An Integrated Discovery Platform for Accelerating Oligonucleotide Therapeutics via Advanced Conjugate Delivery Systems

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Abstract

Oligonucleotide therapeutics represent a promising modality for treating metabolic disorders, CNS diseases, muscular dystrophies, and other conditions. However, their clinical advancement is significantly hindered by the lack of efficient and specific delivery strategies. Recent progress in conjugate-based delivery systems offers potential solutions for improving the safety and targeted delivery of oligonucleotides to various tissues and organs.

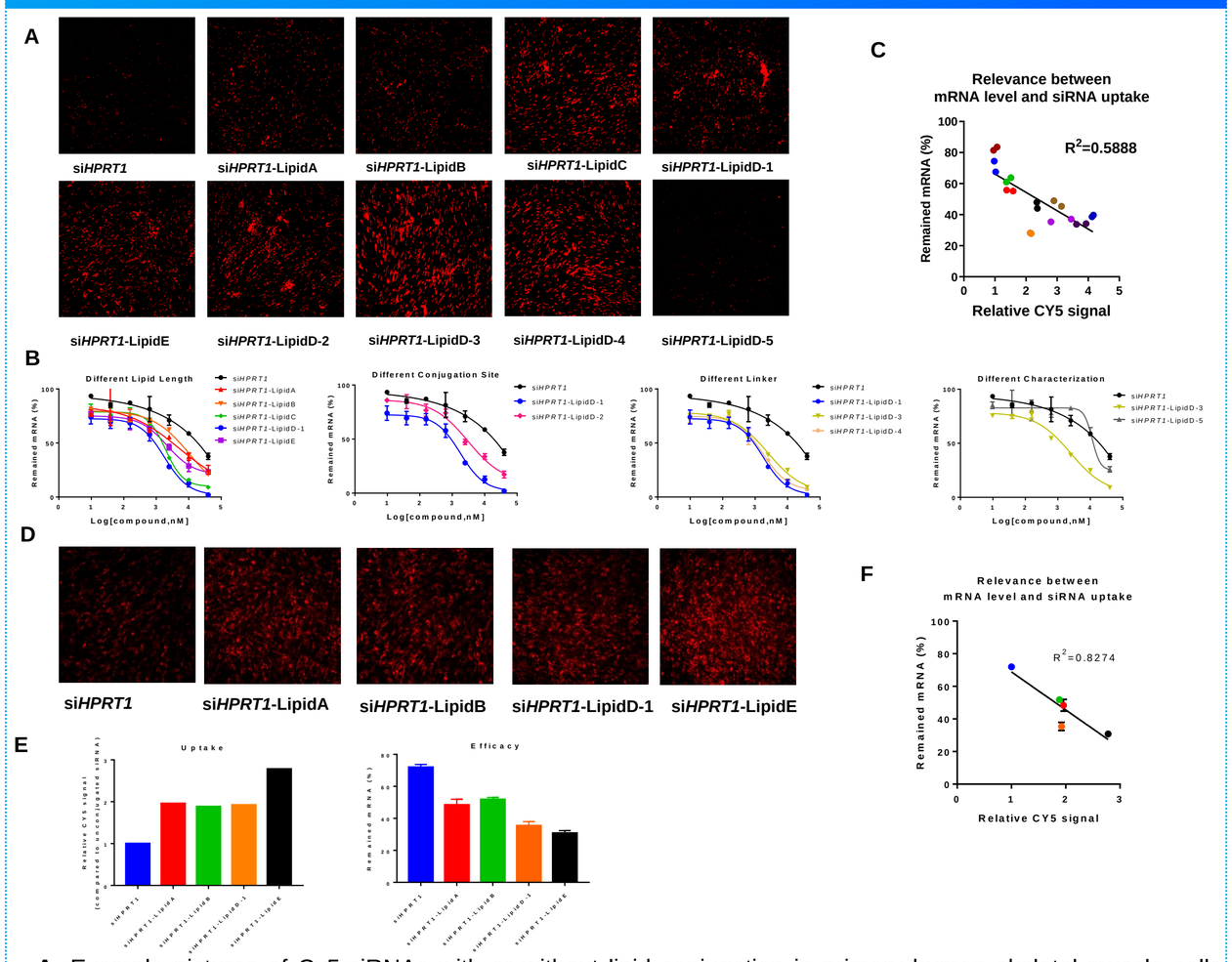
To accelerate the discovery of oligonucleotide drugs, we have established an integrated service platform. This platform encompasses oligonucleotide sequence design and optimization, in vitro screening using primary human cell models, discovery and evaluation of delivery tools, and assessment of in vivo tissue distribution and efficacy in animal models. Our peptide profiling platform also provide comprehensive in vitro testing services to evaluate drug toxicity, permeability and functionality facilitate the development and validation of peptide, using flow cytometry and Confocal image. Moreover, the peptide penetrability of blood brain barrier (BBB) was analyzed by in vitro Human or Mouse BBB transwell models. Both TfR1 target and non-target penetration efficiency were also evaluated as high efficiency (Papp＞1E-6 cm/s) in our candidate peptides. In order to mimic the morphology and function of real intestinal organ, we setup the intestinal organoids with three-dimensional (3D) structure cultured in vitro which is highly similar to that of the tissue and organ, and successfully evaluate the peptide penetrability.

For Lipid-Oligonucleotide Conjugates (LOCs), we synthesized siRNAs conjugated to structurally diverse lipids (varying chain lengths, linkers, chemical groups, and conjugation sites) and labeled with a CY5 fluorophore. Utilizing high-content imaging, we demonstrated that most LOCs exhibited significantly increased cellular uptake and target knockdown efficacy in primary human adipocytes (PHA) and human skeletal muscle cells (HSKMC) compared to unconjugated siRNA controls. Subsequent in vivo biodistribution studies in mice using IVIS imaging revealed that lipid conjugation significantly enhanced siRNA retention time in both hepatic and extra-hepatic tissues, correlating with improved target knockdown efficacy.

For Peptide-Oligonucleotide Conjugates (POCs), we utilized cells expressing high, medium, and low levels of specific peptide receptors. High-content imaging was employed to quantitatively assess POC uptake efficiency. Different cell model proved effective for evaluating POC knockdown efficacy. Critically, peptides identified through our mRNA display platform demonstrated binding affinity and knockdown efficiency comparable to positive control peptides.

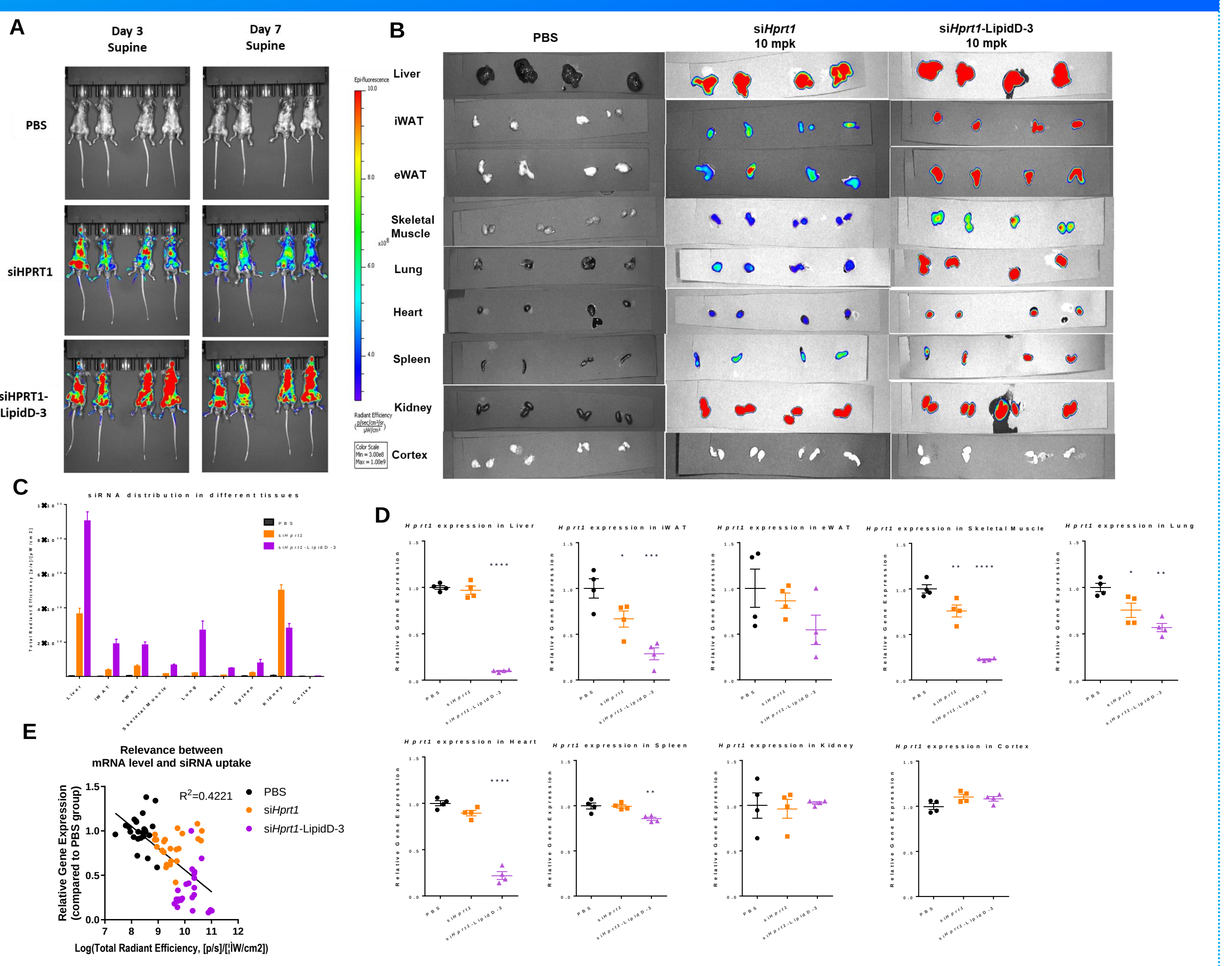
These results underscore the capability of our integrated platform to support the development of both LOC and POC delivery strategies. This platform empowers potential clients by accelerating the discovery and development of novel oligonucleotide therapeutics.

Uptake and efficacy of lipid-conjugated siRNA in cell



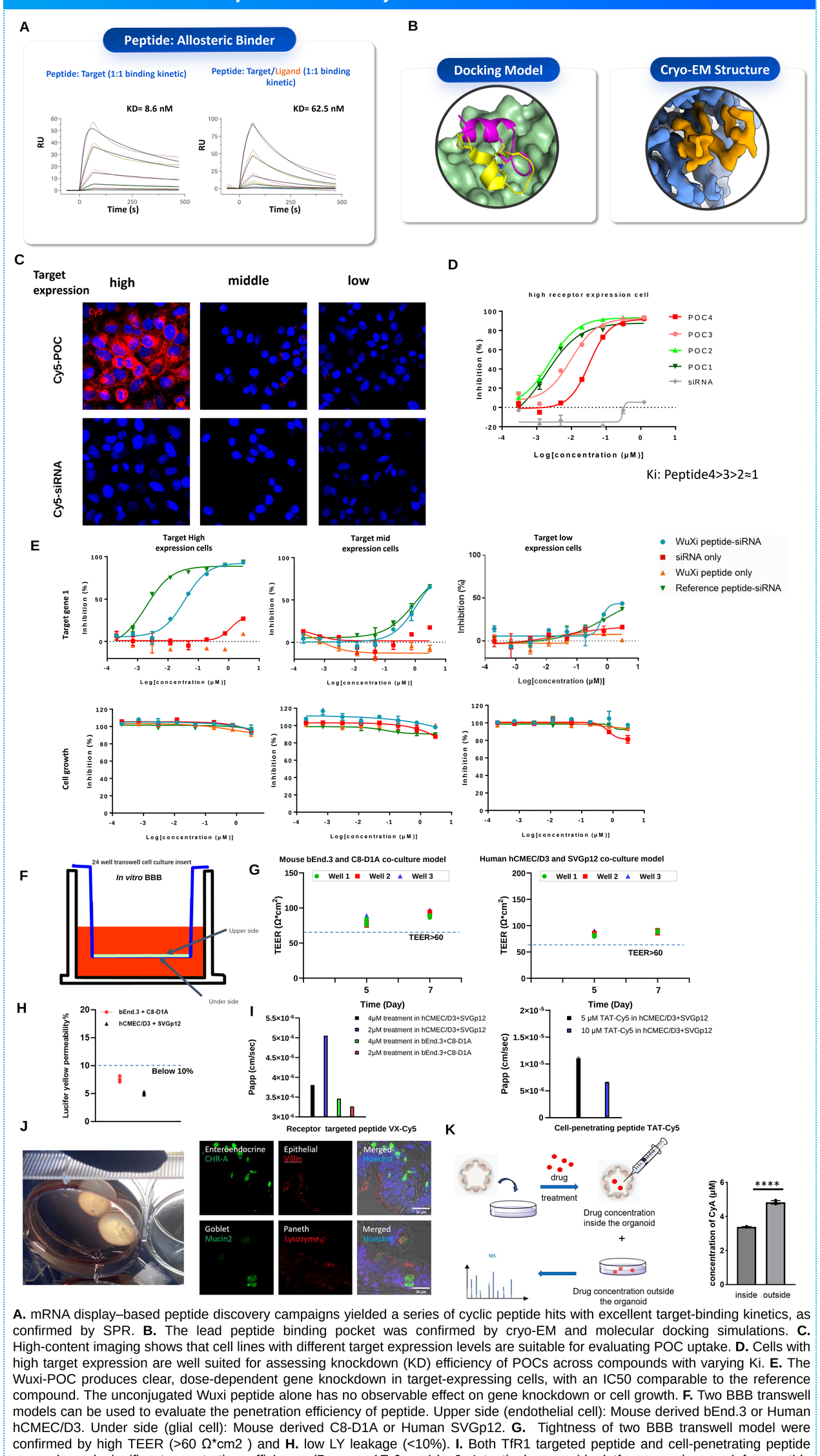
A. Example pictures of Cy5-siRNAs with or without lipid conjugation in primary human skeletal muscle cells (HSKMC). **B.** Dose-response curves of Cy5-siRNAs conjugated with different lipids in HSKMC. **C.** Negative correlation between remained target mRNA level and Cy5-siRNA signal in HSKMC. **D.** Example pictures of Cy5-siRNAs with or without lipid conjugation in the primary human adipocytes (PHA). **E.** The relative Cy5 fluorescence signal (left) and remained target mRNA level (right) in the PHA after the treatment with indicated Cy5-siRNAs. **F.** Negative correlation between remained target mRNA level and Cy5-siRNA signal in PHA.

Uptake and efficacy of lipid-conjugated siRNA in mice



A. Live imaging of mice with single s.c. injection of Cy5-siRNA by IVIS. **B.** Imaging of tissues from mice 7 days after Cy5-siRNA s.c. injection by IVIS. **C.** Relative Cy5 fluorescence signal compared to PBS-treated group**. D.** The remained target mRNA level in different tissues (PD) after s.c. injection with indicated Cy5-siRNAs. **E.** Negative correlation between remained target mRNA level and Cy5-siRNA signal in tissues (except cortex, kidney and spleen).

Peptide discovery and POC evaluation



A. mRNA display–based peptide discovery campaigns yielded a series of cyclic peptide hits with excellent target-binding kinetics, as confirmed by SPR. **B.** The lead peptide binding pocket was confirmed by cryo-EM and molecular docking simulations. **C.** High-content imaging shows that cell lines with different target expression levels are suitable for evaluating POC uptake. **D.** Cells with high target expression are well suited for assessing knockdown (KD) efficiency of POCs across compounds with varying Ki. **E.** The Wuxi-POC produces clear, dose-dependent gene knockdown in target-expressing cells, with an IC50 comparable to the reference compound. The unconjugated Wuxi peptide alone has no observable effect on gene knockdown or cell growth. **F.** Two BBB transwell models can be used to evaluate the penetration efficiency of peptide. Upper side (endothelial cell): Mouse derived bEnd.3 or Human hCMEC/D3. Under side (glial cell): Mouse derived C8-D1A or Human SVGp12. **G.** Tightness of two BBB transwell model were confirmed by high TEER (>60 Ω*cm2) and **H.** low LY leakage (<10%). **I.** Both TfR1 targeted peptide and cell-penetrating peptide were showed significant penetration efficiency (Papp > 1E-6 cm/s). **J.** Intestinal organoid platform can be used for peptide penetrability measurement. Intestinal organoid contains multiple cells indicated by the immunofluorescence staining of cell markers, which can better stimulate the physiological environment. **K.** Drug penetrability measurement through MS method. CyA is a peptide known to penetrate the intestinal barrier.

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