Gold Nano-Probes as Targeted CT Contrast Agents for In-vivo cancer Imaging

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ABSTRACT

This paper describes a new class of immuno-targeted and cancer specific CT contrast agents, which enable CT imaging of cancer at cellular and molecular resolution. The method is based on the development of gold nano-probes that specifically and sensitively target *in vivo* tumor specific antigens, while inducing distinct contrast in CT imaging (increased x-ray attenuation). The overall goal of this research is to develop a novel cancer imaging tool, which may lead to improvements in therapy due to early detection, accurate staging and micro-tumor identification.

Keywords: CT contrast agents, in-vivo cancer imaging, gold nanoparticles.

1. INTRODUCTION

Cancer treatment and potential cure strongly depend on early detection, accurate staging, radiation planning and the ability to evaluate treatment efficiency. Current imaging modalities that perform these tests, such as CT, MRI, and US, can be categorized as structural imaging modalities; structural imaging refers to the ability to identify morphologic features and anatomical patterns of normal tissues and organs, and of malignant lesions within these structures. Although structural imaging modalities play a critical role in cancer diagnosis and treatment, structural imaging modalities *alone* are incapable in diagnosing tumors and metastases that are smaller than 0.5 cm, and they can hardly distinguish between benign and cancerous tumor. Additionally, they cannot provide the clinician or researcher with molecular information that is necessary to fully characterize or monitor cancer or the risk for cancer.

Molecular imaging is an emerging field that strives to image and characterize biological components and processes at the molecular and cellular level, even prior to anatomic changes. Various molecular imaging techniques are currently in use, mainly for research applications. In clinical use the existing molecular imaging techniques include; diffuse optical tomography (DOT), single-photon-emission computed tomography (SPECT), and positron emission tomography (PET) [1]. Most of the existing contrast imaging probes that are being utilized in molecular imaging are nonspecific to distinct cells or diseases. They are only specific to molecules that are associated with general metabolism processes and biological functions.

CT is one of the most useful diagnostic tools in hospitals today in terms of availability, efficiency and cost. Currently, CT is not a molecular imaging modality, since suitably targeted and molecularly specific contrast agents have not yet been developed. Present CT contrast agents are predominantly based on iodine based molecules, which are effective in absorbing X-rays; however, they are nonspecifically targeted since they cannot be conjugated to most biological components or cancer markers, and they allow only very short imaging times due to rapid clearance by the kidneys.

Gold nano-particles have unique physical and chemical properties, which make them an ideal candidate for CT contrast agents. The CT ability to distinguish between different tissues is based on the fact that different tissues provide different degrees of X-ray attenuation, while the attenuation coefficient is determined by the atomic number and electron density of the tissue; the higher the atomic number and electron density, the higher the attenuation coefficient. The atomic number and electron density (79 and 19.32 g/cm^3, respectively) of gold are much higher than currently used iodine (53 and 4.9 g/cm^3). In addition, gold nano-particles provide long circulation times, nontoxicity and biocompatibility in-vivo [2, 3], and can be readily conjugated with bio-molecules for targeting applications [4]. Recently, Hainfeld et al [5] have demonstrated the feasibility of gold nanoparticles as non-targeted CT contrast agents

for in-vivo imaging. Hybrid nanoparticles such as antibiofouling polymer-coated gold nanoparticles [6], polymer-coated Bi_2S_3 nanoparticles [7], and PEG coated nanoparticles [8] have been developed as blood-pool CT contrast agents.

Head and neck cancer is the fifth most common cancer worldwide. In the United States, annually, an estimated 45,660 Americans develop head and neck cancer and 11,210 die from this disease [9]. One of the major diagnosis challenges in head and neck cancer today is a reliable detection of involved lymph nodes, since their status is one of the most important prognosis predictors, and is also pivotal for appropriate treatment. However, assessment of lymph nodes based on structural imaging features is limited in sensitivity and specificity and fails to distinguish between non-neoplastic and malignant processes. These limitations lead to the routine performance of prophylactic procedures such as extensive neck dissection and radiation, and, on the other hand, a lack of treatment for undiagnosed small metastasis. Hence, the development of more sensitive in-vivo imaging techniques is of major importance, and could substantially improve head and neck cancer treatment and potential cure.

Squamous cell carcinoma (SCC) of the head and neck is presently the 6th most common cancer world wide, with dismal 5 year survival rates that range from 14-40%, depending on tumor site, stage, and treatment [9, 10]. An important variable for delivery of the nanoparticles to the tumor is to use a marker that is capable of targeting. It has been demonstrated [11, 12] that the overwhelming majority of SCC tumors are positive for A9 antigen, that this antigen is frequently overexpressed, and that there is a strong correlation between A9 expression and metastatic behavior [11]. It has also been demonstrated that the UM-A9 antibody can home onto SCC tumors in vivo [13].

2. EXPERIMENTAL

Gold nanoparticles in a nanorod shape were prepared using seed mediated growth method [14]. Particles dimensions were measured using transmission electron microscopy and found to be 45x15 nm and the gold concentration was 2.5 mg/mL.

Antibody conjugation: The bio-conjugation of the AuNP to the UM-A9 antibody was achieved according to the method described in reference [15]; briefly, a layer of polyacrylic acid (PAA) was absorbed on the surface of the AuNP to provide COOH functional groups, which undergo an amidation reaction with the -NH_2 group in the IgG2a antibodies to yield the conjugation. Then, the nanoparticles solution was mixed with a UM-A9 antibody solution (20μg AB: 1 mL nanoparticles) and allowed to interact for 30 min at room temperature. The solution was centrifuged twice at 5000 rpm for 2 h to wash unbound antibodies.

SCC cell culture and gold nanoparticle conjugation: One milliliter of UM-SCC-1 cell suspension (4.2 x 106 cells/mL) was mixed with 1mL of the A9 antibody-coated gold nanoparticle solution, and allowed to interact for 90 min at room temperature. Then, the solution was 3 times centrifuged at 1000 rpm for 5 min, to wash unbound nanoparticles-antibody complex; after each centrifuge the SCC-nanoparticles pellet was redispersed in PBS solution (1 mL total volume).

Control experiments were performed by introducing to the same SCC cells gold nanoparticles that were coated with non-matching antibodies (control 1); introducing the same gold nanoparticles that were coated with the UM-A9 antibody to non-cancerous cells (control 2); SCC cancer cells in a suspension (control 3), a solution of gold nanoparticles (control 4) and water.

3. RESULTS AND DISCUSSION

CT imaging were performed to all samples (experiment and control). The attenuation values in Hounsfield units (HU) are shown in figure 1.

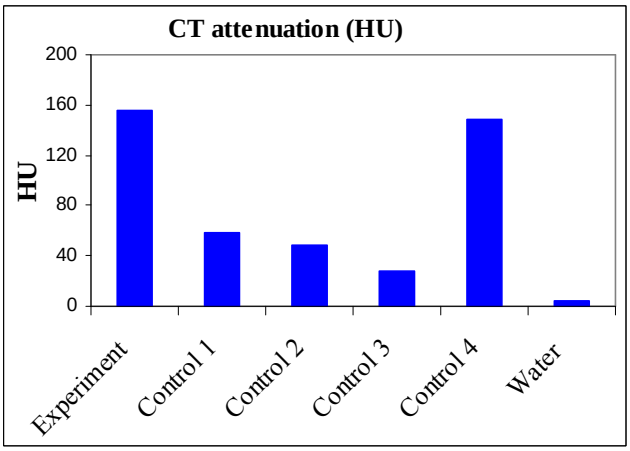

Figure 1: CT attenuation values

As shown in figure 1, the attenuation coefficient of the SCC head and neck cancer cells that were targeted with the UM-A9 antibody coated gold nanoparticles is more then 5 times higher than the untargeted SCC cancer cells (28 HU Vr. 156 HU). This demonstrates that the gold nanoparticles were attached to the cancer cells with high density, which yields a distinguishable CT attenuation number that is not typical to cells or tissue, making the targeted cells distinct and easy to

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diagnose. The attenuation values observed for the control samples revealed some non-specific binding (56 HU for control 1 and 48 HU for control 2). The attenuation values of the water (4HU) and of the gold nanoparticles solution (148 HU) were as expected.

These proof of principle preliminary results showed that the immuno-targeted gold nanorods can provide a significant contrast enhancement for CT imaging. Further research and successful optimization of this molecular imaging modality has the potential to lead to solutions for the combined challenge of cancer research, diagnosis, treatment design, and therapy guidance.

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