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Prostate tumor therapy advances in nuclear medicine: green nanotechnology toward the design of tumor specific radioactive gold nanoparticles

K. V. Katti[1] · M. Khoobchandani[2] · V. C. Thipe[2] · A. Y. Al-Yasiri[3] · K. K. Katti[2] · S. K. Loyalka[3] · T. M. Sakr[4] · A. B. Lugão[5]

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Abstract

We report herein an innovative approach to prostate tumor therapy using tumor specific radioactive gold nanoparticles (^{198}Au) functionalized with Mangiferin (MGF). Production and full characterization of MGF-^{198}AuNPs are described. In vivo therapeutic efficacy of MGF-^{198}AuNPs, through intratumoral delivery, in SCID mice bearing prostate tumor xenografts are described. Singular doses of the nano-radiopharmaceutical (MGF-^{198}AuNPs) resulted in over 85% reduction of tumor volume as compared to untreated control groups. The excellent anti-tumor efficacy of MGF-^{198}AuNPs are attributed to the retention of over 90% of the injected dose within tumors for long periods of time. The retention of MGF-^{198}AuNPs is also rationalized in terms of the higher tumor metabolism of glucose which is present in the xanthanoid functionality of MGF. Limited/no lymphatic drainage of MGF-^{198}AuNPs to various non-target organs is an attractive feature presenting realistic scope for the clinical translation of MGF-^{198}AuNPs in for treating prostate cancers in human patients. The comparative analysis of MGF-^{198}AuNPs with other radioactive gold nanoparticles, functionalized either with epigallocatechin gallate or the Gum Arabic, has revealed significantly superior tumoricidal characteristics of MGF-^{198}AuNPs, thus corroborating the importance of the tumor-avid glucose motif of MGF. Oncological implications of MGF-^{198}AuNPs as a new therapeutic agent for treating prostate and various solid tumors are presented.

Keywords Prostate tumor · Nuclear medicine · Green nanotechnology · Radioactive gold nanoparticles

Introduction

Radiation Therapy of Solid Tumors: When patients present limited clinical options for surgical resection of solid tumors or in instances when solid tumors are deemed inoperable, treatment through singular or multimodality radiations are used [1, 2]. For example, ionizing radiation transfers energy to tumor cells/tissues ejecting electrons from tumor tissue and cellular components including DNA resulting in structural breaks to stop replications through cell cycle. Ionizing radiations present additional advantages due to their capability to interact with water to produce reactive hydroxyl radicals which in close proximity to DNA cause extensive apoptosis thus rendering efficient therapy [3, 4]. Ionizing radiations, through external beam radiation therapy, is delivered to tumor patients from a distance of 80–100 cm from the body [2, 5]. Generally electromagnetic x-rays of appropriate energy and gamma rays are used in this modality. Another approach to radiation therapy uses particles beam radiation which consists of electrons, protons, alpha particles

✉ K. V. Katti
kattik@health.missouri.edu

1 Department of Radiology, Physics, Institute of Green Nanotechnology, University of Missouri Research Reactor (MURR), University of Missouri, One Hospital Drive, Columbia, MO 65212, USA

2 Department of Radiology, Institute of Green Nanotechnology, University of Missouri, One Hospital Drive, Columbia, MO 65212, USA

3 Nuclear Science and Engineering Institute (NSEI), University of Missouri Research Reactor (MURR), University of Missouri, Columbia, MO 65211, USA

4 Radioactive Isotopes and Generator Department, Hot Labs Center, Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt

5 Nuclear and Energy Research Institute – IPEN/CNEN/Sao Paulo, São Paulo, Brazil

which are produced through high speed negatively charged accelerating electrons [2, 6].

Over nearly three decades, interstitial and intratumoral brachytherapy has been used as adjuvant treatment for metastatic prostate, breast, liver and malignant brain tumors in both prospective clinical trials and as part of standard therapy [7, 8]. Radioactive gold grains have also found applications in the treatment of patients with choroidal malignant melanoma. Treatment protocols have involved placement of radioactive sources into surgically constructed scleral pockets. Typical radiation doses ranged from 30 Gy to 120 Gy at the apex of the tumor [9]. There is a strong consensus within the oncological community on the relevance and significant benefits to patients from brachytherapy (also known as interstitial radiation, intracavitary radiation, and internal radiation therapy). Brachytherapy is a cancer treatment modality in which radiation therapy is achieved through a radiation source or radiation device placed within and/or in close proximity to the target site. Solid tumors from breast, prostate or within brains that are sufficiently localized are most suited for treatment by interstitial brachytherapy. A variety of radioactive isotopes are employed in brachytherapy including lower energy sources usually for permanent implantation (such as Palladium-103 and Iodine-125, as well as higher energy sources such as Iridium-192, Gold-198/199, and Cesium-137) that are used for coating on seeds for subsequent intratumoral delivery [10–12]. The MammoSite Radiation Therapy System developed by Proxima Therapeutics, Alpharetta, GA, is an alternative to interstitial brachytherapy that uses either seeds or needles, to treat the intact breast lumpectomy site. With the MammoSite technique, the lumpectomy cavity is dilated by a balloon and a single high-dose radiation source is positioned within the central portion of the balloon to deliver a uniform dose to the walls of the lumpectomy cavity [13, 14]. Although the MammoSite technique may offer a more uniform dose distribution over older techniques of interstitial brachytherapy implants, it has not been shown to offer any appreciable improvement in dosimetry over conventional external beam radiation. Another system that has been approved by the FDA for interstitial breast brachytherapy is the MammoTest Breast Biopsy System developed by Fischer Imaging Corporation, Denver, CO. Analysis of clinical results clearly indicate that brachy therapeutic treatment provides an overall improvement in median survival for cancer patients [15, 16]. Even the most difficult to treat glioblastoma multiforme, recurrent malignant glioma, brain metastases and possibly low grade gliomas seem to benefit from brachy therapy. While Iodine-125 (^{125}I) remains the most popular radionuclide for brachytherapy, there is a recent move away from temporary high-activity implants to permanent low-activity implants which include radioactive gold and palladium-based brachy therapeutic agents [17]. As one example, low dose brachytherapy is the conventional method whereby radioactive seeds are placed permanently into the prostate gland. In prostate cancers, the commonly used seeds are embedded with radioactive iodine or palladium. These radiation therapy approaches deliver a low dose of radiation over a period of several months. The seed-based brachy agent therapeutic modalities are inherently heterogeneous and may result in mild to severe clinical complications. Because of the high heterogeneity of radioactive seeds, oncologists have developed a consensus that a majority of prostate cancer patients receiving real time brachytherapy will experience the following post treatment symptoms ranging from mild severe side effects to severe clinical complications. Side-effects that have been reported include: (a) reduced urine flow due to swelling of the gland in the first few months. All patients are put onto a medication to counter this problem; (b) Increased urgency and frequency of urination due to radiation irritating the sensory nerves in that region; (c) Pelvic pain especially when seeds are placed too laterally; (d) Urinary retention, fortunately not common, that is a nuisance to manage and can have a profound effect on the patient. Many will improve once the prostate swelling resolves; (e) Loose, frequent stools from rectal mucosal irritation; (f) Reduction in ejaculate volume with time as the prostate gland fibroses [18, 19]. In addition to the above side effects, intratumoral incorporation of radioactive brachy seeds within the prostate tumor results in a myriad of clinical complications which include: (a) Orgasmalgia which is a pain associated with orgasm; bloody semen (haematospermia); and reduced intensity of orgasm that occurs fairly frequently in patients treated with radioactive brachy seeds; (b) Accelerated loss of erectile function compared with other ageing males who have not received radiotherapy. Figures between 52 and 76% potency rates are reported at 6 years after treatment. It would seem that patients who are going to get erectile dysfunction will already be reporting this issue by 24 months after the procedure. Older men receiving the radiation tend to have a greater fall-off in erectile function; additional complications caused by the highly heterogeneous brachy seeds within the prostate glands include: (c) Severe complications such as rectal fistulas, urethral stricture, incontinence and penile irradiation that are associated with pre-planning techniques and overdose of radiation to the involved organs.

There are also logistical and operational problems associated with brachy therapy seed placement that has impeded the successful implementation of this therapeutic modality. For example, in patients with prostate tumors, seeds are placed via indirect visualization of the prostate gland. The position of placement is determined from X-ray imaging of the bladder base rather than direct visualization. A system designed by Stock and Stone of the USA improved the method by introducing rectal imaging and visualizing the needle insertion into the prostate gland. Their system was

commercialized, but is associated with major and clinically relevant delivery issues including problems associated with source insertion that results in inaccurate placement of seeds and often times missing the target. Surgeons who do salvage prostatectomies on patients treated with this technique report that the seeds are very erratically placed. Therefore, readily injectable liquids which are inherently homogeneous radioactive probes, which are also tumor specific, will provide significant clinical advances in Brachy therapy of various tumors.

Drawbacks of Brachy therapy from tumor biology considerations

Over 85% of all cancers in human patients belong to solid tumors [20]. The limited blood supply and morphological heterogeneity juxtaposed with interstitial barriers manifested in solid tumors create significant challenges in achieving optimum payloads of both the diagnostic and therapy agents at the tumor sites [21, 22]. Although the leakiness and porosity of solid tumors allows highest accumulation of drugs on the peripheral walls of the tumor, closest to vasculature, and distributing to well-perfused areas, the inability of diagnostic and therapeutic agents to reach most/all parts of tumors has remained to be an unmet clinical need in chemo/radiation therapy of most solid tumors. For example, the currently used brachy therapy agents including, iodine-125 or palladium-103 radioactive seeds and, ^{90}Y immobilized glass microspheres (TherasphereTM),–all utilize microspheres of 10–500 µm in size to achieve selective internal radiation therapy (SIRT) [23, 24]. The limited natural affinity of these microspheres toward tumor vasculature, coupled with significantly larger sizes of brachy seeds (as compared to the porosity of tumor vasculature 250–500 nm), results in limited retention and significant leakage of radioactivity away from the tumor site. Such clinical problems have resulted in decreased efficacy and lower tumoricidal activity of brachy agents [25]. Effective cancer treatment requires interaction of therapeutic agents at the cellular level exposing therapeutic payloads to all the areas of the tumor. Destroying just the outer cells of solid tumors leaves the overall tumor machinery intact and such peripheral partial therapy makes tumors resistant to therapeutic interventions. Therefore, radioactive gold nanoparticles of ^{198}Au and ^{199}Au engineered to sizes in the range 15–30 nm (and hydrodynamics sizes in the 30–85 nm) would be highly attractive for penetrating tumor vasculature to provide optimum therapeutic payloads to solid tumors for complete remission of primary prostate tumors (Fig. 1). Such an approach has the potential to minimize/stop metastases because once the primary prostate tumor is destroyed; it stops the recruitment of proliferating tumor cells into the bone marrow. The following sections provide details on: (i) Production of radioactive gold nanoparticles, (ii) Transformation of radioactive gold nanoparticles into laminin receptor-specific agents for treating prostate cancers and (iii) Overall oncological implications of functionalized radioactive gold nanoparticles in cancer therapy.

Experimental

Production of radioactive gold nanoparticles

^{198}Au was produced by direct irradiation of natural gold foil or metal ^{197}Au (n, γ). In this process, only a small fraction of the gold atoms are converted to ^{198}Au, which leaves the

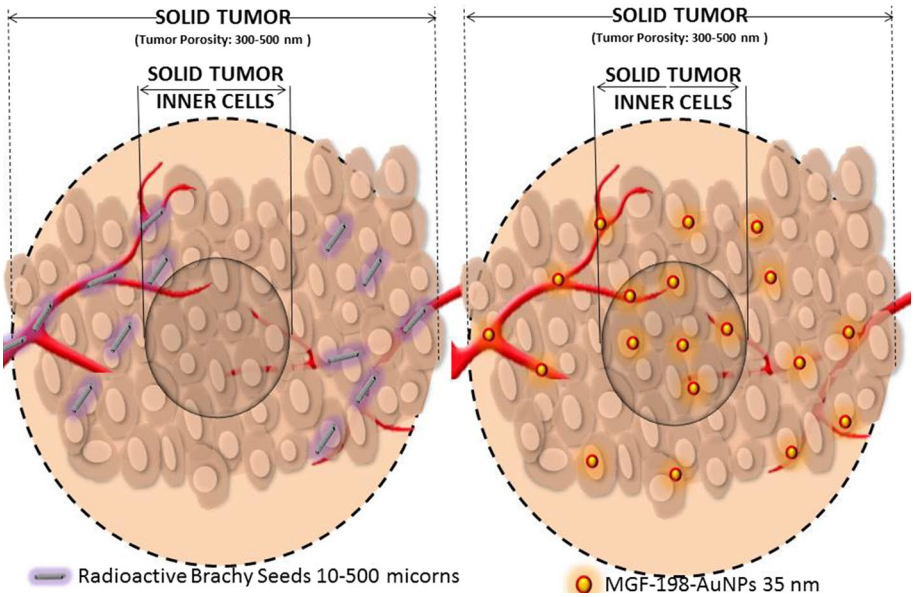

Fig. 1 Tumor penetration: radioactive brachy seeds versus MGF-^{198}AuNPs

majority of the target material comprised mostly of non-radioactive gold. Radioactive gold and other radionuclides are produced by indirect methods such as neutron capture followed by beta decay of the parent radioisotope to the desired daughter radioisotope. For example, neutron activation of enriched ^{198}Pt produces ^{199}Pt ($t½ = 30.8$ m), which is followed by beta decay to ^{199}Au, ^{198}Pt (n, β) ^{199}Au. When the indirect method of production is used, separation of the daughter material from the parent is possible, with the distinct advantage that nearly all of the gold atoms produced are radioactive. A higher specific activity, i.e. higher percentage of radioactive atoms to cold atoms will enable the incorporation of more radioisotopes on each targeting molecule and thereby will increase radionuclide delivery and dose to the tumor. Lower specific activity radionuclides such as ^{198}Au contain a high percentage of cold atoms and therefore deliver a lower dose to tumor cells. Optimized process for the production of ^{198}Au: Gold foil 5–30 mgs was irradiated at a flux of 8×1013 n/cm^2/s. The radioactive foil was dissolved with aqua regia, dried down and reconstituted in 0.05–1 mL of 0.05 N HCL to form $HAuCl_4$. The radioactive gold (50–100 mL) was added to aqueous solutions (6 mL) containing reducing agents. For example, we have extensively used a reducing agent consisting of a trimeric alanine conjugate, THPAL ($P(CH_2NHCH(CH_3)COOH)_3$) —referred to as 'Katti Peptide' for reducing gold to form radioactive nanoparticles of well-defined particle sizes (15–20 nm) in the presence of nanoparticles stabilizing agents such as arabinogalacatan (Gum Arabic, GA) [26–28]. Our experiments have involved addition of THPAL ($P(CH_2NHCH(CH_3)COOH)_3$), (20 mL of a solution containing 0.0337 g of THPAL per 1.00 mL of water) for reducing gold-198 to form radioactive nanoparticles of well-defined particle sizes (15–20 nm). Solutions were evaluated by spectrophotometry to confirm the formation of AuNPs. The change in color from pale yellow to purple is diagnostic of plasmon–plasmon transition present in nanoparticle gold. A number of separations have been investigated to provide high specific activity separations of radioactive gold-198 nanoparticles.

Our most recent studies have focused on the production of radioactive gold nanoparticles using phytochemical-based reducing agents such as epigallocatechin gallate (EGCG) from tea or Mangiferin (MGF) from mango [29–31]. These phytochemicals are electron rich and our discoveries and

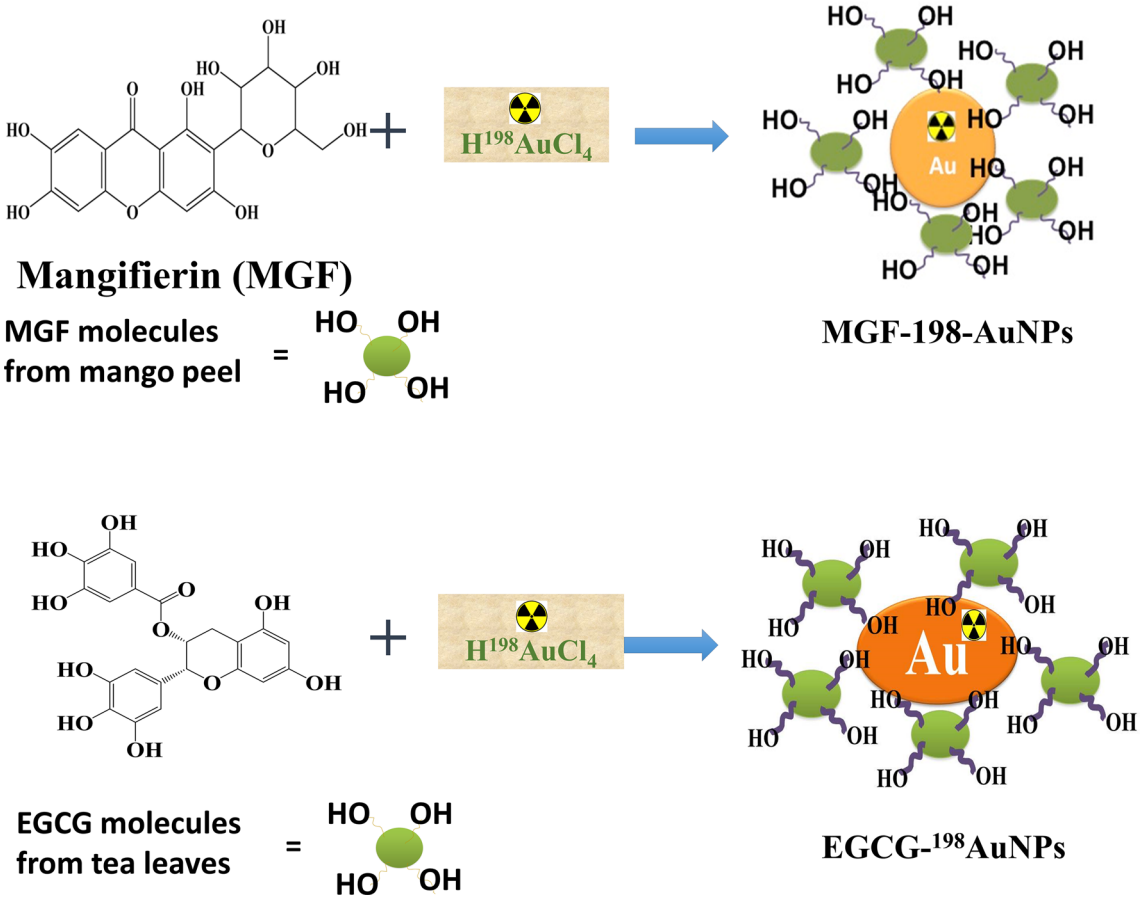

Fig. 2 Production of EGCG-^{198}AuNPs and MGF-^{198}AuNPs

extensive research have shown that they transform radioactive gold-198 precursors into the corresponding phytochemical-encapsulated radioactive gold nanoparticles (EGCG-^{198}AuNPs and MGF-^{198}AuNPs) as shown in Fig. 2 [30–32].

Production of radioactive Mangiferin-functionalized MGF-^{198}AuNPs for prostate cancer treatment

MGF-^{198}AuNPs were synthesized by the addition of 1.55 mg of Mangiferin (MGF) to a glass vial (20 mL scintillation vial), followed by the addition of 2 mL of milli-Q water. Next, the vial was placed on a hot plate and stirred vigorously and continuously and brought to a rolling boil (99–100 °C) with vigorous stirring. At this temperature, Premix (^{198}Au & $NaAuCl_4$) solution with measured amount of radioactivity was added to the Mangiferin solution which resulted in an immediate color change from pale yellow to red–purple. The heating mantle was then removed, and stirring was continued for an additional 1 h. ^{198}Au which is used for the production of ^{198}AuNPs was produced by direct irradiation of natural gold foil or metal according to the nuclear equation ^{197}Au (n, γ) ^{198}Au. After the radioactive gold solution (^{198}Au) was prepared, it was mixed with $NaAuCl_4$ to form radioactive gold precursor.

Stability Studies of MGF-^{198}AuNPs: In vitro stability measurements of MGF-^{198}AuNPs were conducted to evaluate the stability of nanoparticles. The stability study procedure of radioactive Mangiferin gold-198 nanoparticles was performed first by raising the pH of nanoparticles solution to 7 by addition of NaOH and DPBS. Then, the nanoparticles solutions were subjected to stability measurements over a period of 7 days by measuring λ_{max}, as well as performing TLC.

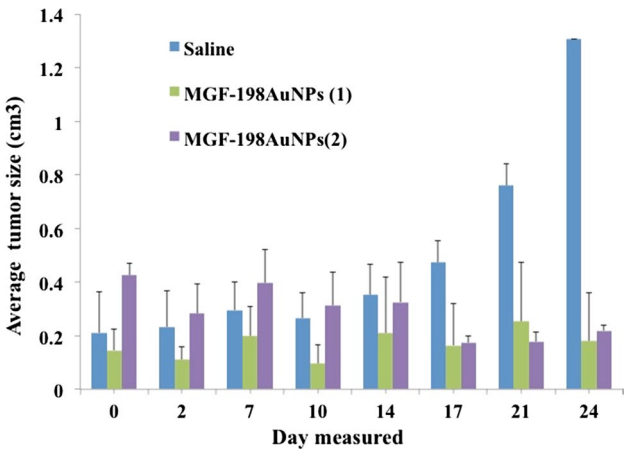

Fig. 3 Therapeutic efficacy data of nanotherapeutic agent MGF-^{198}AuNPs in SCID mice

Therapeutic efficacy studies of MGF-^{198}AuNPs in treating prostate cancer in SCID mice

In order to validate therapeutic targeting abilities of MGF-^{198}AuNP, we have performed in vivo therapeutic efficacy studies of MGF-AuNPs in SCID mice implanted with prostate tumor (PC-3) xenografts. The therapeutic efficacy data of MGF-^{198}AuNPs, as shown in Fig. 3 corroborate its ability to induce apoptosis because tumors harvested from the treatment group consisted largely of apoptotic cells, indicating extensive programmed tumor cell death. The tolerability of the MGF-^{198}AuNP in vivo has been established by monitoring the body weight and blood parameters in this SCID mouse study in both treated and control groups of animals. The treatment group showed only transient weight loss with recovery to normal weight without any early terminations. White and red blood cells, platelets, and lymphocyte levels within the treatment group resembled those of the control mice without tumors. The overall health status and blood measures of the MGF-^{198}AuNP-treated animals indicated that this new therapeutic modality was not only effective, but also well tolerated. These findings support the effectiveness of intralesional therapy of prostate cancer using MGF-^{198}AuNPs in managing the primary tumor location.

Results and discussion

Development of a minimally invasive treatment regimen that controls the growth and proliferation of advanced stages of prostate cancer is of profound importance in the overall treatment and management of this debilitating, life-shortening, and pervasive disease. At present, surgery, chemotherapy, and radiotherapy, alone or in various combinations, have fallen short of effectively making tumors static and result in significant morbidity. We have developed a fundamentally sound and original approach to achieve prostate cancer staticity through the utility of nanoparticles derived from ^{198}Au which are inherently therapeutic, possessing ideal beta energy emission and half-life for effective destruction of tumors. Our approach of intratumoral delivery of Gum Arabic (GA) [26], epigallcatechin gallate (EGCG) [30] and Mangiferin (MGF) [31] functionalized therapeutic gold-198 nanoparticles that penetrate tumor vasculature is innovative because it utilizes a novel local therapy to manage bulky disease allowing complete destruction of primary prostate tumors and thus providing a vitally important unmet clinical means of stopping propagation of tumor cells to other organs. Our strategy to address treatment of prostate tumors centers around keeping the prostate tumor disease static to eliminate expensive treatment and surgeries that often result in severe side effects such as sexual dysfunction, incontinence, difficulty urinating, and bowel dysfunction.

The following discussions provide our experimental results that provide compelling proof to reduce/stop metastases by stabilizing the primary tumor site, particularly in patient populations where surgical resection is not an option such as in men with large tumors or tumors with high risk for surgical morbidity.

Innovation

Our approach embodies a non-brachytherapy ("non-seed") which incorporates injectbale radioactive gold nanoparticles as a new modality with a plethora of innovative advancements:

- *Formulation of the Nanotherapeutic agents*: Inherently therapeutic Gold nanoparticles, with hydrodynamic sizes of 45–100 nm, are homogenously distributed within tumor vasculature allowing uniform tumor dosimetry. ^{198}Au possesses a desirable beta energy emission and half-life for effective destruction of tumor cells/tissue (beta max = 0.96 MeV; half-life of 2.7 days). The range of the ^{198}Au β-particle (up to 11 mm in tissue or up to 1100 cell diameters (depends on energy)) is sufficiently long to provide cross-fire radiation dose to cells within the prostate gland and short enough to minimize significant radiation dose to critical tissues near the periphery of the capsule.
- *Bioactive nanoparticle initiators and stabilizing agents:* Formulation of these inherently therapeutic and biocompatible ^{198}AuNP utilizes the redox chemistry of a tumor-specific phytochemical of green tea, EGCG, or MGF, without the intervention of any other toxic chemicals. Surface conjugation of ^{198}Au nanoparticles with non-toxic glycoprotein gum arabic (GA) or the phytochemicals EGCG/MGF results in GA-^{198}AuNP or EGCG-^{198}AuNP, and MGF-^{98}AuNP respectively, which have demonstrated optimum in vivo stabilities [26, 30, 31].
- *Tumor Uptake and retention though receptor mediated endocytosis:* EGCG-^{198}AuNP specifically targets prostate tumors through the high affinity of EGCG for Laminin receptors (Lam-67R), which are over-expressed on human prostate cancer cells (Fig. 4b). Likewise, MGF-^{98}AuNP also targets laminin receptors of prostate tumor cells thus allowing excellent retention within tumor sites (see sections below). Their natural internalization within prostate tumor cells makes our approach innovative as it allows optimal dose delivery and distribution to the primary prostate tumors while minimizing damage to neighboring tissue. Localization of this dose allows systemic chemotherapy to radiosensitize the prostate tumor, but not increase damage to normal tissues.

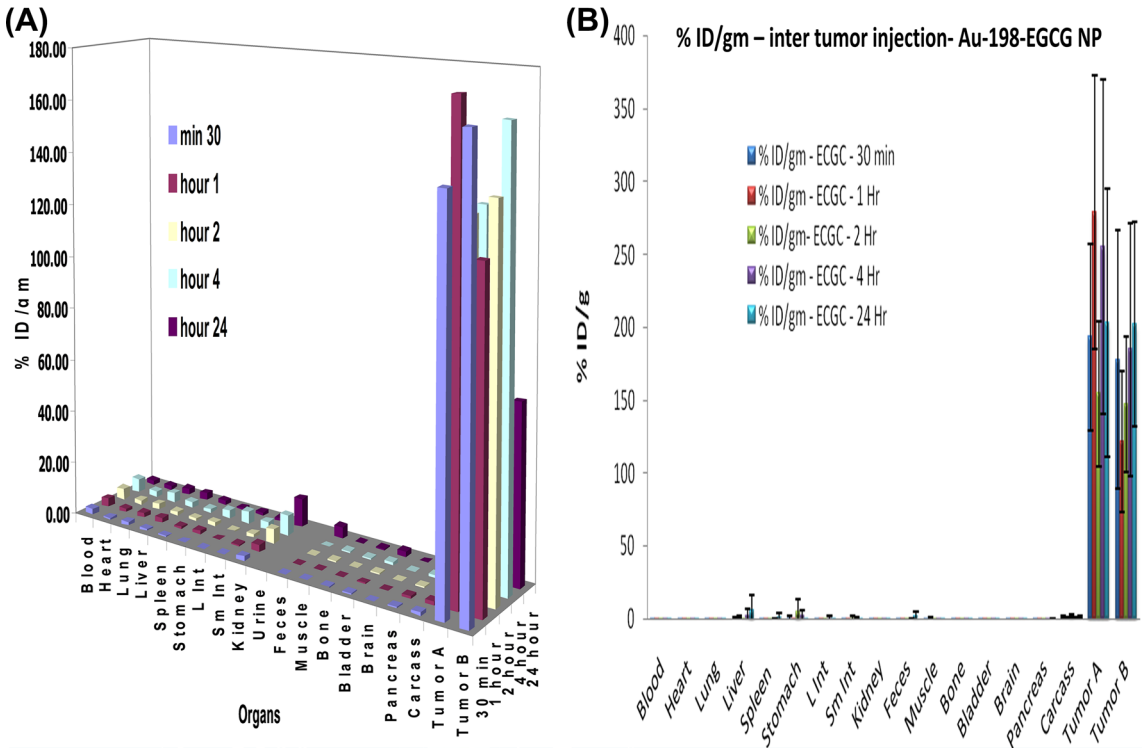

Fig. 4 Biodistribution data of Nanotherapeutic agents in SCID mice **A** GA-^{198}AuNP, **B** EGCG-^{198}AuNP

Our overall approach as discussed below is a significant progress toward intratumorally injectable therapeutic radioactive nanoparticulate agents that will be superior to traditional seed-based radiation therapy that will abrogate the delivery problems leading to inefficacy in treatment and severe toxic side effects.

Our overall research efforts encompassing synthesis, characterization, in vitro/in vivo stability, tumor retention and in vivo therapeutic efficacy are outlined in the following sections:

Synthesis, characterization of nanotherapeutic agents

Our initial studies focused on the development of Gum Arabic (GA) functionalized gold nanoparticles. GA-^{198}AuNP was synthesized by heating (95–100 °C) a mixture of reactor produced $^{198}AuCl_4$ with non-toxic phosphino amino acid $P(CH_2NHCH_3COOH)_3$(THPAL, reducing agent), referred to as 'Katti Peptide' [28] in the presence of 0.2% gum Arabic in water for 2 min. An instant reaction results in the formation of GA labeled radioactive gold nanoparticles with over 98% yields. Epigallacatechin gallate (EGCG) functionalized gold nanoparticles (EGCG-^{198}AuNP) were synthesized by stirring a aqueous solution of EGCG with reactor produced $^{198}AuCl_4$. An instant reaction results in the formation of EGCG conjugated radioactive gold nanoparticles with over 99% yield of EGCG-^{198}AuNP in deionized water without the addition of an external reducing agent. EGCG serves as both reducing and stabilizing agent.

In vitro stability and hemocompatibility studies on nanotherapeutic agents

The in vitro stability of nanotherapeutic agents GA-^{198}AuNPs or EGCG-^{198}AuNPs, and MGF-^{198}AuNPs were determined in their respective non-radioactive surrogates by monitoring the surface plasmon resonance (λ_{max}) and plasmon band width ($\Delta\lambda$) in biologically relevant media (5% NaCl, 0.5% cysteine, 0.2 M histidine, 0.5% HAS (human serum albumin), 0.5% BSA(bovine serum albumin)) for 60 min. These nanotherapeutic agents show high in vitro stability in all biological fluids at physiological pH. Hemocompatibility of the nanoconstructs is assessed by hemolysis assay by direct exposure to freshly drawn whole human blood to non-radioactive surrogates for 2 h at 25 °C. The hemocompatibility studies confirmed that 92% of the RBCs, upon exposure to various concentrations of nanoparticles (GA-AuNPs/EGCG-AuNPs) remained intact. The hemolytic indices of GA-AuNPs/EGCG-AuNPs and MGF-AuNPs, are below the detectable level of equivalence, indicating no occurrence of hemolysis. In addition, complement activation assay showed that GA-AuNPs, EGCG-AuNPs or MGF-AuNPs are highly stable, and biocompatible under in vitro/in vivo conditions. These results clearly indicate that these nanoconstructs are biocompatible and stable for clinical applications.

Biodistribution of nanotherapeutic agents in prostate tumor bearing mice

In order to understand the retention and clearance of radioactive GA-^{198}AuNPs/EGCG-^{198}AuNPs and MGF-^{198}AuNPs within prostate tumors, we have performed detailed in vivo studies of intratumoral administration of GA-^{198}AuNPs (1.5μCi/tumor)/EGCG-^{198}AuNPs (3.5μCi/tumor) to groups ($n = 5$) of SCID mice bearing human prostate cancer xenografts (Fig. 4). GA-^{198}AuNP shows retention of over 154.05 ± 40.7%ID/g within the tumor at 30 min that declined to 87.0 ± 16.9%ID/g by 24 h as shown in Fig. 4a. The uptake of GA-^{198}AuNPs in various organs at different post-injection time points have also been calculated and the results clearly indicate that there is significant accumulation of GA-^{198}AuNPs in the kidneys after 24 h, 10.68 ± 1.72%ID and the main clearance route of ^{198}Au activity was-through urine with 16.77 ± 0.13%ID at 24 h [26, 33]. The EGCG-^{198}AuNPs

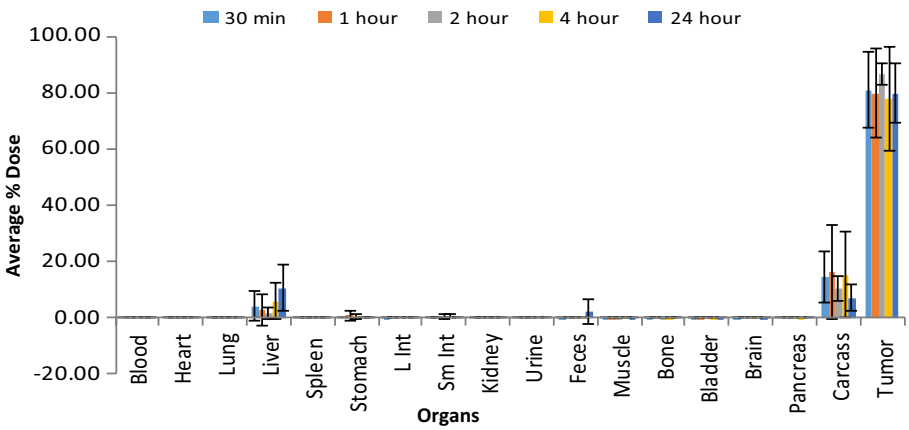

Fig. 5 Retention profile of radioactivity of MGF-^{198}AuNPs in tumors at 30 min, 1, 2, 4, and 24 h after direct injection of single dose of MGF-^{198}AuNPs (4.0 μCi/30 μL) in prostate tumor. In this figure, radioactivity obtained from different organs was calculated as the percentage of injected dose (%ID) of each organ

nanoparticles exhibited slow clearance (leakage) minimal/no leakage into the blood with only 0.06% ID/g at 24 h as shown in Fig. 4b. Lungs and pancreas exhibited low uptake at 24 h with only 0.33% ID/g and 0.22% ID/g respectively. The uptake in the stomach peaked at 5% ID/g at 2 h and decreased to 0.03% ID/g at 24 h. The kidneys and spleen showed slow uptake over time with 0.12% ID/g and 1.56% ID/g at 24 h, respectively. The liver had 0.51% ID/g after 30 min that increased to 6.13% ID/g after 24 h. The gastrointestinal uptake contributed to the feces having 1.71% ID after 24 h. These data revealed that EGCG-^{198}AuNPs clearly exhibit excellent (93%) tumor retention over 24 h. These pharmacokinetics confirmed excellent retention of therapeutic payloads of GA-^{198}AuNPs/EGCG-^{198}AuNPs nanoparticles within prostate tumors.

We have also performed in vivo studies of intratumoral administration of MGF-^{198}AuNP (4 μCi/30μL for each tumor) using SCID mice ($n=5$) bearing human prostate cancer xenografts. The biodistribution and tumor retention characteristics are shown in Fig. 5.

The bio distribution results have confirmed that the percentage of injected dose within tumor (%ID) within prostate tumors at various time points was 80.98 ± 13.39% at 30 min increasing to 86.68 ± 3.58% at 2 h, and remained at 79.82 ± 10.55% at 24 h. Overall; there was minimal/no leakage of MGF-^{198}AuNPs in the liver. The (%ID) in liver was 10.65 ± 8.31% at 24 h, indicating that a small amount of nanoparticles were cleared by the reticulo endothelial system to the liver. Also, there was very low leakage of injected dose into stomach and feces, 0.10 ± 0.16% of injected dose in stomach at 30 min decreasing to 0.02 ± 0.02% at 24 h, and 0.00 ± 0.00% of injected dose in feces at 30 min increasing to 2.20 ± 4.51% at 24 h. These results show that the main route of clearance is via the digestive system through the feces. In contrast, there was no noticeable leakage into blood and lung and other organs. Therefore, our results of intratumoral study showed that MGF-^{198}AuNPs have excellent ability to be retained within the tumor with very minimum leakage to non-target organs [31]. The high tumor retention with concomitant limited leakage are the two main factors to minimize/eliminate systemic toxicity caused by cancer therapy agent.

These data demonstrate that the biodistributions and tumor retention of these nanotherapeutic agents are favorable for primary tumor localization to stabilize primary disease and diminish metastatic tumor cell release for overall disease stabilization. Therefore detailed therapeutic efficacy studies of all the three agents have been undertaken and the results are discussed below:

Therapeutic efficacy data for nanotherapeutic agents GA-^{198}AuNP/EGCG-^{198}AuNP

We have used SCID mice bearing a flank model of human prostate cancer derived from a subcutaneous implant of 10 million PC-3 cells for therapeutic efficacy and pharmacokinetic studies of GA-^{198}AuNP/EGCG-^{198}AuNP. The overall reduction in tumor volume was 80% three weeks after a single dose intratumoral administration of GA-^{198}AuNP (408 μC_i) as shown in Fig. 6a. There was no uptake of

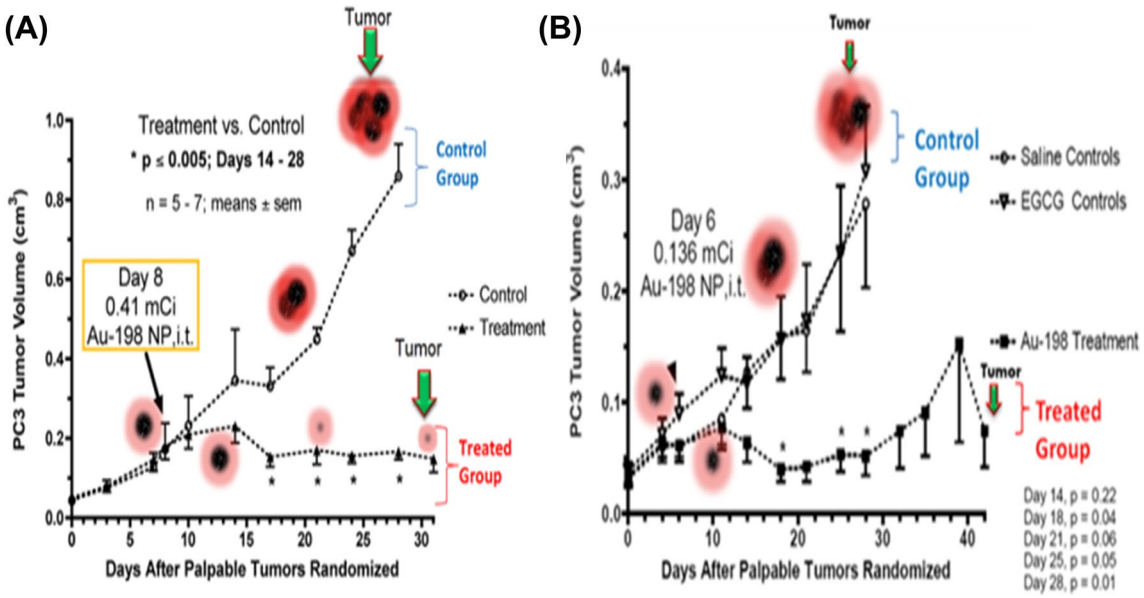

Fig. 6 Therapeutic efficacy data of nanotherapeutic agents in SCID mice **a** GA-^{198}AuNP, **b** EGCG-^{198}AuNP

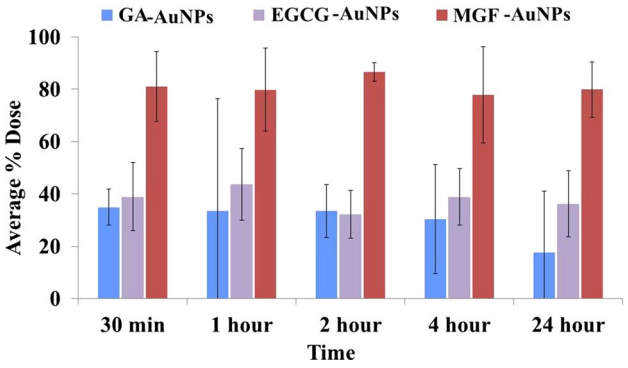

Fig. 7 Retention of therapeutic payloads of Gum Arabic (GA), epigallocatechin gallage (EGCG) and Mangiferin (MGF) conjugated gold nanoparticles in prostate tumor bearing mice

therapeutic payload in non-target organs as the small amount (2–5%) of the injected dose released from the tumor was subsequently cleared through the renal pathway. Figure 6b shows results from the single-dose radiotherapy study of EGCG-^{198}AuNP (136 μCi) in PC-3 bearing SCID mice. The end-of-study biodistribution on Day 42 showed that 37.4. ± 8.1%ID (mean ± sem; $n = 5$) remained in the residual tumor, while 17.8. ± 6.1%ID was noted for carcass and 2.5. ± 1.7%ID was observed for the liver. Retention in other tissues was negligible, with radioactivity near background levels for blood, heart, lung, spleen, intestines, stomach, bone, brain and skeletal muscle. There was a significant reduction in tumor volume (80%), four weeks after a single intratumoral dose of EGCG-^{198}AuNP with minimal uptake in non-target organs.

The therapeutic efficacy data for both GA-^{198}AuNP/EGCG-^{198}AuNP corroborate their ability to induce tumor staticity because tumors harvested from the treatment group

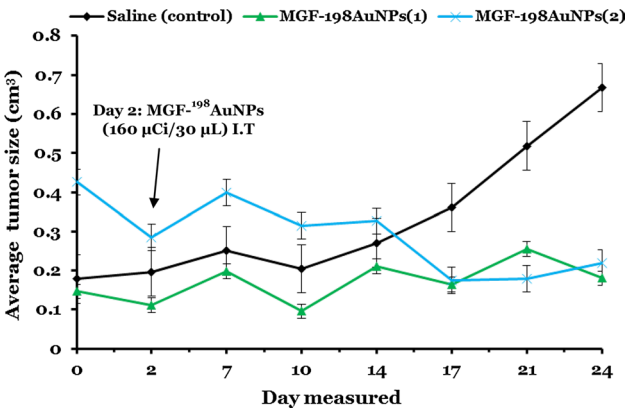

Fig. 8 Therapeutic efficacy studies of MGF-^{198}AuNPs after a single dose intratumoral administration in human prostate cancer bearing SCID mice (mean ± SD). By day 24, treated animal tumors were much smaller than the saline treated control group ($p = 0.04$). The therapeutic effect was maintained over a three-week period

in both cases consisted largely of necrotic tissue, indicating extensive tumor cell kill [26, 30].

Oncological implications

In search of gold nanoparticles capable of targeting primary prostate tumor, we have developed a large number of candidates encapsulated with various proteins, peptides and small molecules [26–30, 34–44]. The tumor retention characteristics of these candidates have been accurately quantified by pioneering the development of the corresponding ^{198}Au radioactive gold nanoparticulate analogs [26–30, 34–44]. The gamma emission of ^{198}Au nanoparticles allows precise estimation of gold within tumor cells/tissues through scintigraphic counting techniques (Fig. 7). For the proposed studies, we have created immunomodulating, The presence of glucose in Mangiferin provides an ideal targeting ligand with excellent prostate tumor-avidity through laminin receptor specificity.

Development of the corresponding ^{198}Au radiolabeled MGF-^{198}AuNPs, followed by in vivo prostate tumor xenograft retention studies, have demonstrated that MGF encapsulation transforms these nanoparticles to be prostate tumor specific with over 90% of the injected dose retained in the tumor for over 24 h (Fig. 7). The tumor retention characteristics of MGF-^{198}AuNPs are an order of magnitude higher as compared to those of GA-^{198}AuNPs and EGCG-^{198}AuNPs. MGF-^{198}AuNPs, because of their size and charge, cross tumor vasculature, and accumulate within prostate tumors by EPR, while the concomitant laminin receptor specificity, and the presence of glucose functionality—leads to efficient endocytosis within prostate tumor cells, thus augmenting tumor uptake and retention (Fig. 7). The therapeutic efficacy data of MGF-^{198}AuNPs as depicted in Fig. 8 unequivocally demonstrate the superior tumor uptake and retention characteristics exerted by Mangiferin's xanthanoid motif which has sugar moiety as part of its chemical strcture (Fig. 2). Our therapeutic efficacy data have shown that a single dose of MGF-^{198}AuNPs completely stops prostate tumor growth with no toxicity associated with the radiation dose to various non-target organs (Fig. 8) [31].

We therefore, conclude here that the glucose unit in Mangiferin allows effective tumor accumulation and retention of MGF-^{198}AuNPs within prostate tumors and thus is an important green-nanotechnology-based 'Pharmaco Motif' for the development of site specific radiopharmaceuticals for applications in oncology.

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