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Liver as a target for oligonucleotide therapeutics

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Summary

Oligonucleotide-based therapeutics are an emerging class of drugs that hold the promise for silencing "un-druggable" targets, thus creating unique opportunities for innovative medicines. As opposed to gene therapy, oligonucleotides are considered to be more akin to small molecule therapeutics because they are small, completely synthetic in origin, do not integrate into the host genome, and have a defined duration of therapeutic activity after which effects recover to baseline. They offer a high degree of specificity at the genetic level, thereby reducing off-target effects. At the same time, they provide a strategy for targeting any gene in the genome, including transcripts that produce mutated proteins.

Oligonucleotide-based therapeutics include short interfering RNA (siRNA), that degrade target mRNA through RISC mediated RNAi; anti-miRs, that target miRNAs; miRNA mimics, that regulate target mRNA; antisense oligonucleotides, that may be working through RNAseH mediated mRNA decay; mRNA upregulation, by targeting long non-coding RNAs; and oligonucleotides induced alternative splicing [1]. All these approaches require some minimal degree of homology at the nucleic acid sequence level for them to be functional. The different mechanisms of action and their relevant activity are outlined in Fig. 1. Besides homology, RNA secondary structure has also been exploited in the case of ribozymes and aptamers, which act by binding to nucleic acids or proteins, respectively. While there have been many reports of gene knockdown and gene modulation in cell lines and mice with all these methods, very few have advanced to clinical stages. The main obstacle to date has been the safe and effective intracellular delivery of these compounds in higher species, including humans. Indeed, their action requires direct interaction with DNA/RNA within the target cell so even when one solves the issues of tissue and cellular access, intracellular/intranuclear location represents yet another barrier to overcome. To date, hepatic delivery of oligonucleotides has been the area with greatest progress, and thus we have focused on liver-targeted therapeutics that have shown promise at the preclinical and/or clinical level.

The liver is the largest internal organ in the body, playing a central role in metabolism, detoxification, synthesis, and secretion of major plasma proteins (carrier proteins, coagulation factors, complement components, hormones, and apolipoproteins), and iron homeostasis. It is therefore not surprising that a large number of disease targets reside in the liver where they are susceptible to modulation by oligonucleotide therapies.

Clinical-stage oligonucleotide therapies addressing liver targets

The number of oligonucleotide therapies targeting liver expressed targets in clinical trials is growing rapidly. The first trials involved antisense MOE/Gapmers; however, they have now been joined by siRNA, locked nucleic acid (LNAs), and morpholinos. MOE/Gapmers are antisense oligonucleotides with phosphorothioate backbone linkages. They have a stretch of nucleotides with deoxy sugars and the remaining nucleotides containing an O′-methyl O′-ethyl substitution at the 2′ position (MOE). siRNA are double-stranded RNA molecules that vary in length from 18 to 30 bp, for therapeutic purposes they are typically chemically modified, e.g., with 2′O-methyl nucleotides to increase stability and to limit their immunogenicity. LNAs are modified RNA nucleotides with an extra bridge connecting the 2′ oxygen and 4′ carbon in the ribose sugar that increases the melting temperature of the molecule. Morpholinos are synthetic molecules with standard nucleic acid bases bound by morphine rings instead of deoxyribose, and linked through phosphoramidite groups. They act by creating steric hindrance after binding to the target site in a molecule.

This area of metabolic disease has seen the greatest advances with multiple compounds in clinical trials in the US and/or Europe. Gene targets for hyperlipidemia, hypercholesterolemia, and diabetes have all been well validated as loss of function targets via human genetic studies. Several oligonucleotide therapeutics targeting liver expressed gene targets have shown promise in recent clinical trials (Table 1). Specifically, in the area of hypercholesterolemia, several programs are progressing through clinical trials.

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Abbreviations: ALAS1, δ-aminolevulinate synthase 1; *ANGPTL3*, angiopoietin-like 3; FVII, factor VII; FXI, factor XI; *GCGR*, glucagon receptor; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HoFH, homozygous familial hypercholesterolemia; *KSP*, kinesin spindle protein; LDL, low density lipoprotein; LNA, locked nucleic acid; miR, microRNA; MOE, 2′-O-methoxyethyl; MTP, microsomal triglyceride transfer protein; *PCSK9*, proprotein convertase subtilisin/kexin type 9; *PLK1*, Polo like kinase 1; RISC, RNA induced silencing complex; SAA, serum amyloid A; siRNA, short interfering RNA; *TMPRSS6*, transmembrane protease serine 6; *TTR*, transthyretin; UTR, untranslated region; *VEGF*, vascular endothelial growth factor.

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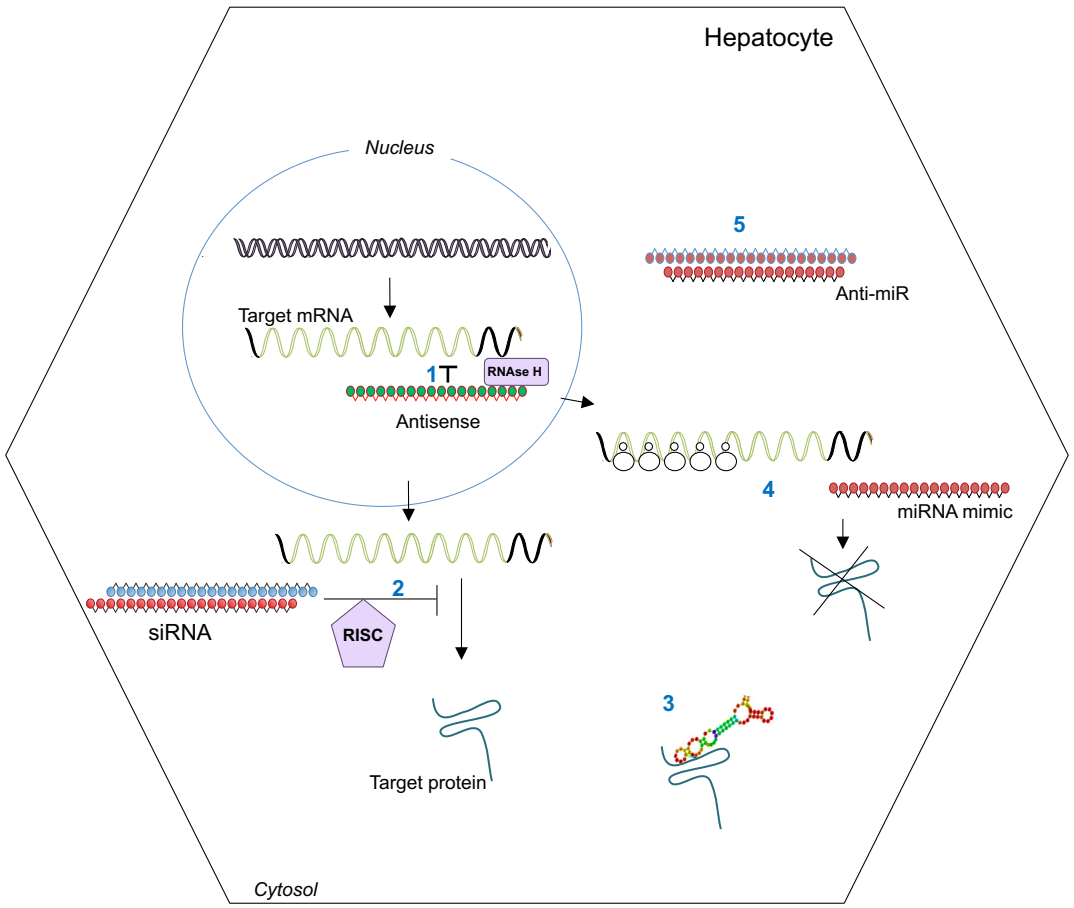

Fig. 1. Proposed mechanism of action for oligonucleotide based therapeutics. (1) RNase H mediated mRNA decay by antisense molecules in the nucleus; (2) siRNA mediated target mRNA decay exploiting the naturally occurring RNA induced silencing complex (RISC); (3) inhibitory binding of oligonucleotide based aptamer to target protein; (4) regulation of mRNA translation mediated by binding of miRNA mimics to miRNA binding sites in target mRNA; (5) anti-miR binding to miRNAs in the cytoplasm, rendering them ineffective for binding their targets.

Apolipoprotein B (ApoB) is one of the primary components of circulating atherogenic lipids. Human genetic studies have shown that mutations in ApoB, which reduce their affinity for the Low Density Lipoprotein receptor (LDLR), cause marked hypercholesterolemia. Complete loss of function in animal models, however, results in lower total and low density lipoprotein cholesterol (LDL-C). While being a genetically validated target, loss of ApoB has also been associated with on-target liabilities, such as liver steatosis [2].

Currently, the most clinically advanced oligonucleotide therapeutic against a liver gene is an antisense molecule (mipomersen) targeting ApoB (NCT01414881). In phase 3 trials, mipomersen was recently shown to lower LDL-C in heterozygous and homozygous familial hypercholesterolemia (FH) patients. In heterozygous FH, mipomersen treatment resulted in a −28% *vs.* +5% (p <0.001) reduction of LDL-C with almost half of the patients (~45%) achieving a target LDL-C of <2.6 mmol/L (100 mg/dl) [3]. Of note, however, 10.8% of the patients receiving mipomersen withdrew from treatment due to adverse effects such as injection site reactions and liver enzyme elevations, as compared to no patients in the placebo arm. Mipomersen has been approved by the FDA, but not the EMA, as a treatment to reduce LDL and total cholesterol in patients with HoFH. A phase 1 trial with an ApoB siRNA formulated in a first generation lipid nanoparticle (LNP) [4] molecule was carried out by Tekmira Pharmaceuticals; the trial was halted during dose escalation in 2009 (NCT00927459).

Proprotein convertase subtilisin kexin 9 (PCSK9) was originally identified in a human genetic study which linked it to severely elevated LDL-C. More specifically, loss-of-function mutations in PCSK9 in humans resulted in lower LDL-C levels and protection from cardiovascular disease [5]. Consistent with this observation, studies in animal models demonstrated that loss of PCSK9, a protease that physiologically downregulates the LDL receptor (LDLR), leads to an increase in LDLR levels on the hepatocyte, resulting in increased LDL-C clearance. The RNAi therapeutic ALN-PCS, targeting PCSK9, has been evaluated in a phase 1 clinical study (NCT01437059) [4,6,7]. It was safe, well-tolerated and showed dose-dependent reduction in PCSK9 and LDL-C. A similar phase 1 single ascending dose of a LNA targeting PCSK9 by ISIS/BMS was halted during dose escalation due to safety concerns [8].

Apolipoprotein C-III (ApoC3) is a component of very low density lipoprotein (VLDL), and is thought to be an active inhibitor of lipoprotein and hepatic lipases, both of which are involved in the processing and uptake of triglyceride-rich particles. Mutations in ApoC3 in humans result in lower circulating triglycerides, and a lowering risk for the development of cardiovascular disease [9]. Phase 1 studies of an antisense molecule targeting ApoC3 (ISIS Pharmaceuticals, NCT01529424) have shown promise in