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Formulating Targeted Bismuth Oxide Nanoparticles for Prostate Cancer Imaging & Therapy.

INTRODUCTION

Today, prostate cancer, PCa, is the second leading cause of cancer-related death among men in the United States, with a mortality rate that is twice as high in the African American population. Accurate staging for prostate cancer is essential in selecting appropriate treatment options. Current clinical guidelines recommend the use of bone scan and abdominal/pelvic CT scan for staging. MRI has also been routinely utilized to help identify extraprostatic disease as well as lymph node metastases. However, these imaging studies have been inaccurate and largely ineffective with reported accuracy ranging from 60 to 80 percent. A new MRI technique using lymphotropic superparamagnetic nanoparticles (Combidex®) to detect lymph node cancer is pending FDA approval. Combidex, which is currently in Phase III clinical trials, has been shown to have 90.5% sensitivity and 97.8% specificity for prostate cancer nodal disease. The limitations of Combidex relate to the relatively unstable surface coating and the lack of cancer targeting outside of lymph nodes. In contrast, a targeted molecular imaging technique, capromab pendetide (Prostascint), which targets the intracellular domain of the PSMA protein on the surface of prostate cancer cells, held great promise as a highly sensitive and specific imaging modality for prostate cancer staging. However, clinical results for Prostascint have been mixed in part because of the lack of sensitivity attainable with a radioimmunoconjugate. Furthermore, Prostascint requires cancer cell lysis to expose the intracellular domain of the PSMA further decreasing its imaging sensitivity. Another challenge lies in disease monitoring after definitive therapy. Rising serum prostate specific antigen (PSA) levels are worrisome for recurrent local or metastatic disease. Diagnosis of metastatic disease is critical as it greatly influences the choice between local salvage treatments or systemic therapy. However, identification of local recurrence is often very difficult to make based on current practice using CT or MRI, as significant anatomic changes may occur after local therapy. We believe that aptamer-based targeted imaging agents can improve prostate cancer imaging and staging. These agents will allow us to detect local and distant prostate cancer more accurately, which will help with clinical decision-making, and improve treatment outcomes.

Current mainstay of therapy for localized prostate cancer is surgical resection or radiotherapy, both of which have demonstrated good clinical outcome. In approximately 15–30% of patients after radical prostatectomy, the disease recurs, suggesting undetected disease dissemination and micro-metastasis prior to surgical resection. For disseminated prostate cancer, the treatment often involves androgen ablation or anti-androgen therapy, which takes advantage of the androgen-dependence of PCa cells for growth. Unfortunately, virtually all hormone responsive cancers become resistant to androgen ablation or anti-androgen therapy within an average of 18 months and there is no effective treatment for metastatic hormone refractory prostate cancer. Single or combination chemotherapy using cyclophosphamide, estramustine, vinblastine, doxorubicin, rapamycin, cisplatin, etoposide, docetaxel, and paclitaxel have also been tried with limited clinical efficacy. Therefore, there is an unmet medical need for novel approaches, such as targeted delivery and controlled release of drugs, to decrease the adverse effects and improve the desired effects of current chemotherapeutic modalities for relapsing or metastatic disease where current treatments are largely ineffective.

Despite the recent success of monoclonal antibodies as drugs, this class of molecule continues to exhibit important suboptimal properties. Foremost, antibodies have a hydrodynamic size of ~20 nm that can increase the size of the nanoparticles in proportion to the number of antibodies functionalized on the nanoparticles. In addition, the biological production of monoclonal antibodies can be difficult and unpredictable; and the performance of antibodies can vary from batch to batch, in particular with large-scale productions. It would be desirable to use another class of targeting molecules for the delivery of nanoparticles that like monoclonal antibodies bind with high affinity and specificity but avoid some of the problems associated with the use and production of monoclonal antibodies. Nucleic acid ligands (aptamers) are a novel class of ligands, which have the potential to rival the current antibody-based targeting approach. Aptamers have many favorable characteristics, including small size, lack of immunogenicity and ease of isolation, which together have resulted in their rapid progress into clinical practice today. These characteristics may make aptamers an ideal class of ligands for the targeted delivery of imaging and therapeutic nanoparticles and we have already begun to exploit them for these applications. The Langer Laboratory was the first group to demonstrate proof of concept for the use of aptamer for delivery of drug-encapsulated nanoparticles.

The Langer Laboratory has previously developed nanoparticle-aptamer bioconjugates using controlled release polymer systems (PLGA-PEG and PLA-PEG). Using subcutaneous xenograft mouse model of PCa, they have demonstrated that a single intratumoral administration of bioconjugates is remarkably more efficacious in tumor reduction as compared to non-targeted nanoparticles and controls. We anticipate a similar enhanced uptake of the aptamer-SPION conjugate by PCa cells, which is particularly valuable as the higher concentration of contrast material in the target lesion may translate into better lesion-tissue contrast by MRI imaging.

Langer Lab has also formulated a novel strategy for targeted delivery of doxorubicin (Dox) to cancer cells via formation of aptamer–Dox physical conjugate. Dox is known to intercalate within the DNA strand due to the presence of flat aromatic rings of this molecule. We were able to demonstrate the formation of aptamer-Dox physical conjugate, and the targeted delivery of Dox via the physical conjugate *in vitro*.

The success of this research can result in the first set of nanoscale cancer diagnostic and therapeutic agents. It will also validate aptamers as important targeting molecules in cancer imaging and therapy. Furthermore, similar strategies can also be used to generate diagnostic and therapeutic agents for other diseases. Therefore, this research has broad significance and may influence a myriad of important human disease.

NATURE OF RESEARCH

The central aim of this project is to develop novel targeted nanoparticles for combined prostate cancer (PCa) imaging and therapeutic applications. Our research has three specific aims: 1) development of aptamer-bismuth oxide nanoparticle bioconjugates targeted against prostate cancer, 2) *in vitro* and *in vivo* evaluation of aptamer-bismuth oxide nanoparticle bioconjugates in prostate cancer imaging, 3) *in vivo* evaluation of Doxorubicin-aptamer-bismuth oxide nanoparticle conjugates as targeted therapy.

SPECIFIC AIM 1:

To examine and demonstrate the feasibility of our approach, we propose to formulate novel targeted bismuth oxide nanoparticles as CT contrast agents for prostate cancer imaging and therapy. We aim to generate nanoparticles (~20 nm) with decreased immunogenicity, improved pharmacokinetics, favorable toxicity profiles, and enhanced tissue targeting and retention. To achieve our aim, we will first modify the nanoparticle surface with polyethylene glycol (PEG) to form a polymeric monolayer. The PEG molecules will have a carboxyl group terminal end, which is available for further reaction. We will then conjugate the 2'-F modified nuclease stable RNA aptamer that targets the extracellular domain of the prostate specific membrane antigen (PSMA) protein on PCa cells to the PEG through the formation of a stable amide bond. The resulting bioconjugates will be able to target prostate cancer cells specifically and are detectable by CT. Furthermore, they are capable of delivering doxorubicin to prostate cancer cells.

SPECIFIC AIM 2:

The broad goal of this aim is to evaluate the efficacy of the aptamer-bismuth bioconjugates in their capacity to detect PCa by CT. We will first optimize the aptamer-bismuth oxide bioconjugates for the ability to escape macrophage clearance after systemic administration while being able to be differentially taken up by PCa cells. We will then evaluate the aptamer-bismuth oxide conjugates as a targeted CT contrast agent *in vitro* and *in vivo*.

SPECIFIC AIM 3:

Based on our preliminary data that Dox and aptamer can form physical conjugates, we hypothesized that a formulation of Dox-aptamer-bismuth oxide conjugates may result in novel combination targeted imaging and therapeutic modality. We will first confirm the specific binding of Dox-aptamer-bismuth oxide to prostate cancer cells *in vitro*. We will then evaluate the Dox-aptamer-bismuth oxide conjugates *in vivo* for their 1) specificity in targeting tumor and 2) efficacy against tumor growth in murine xenograft models of PCa.

PERSONAL STATEMENT

My long-term career goal is to become an academic bioengineer. I plan to conduct research that is clinically relevant, and may one day influence clinical care. To prepare myself for this goal, I plan to enroll in a graduate program in chemical/biologic engineering upon finishing my undergraduate studies. This project will allow me to gain valuable research experience. I will learn the essential skills to conduct academic research: experimental design, trouble shooting, manuscript and grant writing, and independent thinking. Dr. Wang will provide me the mentorship for me to succeed. I believe that this program will provide me the necessary support to achieve my goals.