Lipid-substituted Polyethylenimine Conjugate Mediated STAT5A siRNA Reduces the Growth of Acute Lymphoblastic Leukemia *in vivo*

Mohammad Nasrullah[1,2], Remant KC[1], Hasan Uludağ[1,2,3]

[1] Department of Chemical & Materials Engineering, Faculty of Engineering,
[2] Faculty of Pharmacy & Pharmaceutical Sciences, and [3] Department of Biomedical Engineering, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta

Introduction

- Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children, teens and younger adults [1]
- It originates from the immature lymphocytes and progresses very quickly and aggressively. **Due to high relapse rate and drug resistance [2]**, there is an urgent need to develop alternative treatments with higher efficacy and safety
- STAT5A is a major transcription factor in ALL that causes leukemic growth by activating downstream oncogenes [3]. Thus, silencing STAT5A by siRNA can be a key player to therapeutic development in ALL
- siRNAs are highly **unstable in physiological fluids** and their **anionic nature prevents them from traversing cellular membranes**. Therefore, the success of siRNA therapy will be governed by the efficiency of the delivery system
- Here, we explored the use of lipid-substituted polyethylenimine **(PEI)** to deliver siRNA
- The aim of this study is to **assess the siRNA delivery efficiency of this non-viral carrier into ALL cells and the potential effects on *in vivo* tumor growth inhibition**

Materials & Methods

- We prepared lipopolymer (PEI-A) by substituting lipid A onto low molecular weight PEI (MW 1.2kDa). The preparation was described previously [3,4]. The name of the lipid is not mentioned due to IP issues
- Acute lymphoblastic **RS4;11 cells** was purchased from American Type Culture Collection (ATCC) (Rockville, MD) and used for further studies

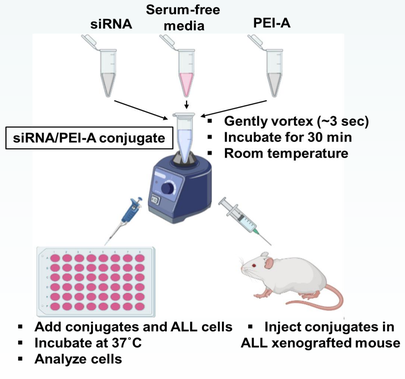

Figure 1: *in vitro* and *in vivo* siRNA transfection procedure. siRNAs (~10μM) were diluted in serum free media (RPMI) and mixed with PEI-A at different siRNA/PEI-A weight ratios. The mixtures were gently vortexed and incubated for 30 min at room temperature for preparing the conjugates

- The conjugates are ready to use for both *in vitro* and *in vivo* studies

Materials & Methods

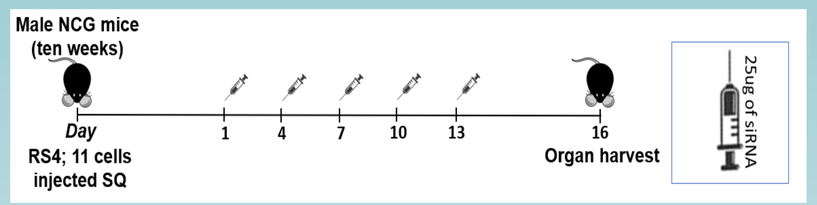

Figure 2: Xenografting RS4; 11 cells and monitoring tumor growth. Triple immunodeficient (mature T, B, and NK cells deficient) male NCG mice were obtained from Charles River Laboratories (Laval, QC). The RS4;11 cells (**2.5 x 10^6 cells** suspended in 100 μL 60:40% RPMI: Matrigel) were administered subcutaneously (SQ) into the left flank and sufficient time was given for establishment of human xenografts in mice. The protocol was approved by the Health Sciences Laboratory Animal Services, University of Alberta and performed according to its guidelines

- The tumor volume was measured every 3 days and subjects were recruited to the study when the tumor size reached ≥100 mm^3
- 25μg of STAT5A siRNA **(siSTAT5A)** complexed with PEI-A were injected SQ every 3 days for 5 times. Scrambled siRNA **(CsiRNA)** were injected as negative control
- The mice were typically monitored within 24 hours post-injection. After 3 days of final dose, mice were euthanized, tumors and vital organs were collected for

Results

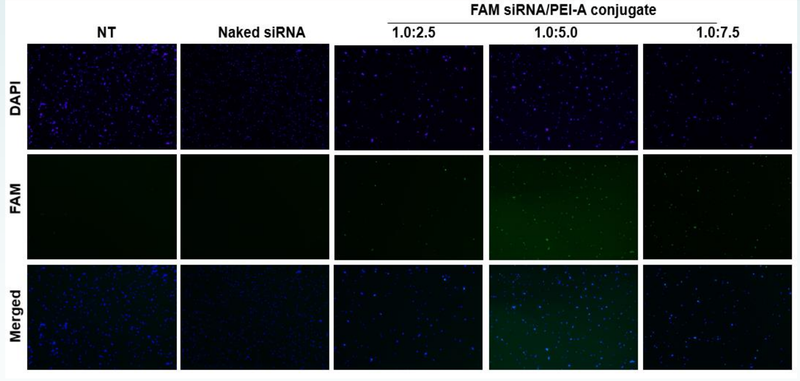

Figure 3: Cellular uptake of FAM labeled siRNA into RS4; 11 cells. siRNA transfection efficiency of PEI-A was measured by fluorescence imaging. (NT: No treatment; FAM: Fluorescein amidites dye; DAPI: 4′,6-diamidino-2-phenylindole dye)

- **FAM siRNA/PEI-A conjugates** were prepared at 3 different ratios (2.5, 5.0 and 7.5, w/w) and transfected into RS4; 11 cells
- After 24 hrs of transfection, cells were prepared for microscopy analysis. Green fluorescence signals (FAM siRNA) were **distinctly visible in PEI-A ratio 5.0 and 7.5** treated cells

Results

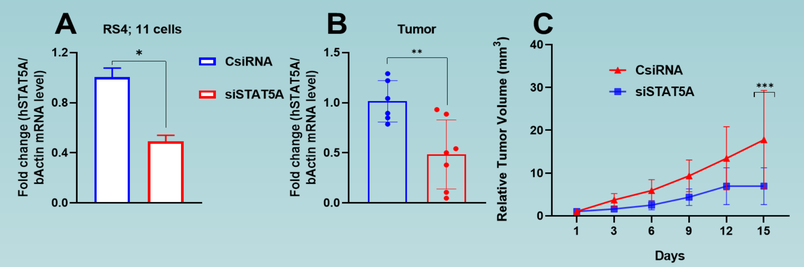

Figure 4: Relative expression of STAT5A in RS4; 11 cells and tumor sample. Fold changes in STAT5A expression were analyzed by normalizing to reference gene (β-actin). Significant difference was determined by using parametric unpaired t-test. Data are presented as mean ± SD (*p<0.05; **p<0.001; and ***p<0.0001)

- **(A)** PEI-A complexed siSTAT5A induced **significant *in vitro* STAT5A knockdown (p=0.0142)** compared to the CsiRNA group
- **(B) In xenografted tumors,** human STAT5A expression **significantly downregulated (p=0.0073) in siSTAT5A group**

Comparison of relative tumor growth between CsiRNA and siSTAT5A group. Significance was analyzed by two-way ANOVA and Sidak's multiple comparisons test ((*p<0.05; **p<0.001; and ***p<0.0001). Error bars indicate standard deviation

- **(C)** siSTAT5A treatment **significantly reduced (p=0.0005) the tumor volume**

Conclusions

1. Lipid substituted Polyethylenimine efficiently transfected siRNAs into ALL cells and helped to significantly knockdown the STAT5A expression both *in vitro* and *in vivo*
2. This polymer can be used as an effective non-viral carrier to deliver RNA for therapeutic purpose in ALL
3. More studies are required to evaluate the immune response levels and changes in downstream targets

References & Acknowledgements

[1] Caron M et al., J Imms. 2015;422:118-124. Sci Rep. 2020;10(1)
[2] Li B et al., Blood. 2020;135(1)
[3] Mohseni et al., PLoS One. 2021;16(6)
[4] Remant KC et al., J Biomed Mater Res A. 2020;108(3):565-580

MN was supported by a fellowship from MITACS Accelerate and RJH Biosciences Inc. RK and HU declare conflict of interest due to IP ownership in the described material.