Development of a Peptide-Modified siRNA Nanocomplex for Targeting Delivery to Hepatic Stellate Cells
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Purpose
Liver fibrosis is a wound healing process characterized by the accumulation of excess extracellular matrix (ECM) in the liver. It is induced by chronic liver injuries caused by nonalcoholic steatohepatitis, hepatitis, alcohol abuse, and metal poisoning and there are currently no standard treatments for liver fibrosis. The expression of ECM increases dramatically when quiescent hepatic stellate cells (HSCs) are activated to myofibroblast-like cells. Although HSCs only constitute approximately 5-8% of total liver cells, they are the major contributors for liver fibrosis and are able to cover the entire microcirculatory network of hepatic sinusoidal by contacting with sinusoidal endothelial cells and hepatocytes. Targeted delivery of antifibrotic agents to HSCs is a major challenge in liver fibrosis therapy. Therapeutic agents cannot easily reach HSCs because of the excessive accumulation of ECM, the closure of endothelial fenestrae, and the reduced flow exchange between sinusoid blood and liver cells. We recently discovered an IGF2R-specific peptide, peptide-431, using a novel combinatorial biopanning strategy. Peptide-431 and its dimeric form showed high and specific affinity to activated human and rat HSCs. In this study, dimeric peptide-431 was used as a targeting ligand of the siRNA nanocomplex, compared with other two HSC-targeted ligands, Vitamin A and Cholesterol to specifically deliver the nanocomplex to activated HSCs in fibrotic liver.

Methods
Biotin-PCBP2 siRNA and biotinylated ligands (cholesterol, Vitamin A, and IGF2R peptide) were mixed with neutravidin and protamine to form the siRNA nanocomplexes with the optimized N/P ratio, particle size and zeta-potential. Binding affinities, activity study, cellular uptake study, stability study and in vitro cytotoxicity study of the nanocomplexes containing different ligands were evaluated in rat hepatic stellate cells (HSC-T6, primary HSC) and human hepatic stellate cells (LX-2). Liver fibrosis in rats was induced by intraperitoneal injection with the mixture of carbon tetrachloride (CCl4) to perform the in-vivo Biodistribution Study.

Results
Multicomponent siRNA-neutravidin-peptide-protamine nanocomplex (SNPP), siRNA-neutravidin-cholesterol-protamine nanocomplex (SNCP), and siRNA-neutravidin-vitamin A-protamine nanocomplex (SNVP) were formulated (Figure 1). Compared to Vitamin A and cholesterol, the IGF2R-specific peptide exhibited the highest targeting effect to human LX-2 HSC, rat HSC-T6 cell line, and activated primary rat HSCs. Accordingly, the IGF2R-specific peptide coupled nanocomplex demonstrated higher silencing activity of PCBP2 and better inhibition on the migration of activated HSCs. Compared to free siRNA and the nanocomplexes coupled with Vitamin A and cholesterol, the IGF2R-specific peptide coupled nanocomplex showed the highest uptake in the liver and lowest uptake in the lung and kidney of the rats with CCl4-induced liver fibrosis (Figure 2).

Conclusion
On basis of above mentioned results, the IGF2R-specific peptide conjugated PCBP2 siRNA nanocomplex may provide a promising delivery system for liver fibrosis patients.