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Targeted gold nanoparticles enhance sensitization of prostate tumors to megavoltage radiation therapy *in vivo*

Tatiana Wolfe[a], Dev Chatterjee[a], Jihyoun Lee[a,b], Jonathan D. Grant[a], Shanta Bhattarai[a], Ramesh Tailor[a], Glenn Goodrich[c], Patricia Nicolucci[d], Sunil Krishnan[a,*]

[a]*The University of Texas MD Anderson Cancer Center, Houston, TX, USA*
[b]*SoonChunHyang University Hospital, Seoul, South Korea*
[c]*Nanospectra Biosciences Inc., Houston, TX, USA*
[d]*University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazil*

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Abstract

We report potent radiosensitization of prostate cancers *in vitro* and *in vivo* using goserelin-conjugated gold nanorods. Progressive receptor-mediated internalization of conjugated nanorods over time increases the radiation interaction cross-section of cells and contributes to the effects observed *in vitro*. The low concentrations of gold required, the long interval between injection of nanoparticles and radiation, and the use of megavoltage radiation to generate radiosensitization *in vivo* foretell the possibility of eventual clinical translation of this approach.

Key words: Megavoltage radiation therapy; Gold nanoparticles; Tumor targeted delivery; Conjugated; Prostate cancer; Goserelin acetate

Background

The ability of gold nanoparticles (AuNPs) to enhance the effect of physical radiation dose on tumor cells has been previously shown.[1–12] Systemic injection of nanoparticles results in a preferential accumulation in the typically "leaky" tumor vasculature (the "enhanced permeability and retention" [EPR].[6,13,14] The radiosensitization effect of AuNPs is thought to result from an increased number of photoelectric absorption events and low-range electron showers that follow the interaction of photons with the increased number of electrons present in gold. Hainfield *et al.* demonstrated that administration of AuNPs followed by kilovolt (kV) radiation substantially improved the 1-year survival rate of mice bearing subcutaneous mammary carcinoma (86%) over radiation alone (20%).[1] The aforementioned curative effect of combined radiotherapy and AuNPs was observed in mice following intravenous administration of approximately 1.35 mg of gold per gram of body weight. Immediate radiation – 2 min after AuNPs injection – guaranteed that the transient gold content encountered in the tumor, primarily within the vasculature, increased interaction probability with ionizing radiation, leading to enhanced biological effect. The photoelectric absorption is most prominent at kV energies near the binding energies of the lower shells of electrons in gold – a fact that has hindered the translation of this technology to the clinic due to the inherently shallow penetration of kV energies. Attempts at using the clinically-utilized megavoltage (MV) energies and clinically non-prohibitive amounts of gold have shown only modest dose enhancement from AuNPs, <17% *in vitro* as reported by Chitrani et al.[4]

We hypothesized that the MV radiation dose-enhancing effects of AuNPs could be amplified if more particles were taken up by the tumor cells, leading to greater cellular toxicity from the short-range secondary electron cascade. Majority of the successful studies combining AuNPs and ionizing radiation utilize a polyethelene glycol coating (PEGylation) – or similar coating molecules – to prevent nanoparticles from being rapidly eliminated from the body while still staying in the blood stream, ultimately reducing uptake of these particles by the reticuloendothelial cells, consequently enhancing their accumulation

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*Corresponding author at: The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030.
E-mail address: skrishnan@mdanderson.org (S. Krishnan).

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within tumors.[6,10,12,15,16] Modest radiosensitization has been reported with megavoltage irradiation of pegylated AuNPs in vitro with widely varying results based on the cell lines being treated and the treatment conditions where high concentrations of particles remain in the media (are not washed off) during irradiation.[3,4,6] In vivo, most of these PEGylated nanoparticles tend to accumulate in the perivascular space, however, with limited uptake by cells. We sought to further increase the amount and specificity of gold accumulation inside cancer cells by conjugating the nanoparticles to a small peptide targeted to a receptor preferentially overexpressed by tumors.

Radiation dose escalation has been demonstrated to be of clinical benefit in some cancers but not all. Prostate cancer is one instance where there is demonstrable therapeutic value to escalated doses of radiation to the primary tumor while sparing adjacent normal tissues. Recent clinical trial results have shown that overall survival is directly correlated to cumulative tumor dose due to more efficient elimination of radioresistent clones at the primary site. In addition, a cross-sectional analysis of these three major clinical trials revealed a linear correlation between total tumor dose and improvements in biochemical control. Moreover, modest local dose enhancements (between 11-24%) result in great improvements in the overall survival time (from 10% to 200%).[17–19]

Our search for a suitable targeting ligand for prostate cancer led us to choose goserelin acetate, a synthetic luteinizing hormone–releasing hormone (LHRH) analogue that binds to the LHRH receptor overexpressed in the vast majority of prostate cancers[20] and works by reducing the secretion of gonadotropins, which in turn reduces the testicular secretion of testosterone.[21] Concurrent administration of doses of goserelin acetate that result in sustained testosterone suppression to near-castrate levels improves local control and survival of locally advanced prostate cancer patients treated with radiation therapy.[22] However, no supra-additive radiosensitization was observed *in vitro* in human prostate cancer cells treated with low concentrations of goserelin[23] – the concentrations we evaluate in the present study – consistent with the conclusion that testosterone suppression does not sensitize prostate cancers to radiation therapy but the combination causes additive cytotoxicity and growth inhibitory effects that are clinically meaningful.[24] We then reasoned that the affinity of goserelin for prostate cancer cells, given the plentiful expression of type I and type II gonadotropin receptors on the membranes of such cells,[20] could be exploited to enhance the accumulation of gold nanoparticles in prostate cancer cells for radiation therapy, and thus enhance the biological effects of radiation compared with untargeted AuNPs that accumulate in the extracellular and perivascular compartments. Here we describe our successful conjugation of goserelin acetate to the surface of AuNPs (gAuNRs) at a ratio of approximately 60 molecules per nanoparticle. We thereafter report our *in vitro* and *in vivo* investigations into the radiosensitizing effects of targeted gold nanoparticles when applying radiation in the MV energy range.

Methods

A construction of goserelin conjugated AuNPs

Bare rod-shaped gold nanoparticles, gold nanorods (herein denoted AuNRs), were synthesized as described in the supplementary information. Further, these nanoparticles were reacted with thiol-terminated methoxy polyethylene glycol (PEG) to generate PEGylated AuNRs (pAuNRs). AuNRs were conjugated to goserelin via PEG as outlined in the cartoon (Figure 1, *A*). Briefly, goserelin acetate (Sigma) stock solutions were made by dissolving the peptide powder in deionized water at 5 mg/mL (0.8 mM), aliquot and kept at −80 °C. 355 μL of ethyl (dimethylaminopropyl) carbodiimide, EDC (Thermo Scientific), solution at 2 mM was added to 100 μL of 1 mM thiol-PEG-carboxylate (SH-PEG-COOH) solution, immediately followed by the addition of 326 μL of 5 mM sulfo-N-hydroxysuccinimide, NHS (Thermo Scientific), solution resulting in a semi-stable amino-reactive SH-PEG-NHS-ester. Subsequently, 382 μL of 0.8 mM goserelin peptide solution was reacted with this mixture in glass vials gently agitated on ice and then allowed to equilibrate under constant gentle shaking for 30 min at 4 °C. A molar ratio of goserelin to PEG of 1:1 was determined to be optimal for maximizing the number of goserelin molecules linked to the surface of each AuNRs in the next step. After creation of the SH-PEG-goserelin, the vial was again placed on ice and 3 mL of AuNRs solution (at OD = 3) was added drop-wise to the vial. The entire solution was then kept overnight at 4 °C under constant gentle mixing to allow the thiol

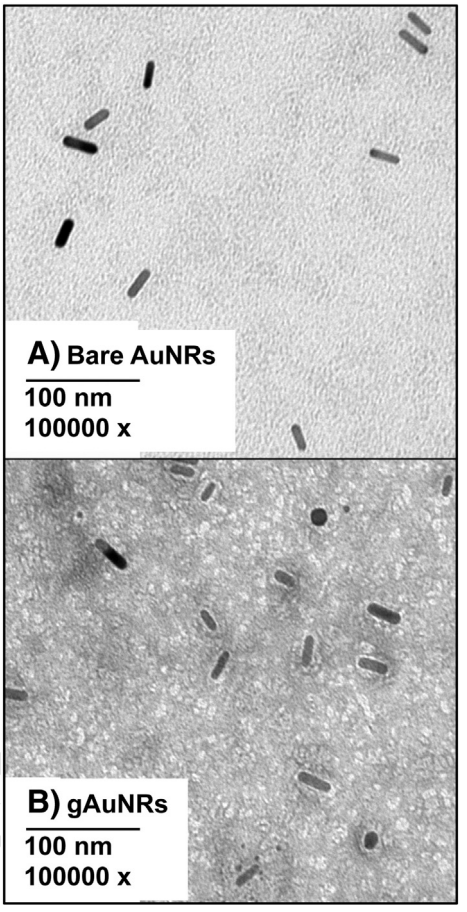

Figure 1. Architecture of goserelin-conjugated gold nanorods (gAuNR). Transmission electron microscopy images of gold nanorods (AuNRs) before conjugation **(A)** and after conjugation (gAuNR) **(B)**. Detailed information for manufacture of gAuNR can be found in the supplementary material.