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Quantitative NanoSIMS provides subcellular concentration and distribution of oligonucleotide therapeutics

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Abstract

Antisense oligonucleotides (ASOs) represent a powerful therapeutic modality that can selectively modulate gene expression. However, ASOs face two major hurdles that restrict their use in the clinic. The first issue is delivery of the ASO to a tissue of therapeutic interest while reducing exposure to unrelated tissues. Additionally, inefficient escape of ASOs from endolysosomal compartments affects their activity since ASOs are unable to reach their intracellular RNA target in the nucleus and/or cytosol. Despites the variety of chemical modifications developed to tackle these delivery issues, it remains challenging to reach particular tissues and/or cell types outside of the liver, and there are still no non-toxic solutions to the endosomal escape problem.

To fully realize the therapeutic potential of this class of molecules, it is crucial to understand the mechanisms underlying how ASOs enter cells and exit the endosomal space. Therefore, this thesis focuses on the use of nanoscale secondary ion mass spectrometry (NanoSIMS), in combination with electron microscopy, to investigate the subcellular distribution and accumulation of ASOs.

It was necessary to develop a NanoSIMS method capable of absolute quantification of the intracellular exposure of ASO. Thus, external standards were developed to quantify several halogenated compounds (iodine, bromine, and fluorine) as well as a sulfur isotope (^{34}S).

Results showed that the uptake of different ASOs was saturable, but conjugation to a *N*-acetylgalactosamine targeting domain enhanced cellular uptake and improved target knockdown. NanoSIMS data also showed that upon colchicine treatment, the uptake and localization of ASOs were affected. It was also possible to quantifying both the targeting domain and ASO components of an engineered glucagon-like peptide 1-ASO conjugate. That highlighted that fine tuning of ASO chemistry can be used to affect the productive uptake of ASOs.

Overall, these findings contribute to a better understanding of the cellular delivery, uptake and trafficking mechanisms of ASOs, which is valuable for the future development of more effective oligonucleotide-based therapeutics.