—peptide complexes, reverse transcription, semiquantitative PCR, and resazurin assay.



1. Transfection with interpolyelectrolyte complexes of primary fibroblast cell culture obtained from skin biopsy of patients with SMA II [3] in the presence of fetal bovine serum. **2.** RNA isolation. **3.** Reverse transcription. **4.** Semi-quantitative fluorescence PCR with primers for amplification of a cDNA fragment from 6 to 8 exons of SMN2 [4]. **5.** Visualization of PCR by electrophoretic separation of PCR products in 6% polyacrylamide gel. Gel staining with ethidium bromide. Estimation of the luminescence intensity of amplification products using the ImageJ. **6.** Calculation of the proportion of full-length *SMN* transcripts (*FL SMN*) to the sum of full-length and truncated SMN transcripts (Δ7 *SMN*) [5].



Resazurin Assay

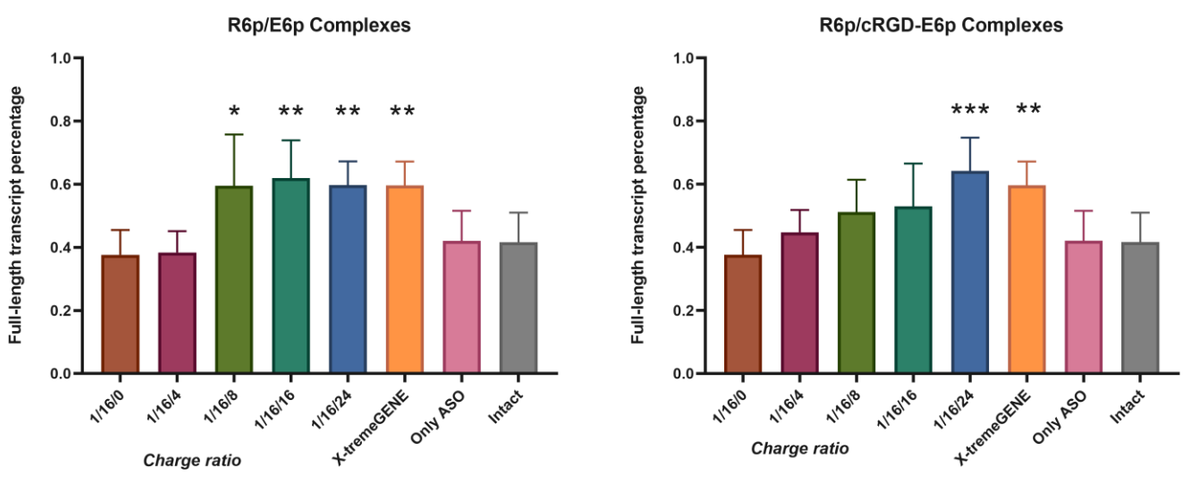
Relative amount of living cells $= \frac{F - F_0}{F_c - F_0}$,

F_c - dye fluorescence intensity in the absence of RNA/carrier complexes,

F_0– dye fluorescence intensity in the absence of cells.

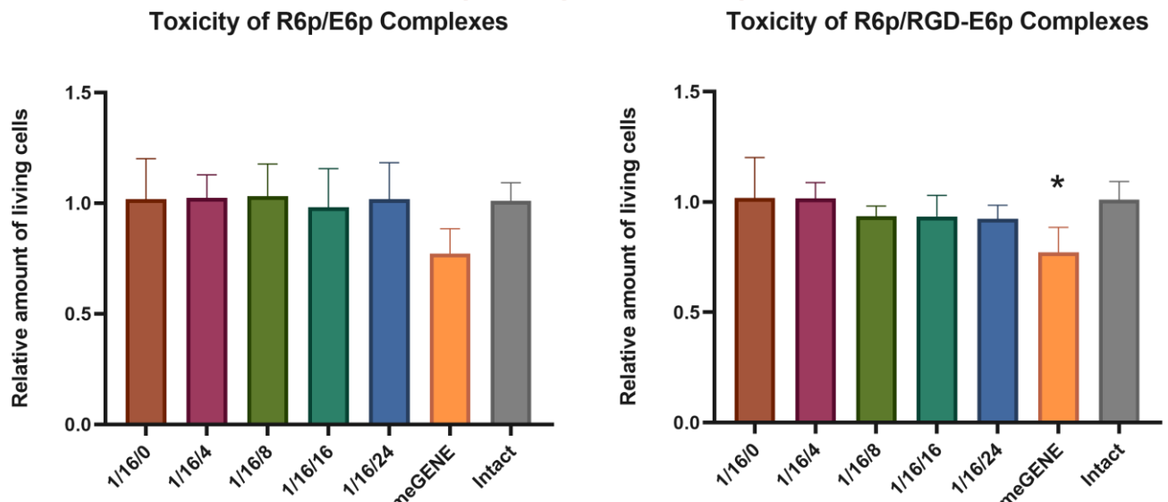
RESULTS & DISCUSSION

Evaluation of the Effectiveness of *SMN2* Gene Splicing Correction



The results are presented as a mean ± SD n = 12 individual samples from the three independent experiments. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.0001$ compared to intact cells.
The level of significance is determined using the Kruskal-Wallis test.

Toxicity Analysis of Complexes



The results are presented as a mean ± SD n = 6 individual samples from three independent experiments. * - $p < 0.05$ compared to intact cells.
The level of significance is determined using the Kruskal-Wallis test.

Developed system demonstrates **low toxicity** and **effectively facilitates transfection** in cell culture, closely resembling **physiological conditions**.

REFERENCES

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