Synthesis of Novel Building Blocks for Nucleic Acid Nano-scale Engineering

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Background

Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disorder resulting in ageing features in childhood. The HGPS cells express progerin, a truncation of the protein Lamin A, that perturbs cellular homeostasis leading to nuclear alterations, genome instability, heterochromatin loss and telomere dysfunction, which leads to premature cellular senescence.[1]

Antisense oligonucleotides (ASOs) based therapeutic strategy has recently been spotted as one of the most promising approaches for the treatment of various diseases. It relies on the capability to specifically knockdown the expression of a target gene at the mRNA level.[2] This could be accomplished by using complementary oligonucleotides (known as antisense oligonucleotides) via sequence-specific Watson-Crick base pairing and consequently blocking expression of the protein encoded by the target RNA. The utilization of chemically modified nucleotides is paramount to optimize the stability, the binding affinity, and the nuclease resistance of ASOs-based therapeutic molecules. Recently promising results have been reported using ASOs, where the telomeric disorder of the progerin in mice could be inhibited. [1] In Addition, two ASOs drug candidates have been approved by the US Food and Drug Administration (FDA), bringing the total number of FDA approved ASOs drugs to four, [3,4] while 79 oligonucleotides drugs are currently undergoing clinical trials.[5]

Aim of the study

This project will enhance our understanding of RNA export, a process that can impact cell physiology and disease in addition to developing a novel possible ASOs-based drug for progeria.

Objectives

Use the organic synthesis for generating novel nucleoside and /or non-nucleoside modifications that can modulate bioactive DNA/RNA structures. In a parallel line of chemical experiments, the synthesized building blocks can also be incorporated into rationally designed modified ASOs. Subsequently, the influence of the modified ASOs on progerin would be studied at the cell level via bioimaging investigation for the effect of the modified ASOs on the stiffening of the nuclear envelope, degree of lobulation of the progeroid cell nuclei, and the quantity transport of RNA though nuclear pore complexes (NPCs). The expression of a targeted RNA in addition to the protein of interest will be estimated using relevant techniques.

Methods

1- Designing, synthesizing and characterizing the proposed monomers and their design for bioactive engineered antisense constructs.
2- Growing the progeria cells and transfecting with the modified ASOs.
3- Studying the morphology change in progeria cells upon the ASOs treatment via confocal imaging.
4- Quantifying the RNA and the protein expression via qPCR and western blots over a specific treatment period.
5- Quantify the transport of RNA through NPCs by cutting-edge quantitative super-resolution imaging and correlate to numbers of lobulation and degree of nuclear lamina stiffening.

References

[1] Aguado, J., Sola-Carvajal, A., Cancila, V. et al. *Inhibition of DNA damage response at telomeres improves the detrimental phenotypes of Hutchinson–Gilford Progeria Syndrome. Nat Commun* **2019,** 10: p.4990.
[2] P. C. Zamecnik, M. L. Stephenson, *Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide.* Proc. Natl. Acad. Sci. USA **1978**, 75: p.280-284.
[3] Y. Y. Syed, *Eteplirsen: First Global Approval.* Drugs, **2016**, **76**: 1699-1704.
[4] D. R. Corey, *Nusinersen, an antisense oligonucleotide drug for spinal muscular atrophy.* Nat. Neurosci., **2017**, 20: p.497–499.
[5] ClinicalTrials.gov. https://clinicaltrials.gov/, August **2020**.