When Oligonucleotide Drugs Clog the RNA Exosome: MS-Based Interactome Profiling Reveals a Novel Toxicity Mechanism

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Background: Protein interactions strongly influence the activity and toxicity of therapeutic oligonucleotides, yet their intracellular interaction networks remain difficult to resolve under pharmacologically relevant conditions. Method: We developed in situ ASO-ID (isASO-ID), a proximity biotinylation workflow in which a functionalized oligonucleotide recruits a recombinant biotin ligase after cellular fixation, without requiring genetic manipulation of the analyzed cells. Proximal proteins are biotinylated, enriched, and identified by quantitative mass spectrometry. This enables direct comparison of protein interactions with pharmacological properties such as efficacy, potency, subcellular localization, and toxicity. Results: We applied isASO-ID to long, chemically stabilized antisense oligonucleotides, including ADARrecruiting RNA-editing oligonucleotides, in an as-yet unpublished study. isASO-ID identified the catalytic RNA exosome subunits DIS3 and EXOSC10 as modification-sensitive interaction partners. Exosome binding was strongest for specific combinations of 2'-ribose modifications and phosphorothioate linkages and correlated with dose-dependent stabilization of promoter upstream transcripts, accumulation of ribosomal RNA processing intermediates, and reduced cell viability. Short oligonucleotides did not inhibit exosome function, supporting a model in which long, nucleaseresistant oligonucleotides act as non-degradable pseudo-substrates that occupy the exosome channel. Systematic variation of oligonucleotide chemistry showed that exosome interference can be substantially reduced through appropriate placement of bulky and sterically demanding 2’-ribose modifications and alternative backbone linkages while preserving efficient ADAR recruitment and RNAediting activity. Conclusion: Taken together, these results show that long, chemically stabilized oligonucleotides can inhibit the RNA exosome in a length- and modification-dependent manner, thereby perturbing RNA processing and reducing cell viability. These findings provide experimentally grounded design principles for the safer development of therapeutic oligonucleotides that preserve ADAR editing while minimizing exosome inhibition and toxicity