Nanotherapy targeting RHAMM^B-positive pancreatic neuroendocrine tumors

Xiang Chen[1,+], Seung Koo Lee[2,+], Tiantian Zhang[1], Ching-Hsuan Tung[2,*], Yi-Chieh Nancy Du[1,*]

[1]Department of Pathology and Laboratory Medicine, [2]Molecular Imaging Innovations Institute, Department of Radiology; Weill Cornell Medicine, New York, NY 10021, USA

[+]Contributed equally, [*]Co-corresponding authors

Abstract

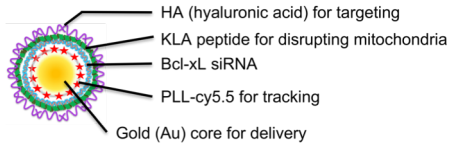
Pancreatic neuroendocrine tumors (PNETs) are the second malignancy of the pancreas. The 5-year survival rate of metastatic PNETs is only about 15%. Sadly, most patients will die of metastatic disease. Novel therapies are urgently needed. We have recently demonstrated that receptor for hyaluronic acid-mediated motility isoform B (RHAMM^B) is upregulated in PNETs and promotes metastasis of PNETs. We have also reported that Bcl-xL accelerates the formation of PNETs with invasive properties. We designed a RHAMM-targeted Combination Therapy (RCT) as a novel therapeutic for PNETs. Using a unique fabrication technology, a stepwise layer-by-layer (LbL) process, several active ingredients, including siRNA against pro-invasive/pro-survival Bcl-xL, mitochondria-fusing peptides, and RHAMM targeting ligand, were assembled into a nanoparticle for effective co-delivery and integrated efficacy.

We found that RCT was efficiently internalized by RHAMM^B-positive PNET cells, but not by RHAMM^B-negative control tumor cells. The encapsulated Bcl-xL siRNA and the mitochondria-fusing peptide were released inside RHAMM^B-positive PNET cells to induce cell death. A synergistic cell killing effect was achieved in cell culture study (> 83% cell death). In a preclinical mouse model, the systemically-injected RCT significantly reduced tumor burden (> 65% reduction) and sustained the blood glucose levels of mice bearing RHAMM^B-positive insulinomas.

In summary, RCT loaded with Bcl-xL siRNA and the mitochondria-fusing peptide could be a promising drug to treat RHAMM^B-positive PNETs. Because RHAMM^B is upregulated in many different cancer types and RHAMM protein expression is restricted in normal adult tissues, we anticipate a board application of RCT which carries multiple functional therapeutics for treating cancers that overexpress RHAMM^B.

Scheme

RHAMM-targeted combination therapy (RCT)



Results

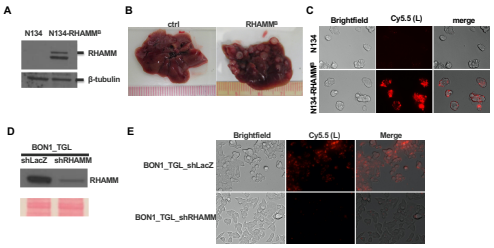


Fig. 1. RHAMM^B promotes liver metastasis of mouse PNET cells and mediates cellular uptake of RHAMM-targeted Combination Therapy (RCT). (**A**) Western blot analysis of human RHAMM in mouse N134 and N134-RHAMM^B PNET cells. (**B**) Representative liver photos from mice injected with N134 or N134-RHAMM^B cells. Cells were injected into NSG mice (n = 5) through the tail vein. Six weeks later, mice were euthanized to survey for metastatic sites and incidence. (**C**) Target-specific uptake of Au/L/HA in N134 and N134-RHAMM^B PNET cells (magnification: × 40). (**D**) Western blot analysis of human RHAMM in human BON1_TGL_shLacZ and BON1_TGL_shRHAMM PNET cells. (**E**) Target-specific uptake of Au/L/HA in BON1_TGL_shLacZ and BON1_TGL_shRHAMM PNET cells (magnification: × 40).

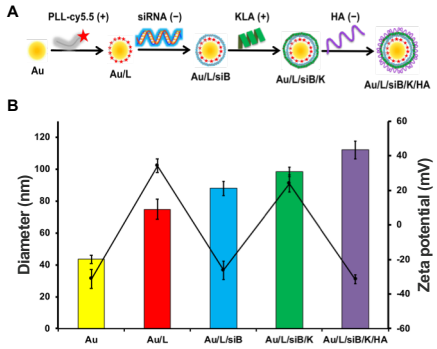


Fig. 2. Characterization of RCT. (**A**) Schematic illustrating the process of preparing multilayered RCT by electrostatic interaction. Au, gold; L, PLL-cy5.5; siB, Bcl-xL siRNA; K, KLA peptide. (**B**) The average size (color bars) and zeta potential (black line) of RCT.

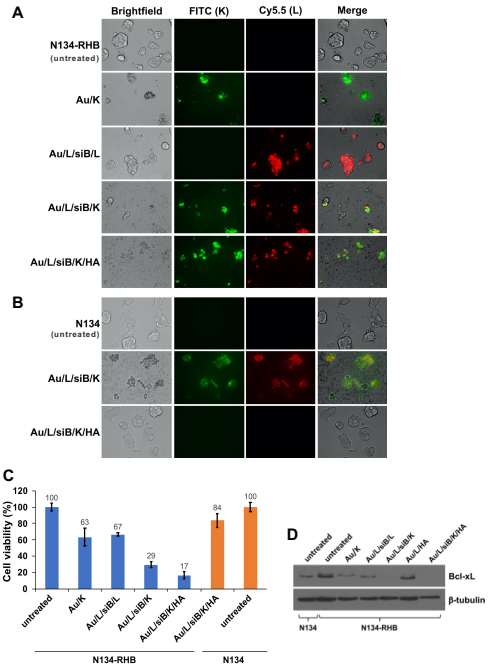


Fig. 3. In vitro functional efficacy of RCT. (**A, B**) To visualize encapsulated components on RCTs, PLL or KLA was conjugated with cy5.5 or FITC, respectively. After incubation with indicated RCTs, N134-RHAMM^B (**A**) and N134 (**B**) Cells were imaged using fluorescence microscopy (magnification: × 40). Both FITC and cy5.5 signals were observed in Au/L/siB/K or Au/L/siB/K/HA treated N134-RHAMM^B cells. However, due to the absence of HA-RHAMM binding, Au/L/siB/K/HA couldn't internalize and showed no signal in RHAMM-negative N134 cells. (**C**) Synergistic cytotoxic effect induced by RCTs in PNET cells. After 12 h incubation of various RCTs, cells were washed with PBS and cultured in complete medium for additional 48 h. The cell viability of the untreated N134-RHAMM^B or N134 cells were set as 100%. The combination of Bcl-xL siRNA and KLA inside RCT gave the best synergistic cytotoxic effect. (**D**) Gene silencing effect of RCTs in RHAMM^B positive PNET cells. Protein expression of Bcl-xL in N134 and N134-RHAMM^B cells treated with various RCTs was evaluated by Western blot. KLA: 1.6 μM, Bcl-xL siRNA: 0.12 μM.

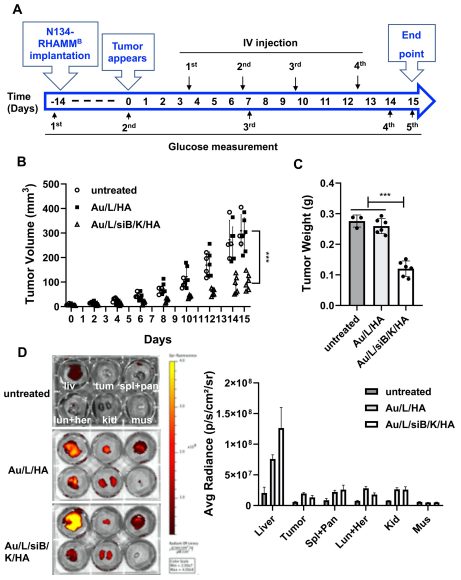


Fig. 4. In vivo therapeutic efficacy and biodistribution of RCT. (**A**) The scheme of primary tumor model. N134-RHAMM^B cells were subcutaneously injected to RIP-TVA mice. When tumors were visible (0.2 x 0.3 cm), either Au/L/HA (template particles) or Au/L/siB/K/HA (therapeutic particles) were injected via tail vein (Bcl-xL siRNA: 0.67 mg/kg, KLA: 2.84 mg/kg), twice weekly for two weeks. (**B**) Tumor size of untreated and Au/L/HA treated control groups versus Au/L/siB/K/HA group. (**C**) Tumor weight of untreated and Au/L/HA treated control groups versus Au/L/siB/K/HA group. The tumor burden in Au/L/siB/K/HA group reduced more than 65% comparing with Au/L/HA template particles group and untreated control. (**D**) Accumulation of RCTs in the tumor and major organs. After sacrifice mice, the biodistribution of RCTs was evaluated by optical imaging in IVIS Spectrum. Tumors from the treatment groups presented intense fluorescence of cy5.5 comparing with tumors from the untreated group. Liv: liver; tum: tumor; spl+pan, spleen and pancreas; lu+hrt, lung and heart; kid: kidney, mus: muscle. ***P < 0.001.

Conclusion

- We have developed biocompatible RHAMM-targeted combination therapy (RCT) as a delivery platform for siRNA combined with other active therapeutics drugs to treat RHAMM^B-positive PNETs.
- RCT, encapsulates anti-apoptotic Bcl-xL siRNA and pro-apoptotic KLA peptide, successfully enhanced the specific Bcl-xL gene knockdown and induced cell death in N134-RHAMM^B cells.
- Systemically injected RCT to RHAMM^B-expressing PNET tumor-bearing mice dramatically suppressed the tumor growth without adverse effects and minimal toxicity.
- Together, RCT is a promising therapeutic approach for cancer treatment by targeting RHAMM^B-overexpressing tumors.

Acknowledgement

We thank Anthony Lin, Jennifer Feng, Anthony Daniyan, Danny Huang, Myung Shin Han for assistance. This study was supported in part by NIH: 1R01CA204916, CA135312, STARR: I12-0043, and DOD: W81XWH-16-1-0619, W81XWH-11-1-0442.