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Targeting prostate cancer cells with PSMA inhibitor-guided gold nanoparticles

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ABSTRACT

Prostate-specific membrane antigen (PSMA) is a notable biomarker for diagnostic and therapeutic applications in prostate cancer. Gold nanoparticles (AuNPs) provide an attractive nanomaterial platform for combining a variety of targeting, imaging, and cytotoxic agents into a unified device for biomedical research. In this study, we present the generation and evaluation of the first AuNP system functionalized with a small molecule phosphoramidate peptidomimetic inhibitor for the targeted delivery to PSMA-expressing prostate cancer cells. The general approach involved the conjugation of streptavidin-coated AuNPs with a biotin-linked PSMA inhibitor (CTT54) to generate PSMA-targeted AuNPs. In vitro evaluations of these targeted AuNPs were conducted to determine PSMA-mediated and time-dependent binding to PSMA-positive LNCaP cells. The PSMA-targeted AuNPs exhibited significantly higher and selective binding to LNCaP cells compared to control non-targeted AuNPs, thus demonstrating the feasibility of this approach.

Prostate cancer is the second most common cancer diagnosed in men globally[1] and remains the second leading cause of cancer mortality in men in the United States.[2] Early stage primary prostate tumors are often successfully treated through standard techniques (e.g., radical prostatectomy, radiation, anti-androgen therapy). However, advanced stage and metastatic prostate cancer generally have poorer treatment prognoses, emphasizing a critical need to develop new techniques to improve patient outcomes. Prostate-specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II (GCPII), is a classic type-II membrane glycoprotein and possesses ideal characteristics as an enzyme-biomarker target due to its unique expression in primary and metastatic prostate cancer cells[3–6] and its proclivity to internalize upon binding targeting ligands.[7–9] Of the chemical scaffolds used for targeting PSMA in prostate cancer research,[10–15] our group developed phosphoramidate peptidomimetic inhibitors of PSMA to deliver an array of imaging[8,16–20] and therapeutic[21–23] agents to prostate cancer cells in vitro and in vivo. Of these, the CTT54 inhibitor core is particularly efficacious as a PSMA targeting molecule due to its high affinity (14 nM), pseudo-irreversible mode of binding,[8,18] rapid uptake, and internalization in PSMA-positive (PSMA+) prostate cancer cells.[8,17,19,20,24]

Nanoparticles represent an emerging technology in medicinal applications due to their unique pharmacokinetic properties, amenability for multi-functionalization, and high loading capacities. Because of these features, nanoparticles are attractive platforms for the development of multimodal theranostic agents.[25,26] Gold nanoparticles (AuNPs) in particular possess distinct and controllable physicochemical properties which offer advantages over other nanoparticle platforms. The gold core is biocompatible and has been directly utilized in imaging (e.g., optical contrast[27] and computed tomography[28]) and therapeutic (e.g., radiotherapy,[29] photothermal ablation,[30,31] mechanical disruption[32]) applications. Additionally, the gold surface can be modified by soft donors (e.g., thiols) tethered to reporting, therapeutic, targeting, or biological stabilizing molecules to generate multifunctional devices for in vitro and in vivo use.[33–36]

The combination of a nanoparticle platform with targeting ligands for tumor cell-surface biomarkers is a promising architecture for achieving selective delivery and uptake into target cells. With respect to PSMA targeting, several types of nanoparticles have been outfitted with various types targeting agents (e.g., antibodies, aptamers, urea inhibitors) demonstrating the utility of this biomarker for in vitro and in vivo prostate cancer applications.[27,31,37–44] Although the PSMA-targeted delivery of AuNPs has been pioneered using anti-PSMA aptamers and antibodies,[27,31,39] the employment of AuNPs with small molecule inhibitors of PSMA has not previously been reported. Employment of

Abbreviations: AuNPs, gold nanoparticles; GCPII, glutamate carboxypeptidase II; ICP-OES, inductively coupled plasma optical emission spectroscopy; PEG, polyethylene glycol; PSMA, prostate-specific membrane antigen; PSMA−, PSMA-negative; PSMA+, PSMA-positive; TEM, transmission electron microscopy; % ID, percentage of the injected dose.

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small molecules may offer several advantages over larger platforms in generating targeted AuNPs including low immunogenicity and reduced scale-up costs. Compared to antibodies,[45] small molecules may be conjugated to nanoparticle surfaces in controllable orientations which do not compromise affinity for the biochemical target. Furthermore, antibodies bound to surface antigens present a barrier for subsequent binding of antibodies at neighboring surface antigens[46,47] and may partially limit the effectiveness of multifunctionlized antibody-targeted nanoparticle platforms. The focus of this study was to explore the feasibility of using a small molecule phosphoramidate peptidomimetic PSMA inhibitor for mediating the delivery of AuNPs to prostate cancer cells. The 1st-generation PSMA-targeted AuNP platform developed for this work employed facile biotin-streptavidin coupling[48–50] to functionalize the nanoparticles.[51–57] We have recently used a biotinylated PSMA inhibitor (CTT54) to promote the PSMA-mediated delivery of other macromolecular conjugates (e.g., streptavidin tetramers[24] and streptavidin-coated magnetic beads[58]) to PSMA+ LNCaP cells. As an extension of this previous work to a nanoparticle system, streptavidin-coated AuNPs were outfitted with biotinylated-CTT54 in the current study (Fig. 1).

The synthesis of the PSMA-targeted AuNP platform was achieved by incubating the biotinylated PSMA inhibitor, biotin-PEG_{12}-CTT54,[24] with commercially available 5 nm AuNPs coated with streptavidin (AuNP-streptavidin; Fig. 1). Following centrifugal filtration to remove excess biotin-PEG_{12}-CTT54, the PSMA-targeted nanoparticles (AuNP-streptavidin:biotin-PEG_{12}-CTT54) were re-suspended and characterized by transmission electron microscopy (TEM). TEM analysis showed monodisperse particles prior to and following conjugation (Fig. S1), indicating the particles remained stable and free from aggregation during preparation.

In vitro assessment of PSMA inhibitor-mediated binding was conducted by incubating the AuNP-streptavidin:biotin-PEG_{12}-CTT54 with PSMA+ LNCaP cells and PSMA-negative (PSMA−) PC3 cells at 37 °C for 1 h followed by removal of excess nanoparticles by washing and centrifugation. The resultant pelleted cells were lysed with aqua regia to dissolve the AuNPs followed by removal of cellular debris by centrifugation. The resulting supernatant was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) to quantify the gold concentration bound to the cells as the percentage of the injected dose (% ID) initially added to the cells (procedural details available in the Supplementary data). To test for non-specific cellular interactions inherent to the AuNP, non-targeted AuNP-streptavidin nanoparticles were also examined under the same conditions. The PSMA-targeted nanoparticles exhibited significantly greater binding to LNCaP cells compared to non-targeted AuNP-streptavidin nanoparticles after incubation at 37 °C for 1 h (Fig. 2). These results supported the concept that small molecule inhibitors of PSMA could mediate the enhanced delivery of AuNPs to prostate cancer cells. In PC3 cells, both AuNP-streptavidin:biotin-PEG_{12}-CTT54 and non-targeted AuNP-streptavidin showed significantly lower levels of binding compared to AuNP-streptavidin:biotin-PEG_{12}-CTT54 to LNCaP cells. These findings suggested that the enhanced delivery of the inhibitor-targeted AuNPs observed in the LNCaP cells was due to inhibitor-mediated PSMA binding rather than to non-specific cell interactions. Further confirmation of the PSMA-specific binding of the PSMA-targeted AuNPs to LNCaP cells was provided by performing a competitive blocking study in which LNCaP cells were saturated first with free CTT54 prior to addition of AuNP-streptavidin:biotin-PEG_{12}-CTT54 to the cells. As expected, the targeted binding of AuNP-streptavidin:biotin-PEG_{12}-CTT54 to LNCaP cells was significantly reduced when first blocked by the unconjugated PSMA inhibitor CTT54 and was similar to that observed for non-targeted AuNPs (Fig. 2).

Based on the selective binding observed for the PSMA-targeted AuNPs in PSMA+ cells, the time-dependent delivery of both targeted and non-targeted AuNPs was examined over 2 h in LNCaP cells. Analysis at three time points (0.5, 1, and 2 h) confirmed an observable increase in the percent of AuNP-streptavidin:biotin-PEG_{12}-CTT54 bound to the cells with the highest uptake at 2 h (Fig. 3). This increased PSMA inhibitor-mediated binding to LNCaP

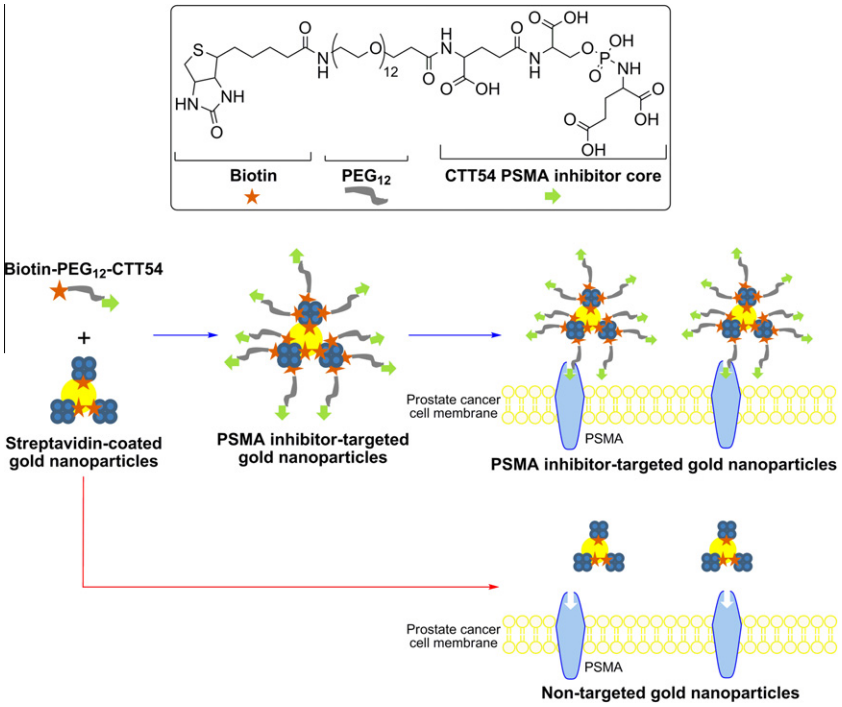

Figure 1. General scheme showing the structure of the biotin-PEG_{12}-CTT54 inhibitor, AuNP functionalization strategy, and PSMA-mediated binding of targeted AuNPs to prostate cancer cells.

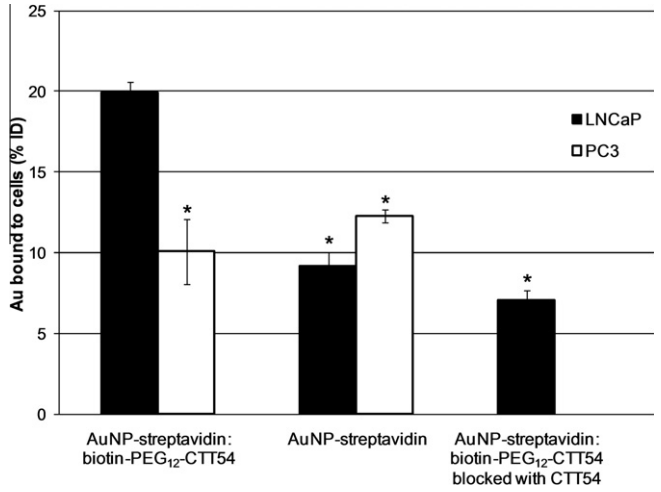

Figure 2. Quantification of AuNP bound to LNCaP and PC3 cells in vitro. Cells were incubated at 37 °C for 1 h with 4.0 nM targeted AuNP-streptavidin:biotin-PEG_{12}-CTT54, 6.6 nM non-targeted AuNP-streptavidin, or 4.0 nM AuNP-streptavidin:biotin-PEG_{12}-CTT54 blocked with CTT54. The total amount of AuNP bound to the cells was quantified by ICP-OES and expressed as the percentage of the injected dose (% ID). Values are the averages of one to two individual experiments (two to three replicate samples per experiment) with the standard deviations represented by error bars. *Indicates a significant difference (P <0.05) compared to AuNP-streptavidin:biotin-PEG_{12}-CTT54 in LNCaP cells.

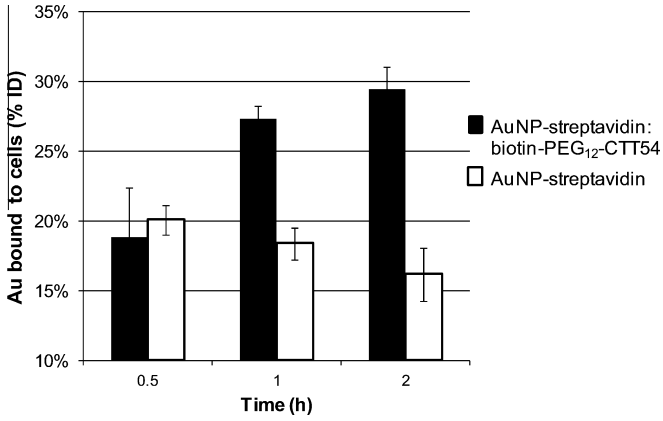

Figure 3. Time-dependent binding of AuNPs to LNCaP cells in vitro. Cells were incubated at 37 °C for 0.5–2 h with 4.7 nM targeted AuNP-streptavidin:biotin-PEG_{12}-CTT54 or 7.0 nM non-targeted AuNP-streptavidin. The total amount of AuNP bound to the cells was quantified by ICP-OES and expressed as the % ID (percentage of the injected dose). Values are the averages of 3–4 replicates with standard deviations represented by error bars. The difference in binding for the targeted and non-targeted AuNPs was significant at 1 h and 2 h (P <0.05).

cells is consistent with the trend observed previously with other CTT54 conjugates.[19,20,24] In contrast, the percent of non-targeted AuNP-streptavidin bound to the cells gradually decreased over 2 h. A separate experiment demonstrated that residual biotin in LNCaP cells was not responsible for the levels of cell binding observed for the non-targeted AuNPs. AuNP-streptavidin nanoparticles which had been incubated with excess biotin (AuNP-streptavidin:biotin) showed similar levels of binding to LNCaP cells as the initial AuNP-streptavidin nanoparticles (Fig. S2). These observations suggest that non-targeted AuNPs may exhibit weak and non-specific interactions with cells that may be transient and decrease over time. Overall, our results support the use of small molecule PSMA inhibitors to effectively deliver AuNPs to PSMA+ prostate cancer cells.

At present, the only reports of PSMA-mediated delivery of AuNPs to LNCaP cells have employed aptamers[27,39] or combinations of aptamers and antibodies[31] as targeting ligands. The relative ratio of PSMA inhibitor-targeted AuNPs to non-targeted AuNPs bound to LNCaP cells observed in this study is consistent with the relative ratio observed previously between PSMA aptamer-targeted AuNPs and non-targeted AuNPs when the total amount of gold delivered to the cells was quantified.[39] However, the inhibitor based PSMA-targeting of AuNPs in this study was achieved through small molecules nearly 1/100th the molecular mass of antibodies. These small molecules with their unique binding properties provide considerable advantages in terms of atom economy and scale-up potential, and thus represent a unique motif for targeting AuNPs to PSMA+ prostate cancer.

In summary, the results herein demonstrate for the first time that AuNPs can be functionalized to selectively target the prostate cancer tumor biomarker PSMA through the deployment of small molecule phosphoramidate peptidomimetic inhibitors. The in vitro results illustrate the significant and specific delivery of PSMA-targeted AuNPs over non-targeted AuNPs to PSMA+ LNCaP cells. These encouraging observations provide the basis for further exploration of CTT54-functionalized AuNPs for PSMA-mediated delivery of imaging and therapeutic combinations.

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Supplementary data

Supplementary data (experimental details for AuNP functionalization, TEM analysis, in vitro cell assays, and ICP-OES quantification) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.015.

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