Optimization of Chemically Modified Oligonucleotides for Site-directed RNA Editing with Endogenous ADAR

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The development of site-directed RNA editing (SDRE) approaches allows selective recoding of RNA nucleobases. One SDRE-catalyzing enzyme is ADAR (adenosine deaminase acting on RNA), which functionally recodes adenosine (A) to inosine (I) (read as guanine (G) by the translational machinery) and is expressed endogenously in humans. Chemically modified oligonucleotides (ONs) are a promising SDRE method that directly guide endogenous ADAR to the desired RNA site without additional components. However, there are no systematic studies on the requirements necessary for efficient, short, and nuclease-resistant ONs. This study uncovered several central requirements for such oligonucleotides.

Using only the commercially available 2'-fluoro, 2'-O-methyl, 2'-H (DNA), and phosphorothioate modifications, new chemically modified ONs were designed based on the previously published RESTORE ON designs (Merkle et al., 2019). These new ONs (termed RESTORE 2.0) resisted serum and lysosomal nucleases for up to 31 days and achieved high editing yields of up to 80% on endogenous mRNA ORF regions. The RESTORE 2.0 designs also displayed two-fold higher efficiency than RESTORE 1.0 ONs and could be shortened to just 32 nt, compared to the 95 nt long RESTORE 1.0 ONs. Efficient RESTORE 2.0 ON symmetries and favored placements of specific chemical modifications also were consistent with known ADAR substrate binding patterns.

Furthermore, fully modified ONs were efficiently administered via clinically relevant delivery routes and corrected multiple disease-relevant G-to-A point mutations. This included a proof-of-concept treatment of an in vivo α-1-antitrypsin deficiency mouse model, where therapeutically relevant levels of the corrected protein were restored. To summarize, the development of the RESTORE 2.0 ON designs provides simple guidelines to further develop chemically modified SDRE ONs for therapeutical purposes.