Aptamer-Directed Delivery of siRNA Therapeutics for Cholangiocarcinoma

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BACKGROUND

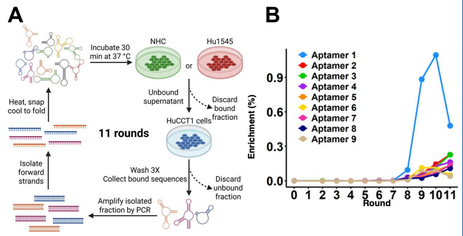
- Cholangiocarcinoma (CCA) is a highly lethal hepatobiliary malignancy with increasing incidence and poor overall survival. (1)
- 1/3 of those who undergo surgical resection experience ≥ Grade 3 or worse post-operative complications and 76% of those who undergo medical treatment experience serious/medically significant side effects. (2)
- We have previously demonstrated that lactosomes can effectively target CCA. (3)
- Aptamers are oligonucleotides with unique 3D structures that can be incorporated into lactosomes to enhance tumor specific targeting. (4)

METHODS

- A CCA-selective aptamer, “Aptamer 1”, was identified using Systematic Evolution of Ligands by Exponential Enrichment (SELEX).
- “Aptamer 1” was characterized by in vitro binding/uptake assays and in vivo biodistribution studies in SB1 tumor-bearing mice.
- Aptamer 1-functionalized lactosomes were loaded with siRNAs targeting *Yap*, *Lck*, and *Wwtr1* (siTriple), and tumor-specific delivery and gene knockdown efficiency were evaluated in vivo.

FIGURE 1.

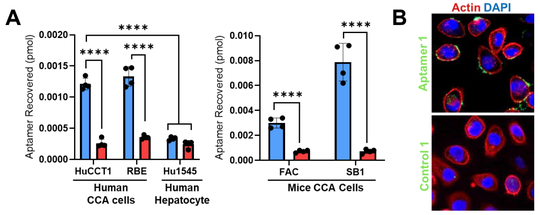
Aptamer selection strategy



(A) Schematic workflow of SELEX, employing negative selection against normal cholangiocytes (NHC) and hepatocytes (Hu1545), followed by positive selection with CCA cells (HuCCT1). **(B)** Relative enrichment (%) of top aptamer candidates identified by deep sequencing after each round.

FIGURE 2.

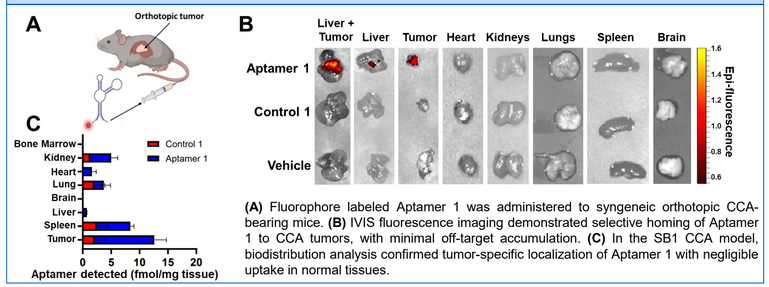
“Aptamer 1” selectively targets human and murine CCA cells in vitro.



(A) Binding analysis showed selective binding of Aptamer 1 to human and murine CCA cells, with minimal binding to Hu1545 cells, confirming tumor-selective targeting over normal hepatocytes. **(B)** Immunofluorescence imaging localized Aptamer 1 binding to the cell surface, highlighting its potential as a targeting ligand for therapeutic cargo delivery.

FIGURE 3.

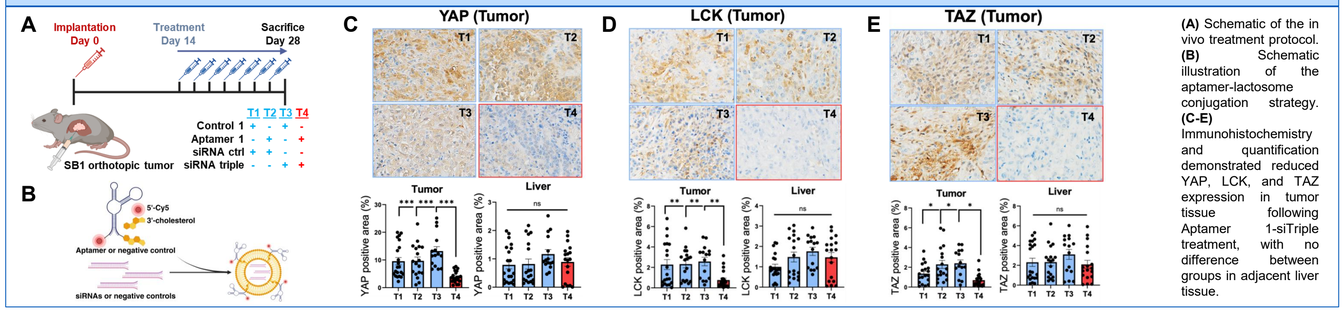
“Aptamer 1” localizes to tumor with minimal accumulation in liver in a mouse model of CCA.



(A) Fluorophore labeled Aptamer 1 was administered to syngeneic orthotopic CCA-bearing mice. **(B)** IVIS fluorescence imaging demonstrated selective homing of Aptamer 1 to CCA tumors, with minimal off-target accumulation. **(C)** In the SB1 CCA model, biodistribution analysis confirmed tumor-specific localization of Aptamer 1 with negligible uptake in normal tissues.

FIGURE 4.

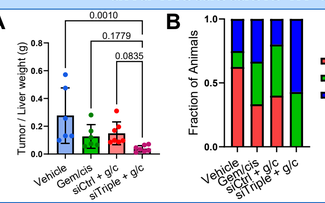
Aptamer 1- lactosomes effectively deliver siRNAs to CCA to cause target knockdown in vivo.



(A) Schematic of the in vivo treatment protocol. **(B)** Schematic illustration of the aptamer-lactosome conjugation strategy. **(C-E)** Immunohistochemistry and quantification demonstrated reduced YAP, LCK, and TAZ expression in tumor tissue following Aptamer 1-siTriple treatment, with no difference between groups in adjacent liver tissue.

FIGURE 5.

siTriple + Gem/Cis reduces tumor burden and metastasis in CCA.



(A) Aptamer 1-siTriple in combination with gemcitabine/cisplatin (g/c) significantly reduced tumor burden in mice and **(B)** reduced metastasis to the lungs.

CONCLUSION

Aptamer 1 functionalizes lactosomes for tumor-selective siRNA delivery to CCA, achieving significant target knockdown with minimal off-target uptake.

REFERENCES & FUNDING

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Lay Summary

Background

- **Current treatments for cholangiocarcinoma (CCA) cause serious side effects because they also affect healthy tissue.**
- **Aptamers are small targeting molecules that act like an “address label,” helping treatment reach tumor cells without going anywhere else.**
- **We used aptamers to guide lactosomes carrying “siRNAs”, molecules that switch off cancer-related genes.**

Results

- **We identified an aptamer that targets CCA cells while sparing healthy tissue.**
- **In mouse models, this targeted treatment reduced cancer gene activity in tumors without affecting nearby normal liver tissue.**
- **When combined with chemotherapy, aptamer guided siRNA loaded lactosomes decreased tumor size in mice and slowed the spread of disease.**

Conclusion

Aptamer-guided siRNA delivery may offer a more precise way to treat cholangiocarcinoma while reducing unintended side effects of current treatments.