Avidity-Driven Targeting of a Novel Biohybrid Nanoscale Carrier Engineered for High Therapeutic Payload and Extended Release of Anticancer Drugs to Treat Small Cell Lung Cancer

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Statement of Purpose: Lung cancer remains the most frequently diagnosed cancer with 1.8 million cases a year. It is also the deadliest, accounting for about a fifth of all cancer deaths worldwide [1]. Small cell lung carcinoma (SCLC) is a subtype of lung cancer, which left untreated, is rapidly fatal within two to four months [2] and with treatment, only 10% of patients survive longer than 5 years. SCLC is treated today almost exactly the same way it was 30 years ago and survival rates have not significantly improved over the last 40 years [3, 4]. We are synthesizing, characterizing, and optimizing a versatile and flexible nanocarrier with controllable loading and release properties for delivery of doxorubicin. Our objective is to investigate the cellular uptake of the carrier and drug using selected SCLC cell lines and to evaluate the efficacy of the targeted delivery system in orthotopic nude mouse models. Long term effects caused by SCLC is metastatic, spreading to other organs, it is our hope to develop novel nanocarriers that can improve or prevent these later stages of SCLC development.

Methods: Anchor DNA was bound to gold nanoparticles (AuNps) using the protocol designed by Mirkin et al. [5]. The anchor DNA chosen has been shown to have a high efficiency for binding to AuNps from previous work in our lab [6]. Using known doxorubicin DNA binding sequences, an aptamer strand was designed containing a drug binding site and a SCLC cell targeting sequence derived from Chen et al. [7]. The cell-specific aptamer will base pair with the anchor DNA bound to the AuNp (see figure 1).

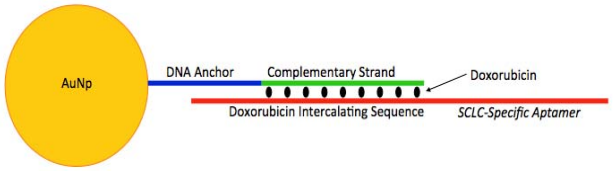

Figure 1. Schematic representation of novel nanoparticle engineered to deliver a high load of intercalating anticancer drugs to specific target cells

Our therapeutic carrier is designed for binding of intercalating agents only in the Watson-Crick base pairing region marked as "complementary DNA strand" shown above, where the double-stranded drug-binding base pairing sequences are located.

Results: We developed a versatile and flexible DNA-derivatized AuNp that can bind approximately 1,100 molecules of daunomycin [8]. "Mutating" nucleic acid strands that hold the drug can modulate its release. To increase the drug payload, we can extend the length and change the sequence of the double-stranded DNA segment of the nanocarrier. Furthermore, the nanocarrier can be programmed to bind either to one type of target or different targets by using an array of aptamers. The latter opportunity is feasible because each nanoparticle is able to accommodate at least 100 aptamer molecules for a 15-nm AuNp using optimized techniques developed in our lab. For instance, we determined that a salt concentration of 0.4 µM NaCl and a DNA concentration of 4 µM for our anchor allowed for a maximum amount of anchor binding to the AuNp [6]. Successful building of our nanoparticle platform is shown in figure 2.

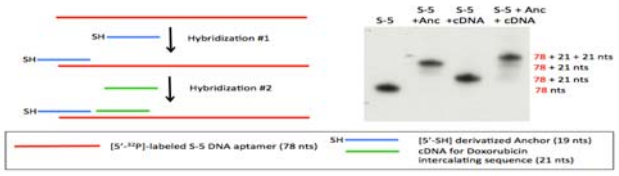

Figure 2. Validation for building of the nanoparticle platform when all components are included

We are currently studying the cellular uptake of our AuNps using transmission electron microscopy (TEM) and inductively coupled plasma mass spectroscopy (ICP-MS). For the cellular uptake test, are using the cultured cell line NCI-H69 used for selection of the SCLC-specific aptamers, in orthotopic nude mouse models containing SCLC recently developed by Isobe et al. [9].

Conclusions: The proposed research involves the first avidity-driven high payload programmable nanocarrier that can be administered either intravenously or intratracheally. Due to the design of the nanocarrier we are able to highly regulate the interactions of the drug to the binding sites and unwanted interactions between the cell-specific aptamer and the drug-binding sequences. In addition, we have been able to optimize the amount of anchor that can bind to the AuNp by adjusting the salt and DNA concentrations. The release rate and quantity of drug can be controlled and manipulated by "mutating" the base pairing in the drug-binding region and by changing the length of the sequence. Preliminary data suggests that our carrier will be able to deliver more drugs per particle than any other AuNp platform known to our knowledge. On going work includes a full analysis and testing of drug efficacy for removal of SCLC from orthotopic nude mouse models using each SCLC-specific aptamer from Chen et al. to determine the most effective aptamer.

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