Lactobionic acid and N-Acetylgalactosamine-conjugated Peptide Nucleic Acids for hepatocyte-targeted delivery

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Purpose
A neutral N-(2-aminoethyl)-glycine backbone of peptide nucleic acid (PNA) ensures enzymatic stability and optimal binding affinity to the complementary DNA or RNA. While PNAs are well-recognized for diverse therapeutic and diagnostic applications, their clinical translation is challenged by non-specific in vivo distribution and rapid elimination. Tris-N-acetylgalactosamine (Tris-GalNAc) mediated asialoglycoprotein receptors (ASGP-R) targeting in hepatocytes has expedited the clinical translation of several nucleic acid drugs. Galactose/lactobioninc acid (LBA) also recognizes the ASGP-R but is not well explored for nucleic acid delivery. We investigated the di-LBA and tris-GalNAc ligands for the liver-targeted PNA delivery for the first time.

Methods
We conducted comprehensive evaluations, including synthesizing di-valent LBA ligand, solution-phase conjugation, and in vitro and in vivo assessment of the PNA conjugates for hepatocyte delivery. As a proof of concept, we synthesized di-LBA and tris-GalNAc conjugated short (8-mer) and full-length (22-mer) anti-miR-122 PNAs targeting microRNA-122 (miR-122). In diverse experiments, we established ASGP-R-mediated liver targeting of PNA conjugates. Finally, efficacy and safety studies of full-length PNA conjugates were conducted in mice.

Results
We established a simple synthesis method for the di-LBA ligand and its conjugation with PNA in solution-phase. Di-LBA and tris-GalNAc conjugated PNAs showed ASGP-R mediated uptake in HepG2 cells, with tris-GalNAC conjugates showing superior uptake. In vivo studies showed PNA conjugates effectively targeted the hepatocytes with maximum liver distribution at ~1h. Liver retention of PNA conjugates depended on the PNA size. Full-length PNA conjugates accumulated 20 and 26-fold higher in the liver than unconjugated PNA. Mir-122, a regulator of fat metabolism, was significantly knocked down (75%) by the full-length PNA conjugates. miR-122 knockdown translated into 1.5-2-fold derepression of its downstream target genes (Aldolase A, Branched-chain ketoacid dehydrogenase kinase, and Glycogen synthase 1) and resulted in positive pharmacological effects (reduced triglycerides, cholesterol, and liver weight). Full-length PNA conjugates showed comparable liver accumulation and in vivo efficacy. In safety evaluation, PNA conjugates were found devoid of toxicity or immune response.

Conclusion
We identified di-LBA as an effective ligand for liver-targeted delivery with a simplified design. Our findings demonstrated significant development toward in vivo PNA delivery and paved the way for broader biomedical applications.

Keywords: liver delivery, lactobionic acid, peptide nucleic acids, tris-N-acetyl galactosamine, microRNA-122