ORIGINAL RESEARCH

Enhanced radiation sensitivity in prostate cancer by gold-nanoparticles

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Abstract

Purpose: Nanotechnology is an emerging field with significant translational potential in medicine. In this study, we applied gold nanoparticles (GNP) to enhance radiation sensitivity and growth inhibition in radiation-resistant human prostate cancer cells.

Methods: Gold nanoparticles (GNPs) were synthesized using HAuCl₄ as the gold particle source and NaBH₄ as the reductant. Either thio-glucose or sodium citrate was then added to the solution separately to bind the GNPs to form thio-glucose-capped gold nanoparticles (Glu-GNP) and neutral gold nanoparticles (TGS-GNPs). Human prostate carcinoma DU-145 cells were exposed to vehicle, irradiation, 15nM TGS-GNPs, or 15nM Glu-GNPs, or GNPs plus irradiation. The uptake assays of GNP were performed using hemocytometer to count cells and the mass spectrometry was applied to calculate gold mass. The cytotoxicity

induced by GNPs, irradiation, or GNPs plus irradiation was measured using a standard colorimetric MTT assay.

Results: Exposure to Glu-GNPs resulted in a three times increase of nanoparticle uptake compared to that of TGS-GNPs in each target cell ($p < 0.005$). Cytoplasmic intracellular uptake of both TGS-GNPs and Glu-GNPs resulted in a growth inhibition by 30.57% and 45.97% respectively, comparing to 15.88% induced by irradiation alone, in prostate cancer cells after exposure to the irradiation. Glu-GNPs showed a greater enhancement, 1.5 to 2 fold increases within 72 hours, on irradiation cytotoxicity compared to TGS-GNPs. Tumour killing, however, did not appear to correlate linearly with nanoparticle uptake concentrations.

Conclusion: These results showed that functional glucose-bound gold nanoparticles enhanced radiation sensitivity and toxicity in prostate cancer cells. In vivo studies will be followed to verify our research findings.

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer-related deaths in American males with similar trends in many western countries.¹ Approximately one in six men, or 230,000 new cases of prostate cancer are diagnosed every year in the United States, with an estimated 27,000 deaths every year.² Since the 1960s, radiation therapy has been the main treatment modality to treat patients with localized advanced prostate cancer.³ Although increasing radiation dose can improve cell kill, early and long-term side effects often restrict its practical usage in patients. Despite recent advances in three-dimensional, intensity modulated external beam radiation therapies, as well as brachytherapy, toxicities to the rectum and bladder remain a major concern.⁴⁻⁵ For these reasons, it is necessary to develop novel approaches for enhancing radiation sensitivity in prostate cancer.

Nanotechnology is an emerging technique for improved cellular targeting and radiosensitivity. Nanoparticles are solid colloidal particles ranging in size from 10 nm to 200 nm that are 100 to 10000 times smaller than human cells.⁶ Nanoparticles smaller than 50 nm can easily pass through cell membrane, and the particles smaller than 20 nm can pass through blood vessel endothelium.⁷ Using different surface modification, nanoparticles can be used as targeted delivery vehicles to carry chemotherapeutic agents or radiosensitizers to malignant cells.⁸ Nanoparticles are widely used to treat cancer. For instance, Abraxane (Abraxis Bioscience) uses nanoscaled particles of albumin to bind paclitaxel. This drug was approved by the Food and Drug Administration in 2005 to treat breast cancers. The National Cancer Institute Alliance for Nanotechnology in Cancer was established in the United States to specifically advanced nanotechnology research for cancers.

When an X-ray source interacts with metallic nanoparticles, free radicals are subsequently generated that can directly damage DNA and indirectly induce cell apoptosis. Animal models have demonstrated that nanoshells improved cell killing using near infrared

light with little or no side effects to normal tissues.⁹⁻¹⁰ Gold nanoparticles (GNPs) have been studied previously to enhance radio-sensitivity in animal models.¹¹⁻¹² Nanoparticles can circulate within the body, target at specific organs, penetrate their cell membranes, and enter the mitochondria, and trigger apoptotic responses.¹³ X-ray sensitive hybrid nanoparticles can bind to targeted cell and then act on transmembrane ligands at the cell surface and cytoplasm. Localized hyperthermia can be induced using external quantum lights.¹⁴ Enhanced cytotoxicity is produced secondary to the photoelectric effect of external beam radiation.¹⁵

Positron emission tomography (PET) imaging has confirmed that most malignant tumors uptake glucose more than normal cells.¹⁶ This unique metabolic characteristic of malignant cells can be used to design nanoparticles for targeted delivery. Nanoparticles with surface bound glucose allow for selective uptake into metabolically active cells.¹⁷

In our previous work,^{17,18} we have developed a series of functional GNPs and modified their surface properties with various active biological reagents for various applications. We have also evaluated how the changes in functional bio-molecule on the GNP surface can affect GNP biological activities in cancer cells.¹⁷ Glucose-capped GNPs (Glu-GNPs), which are designed based on cancer cell metabolism, can be selectively uptaken by cancer cells and localized in the cytoplasm.¹⁹⁻²¹ In this paper, we demonstrate how glucose can enhance cell uptake of GNPs and how GNPs can enhance radiation cytotoxicity in prostate cancer cells.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma-Aldrich (Milwaukee, WI). MTT CellTiter 96 non-radioactive cell proliferation assay kit was purchased from Promega (Madison, WI).

FIGURE 1. . Gold nanoparticles: A. Electric micrograph of gold nanoparticles (Bar = 50 nm); and B. the schematic diagram of naked gold nanoparticle binding with glucose.

Synthesis of Gold Nanoparticles

The general synthesis method for making gold nanoparticles follows three substeps. i) 3.2 ml of 25mM HAuCl_4 solution was added into 60 ml of deionized water in an ice bath with moderate stirring. ii) 4 ml of 26 mM NaBH_4 was then added as a reductant to obtain naked gold nanoparticles. iii) The naked GNPs solution was added into two tubes each containing 22.4 ml of naked GNPs solution. 4 ml of 20 mM 1-thio- β -glucose or 4 ml of 38.8 mM sodium citrate solution was added separately into two gold solutions.

Thio-glucose covalently and sodium citrate electrostatically bind to the GNPs to form functionalized thio-glucose-capped gold nanoparticles (Glu-GNPs) and neutral gold nanoparticles (TGS-GNPs) respectively. (Figure 1A and 1B).

Both the TGS-GNPs and the Glu-GNPs were dialysed for two days to remove any free sodium citrate or thio-glucose from the gold particle solutions before these solutions were provided to the experiments. Both the TGS-GNPs and the Glu-GNPs were characterized using transmission electronic microscopy (TEM), ICP-MS and Kratos Axis 165 X-ray Photoelectron Spectroscopy (XPS) (Kratos Analytical) as described previously.¹⁷

Cell Culture

Human prostate carcinoma cell line DU-145 was used in all the experiments. DU-145 cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 20 mM D-glucose, 100 UI/ml penicillin G, and 100 $\mu\text{g}/\text{ml}$ streptomycin in a humidified incubator with 5% CO_2 in the air at 37°C. DMEM without glucose was used for the cells that were exposed to either Glu-GNPs or Glu-GNPs plus Cytochalasin B (glucose transport inhibitor).

Uptake Assay

The assay was performed in triplicate. A 10ml DU145 cell suspension containing 2×10^6 cells was seeded onto a 100mm-cell culture dish and was cultured overnight. When the cells reached a 70% confluence, the target cells were exposed to the vehicle, 15nM TGS-GNPs, or 15nM Glu-GNPs, respectively at 37°C. After two hours of incubation, the free GNPs in the cell cultures were removed by washing the cells twice with the PBS buffer. The cells were detached with Trypsin-EDTA. After centrifugation and the removal of the supernatant, the cells were resuspended in the PBS with a final volume of 5 ml. The number of

cells in suspension was counted with a hemocytometer. 5 ml of 50% HNO₃ was added to each sample to lyse the cells. The gold mass in the lysis solution was measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The number of gold nanoparticles was calculated via the gold mass, and the number of GNPs in the lysis solution was divided by the number of cells to yield the number of GNPs taken up by cells.¹⁶

Transmission electron microscopy

The cells treated with or without GNPs were collected by centrifugation. The cell pellets were fixed in 4% (v/v) formaldehyde in 0.1 M phosphate buffer (pH 7.2) for four hours at 4°C. After being washed in the same buffer, the cells were resuspended in 1% OsO₄ for one hour at room temperature. They were then washed twice by centrifugation and resuspended in distilled water. The final pellet was resuspended in a small volume of warm 2% (w/v) agarose, poured onto a glass slide, and allowed to cool. When set, the small pieces of gel containing the cells were cut out and dehydrated through a graded series of ethanol solutions. The pieces were then embedded in epoxy resin, and thin sections were cut with an ultramicrotome, stained with uranyl acetate followed by lead citrate and examined in Philips EM301 electron microscope operating at 80 kV.

Irradiation of Cells

All cell irradiation treatments were carried out using a Pautak Therapax 3 Orthovoltage 244 Monitor Units /minute X-ray machine at 200 kVp using a 0.35 CU + 1.5 AL filter. DU145 cells in 100-mm culture dishes were irradiated at room temperature when cultures reached ~ 75% confluence. Cells received either a mock treatment for control or 2 Gy. After irradiation, cultures were returned to 5% CO₂/37°C incubation until they were harvested at the time points required.

MTT Assay

MTT assay is a quantitative colorimetric method to determine cytotoxicity. It utilizes the yellow tetrazolium salt [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] which is metabolized by mitochondrial succinic dehydrogenase activity of proliferating cells to yield a purple formazan reaction product. In the present study, toxicity in mitochondria induced by GNPs with or without radiation was measured by an MTT assay in 96-well plates. The experiments were performed by eight replicates and cells were seeded in 96-well plates (3×10³/well) with 200 µl of culture medium per well. The cells were allowed to grow on the 96-well plates overnight. Cells were then exposed to either TGS-GNPs or Glu-GNPs respectively and different doses of irradiation according to the experimental design. The cell responses were monitored by the MTT assay by following the manufacturer's instructions. After removal of the medium, 100 µl of MTT (0.4 mg/ml) dissolved in medium were added to each well. Following three hours of incubation, the medium was replaced with 100 µl of 0.1 N HCl/isopropanol, and absorbance in each well was assessed at 550 nm using a microplate reader. Absorbance was expressed as a percentage of control. The cell growth inhibitory rate was calculated by the following formula: Inhibitory rate = (1 - average OD_{550nm} of treated group/average OD_{550nm} of control group) × 100%.

Statistical Analysis

In the statistical analysis, differences between the treated and control groups were compared using Student's *t*-tests, with the differences at the *P*<0.05 level considered to be statistically significant.

Results

Uptake of GNPs on DU-145 cells

After exposure to 15 nM naked TGS-GNPs and Glu-GNPs respectively for two hours, the average number

FIGURE 2. DU-145 cell uptakes of GNP. Data are reported as the mean $\pm$ SEM for three separate experiments performed in quadruplicate. The cell uptake values were $(2.01 \pm 0.25) \times 10^4$ /cell for TGS-GNPs and $(6.73 \pm 0.69) \times 10^4$ /cell for GNP-Glu. $P < 0.005$ for GNP-Glu vs GNPs (t test).

of GNPs per cell associated with each DU-145 cell was $(2.06 \pm 0.24) \times 10^4$ for TGS-GNPs, and $(6.73 \pm 0.67) \times 10^4$ for Glu-GNPs (Fig. 2). In contrast, exposure to Glu-GNPs results in a three times increase of nanoparticle uptake compared to TGS-GNPs in each target cell (Fig. 2). $P < 0.005$ for GNP-Glu vs. TGS-GNPs (t test).

Distribution of Glu-GNPs in DU-145 Cells

The distribution of GNPs in DU-145 cells was determined by TEM. Fig. 3 is the micrograph of the cells treated with 15nM Glu-GNPs. The figure shows that most of Glu-GNPs were distributed in the cytoplasm.

Effect of Gold-particles on DU-145 cell growth

Compared to the control, the results in Fig.4 show that DU-145 cell growth was decreased by 13.52% with TGS-GNPs and 17.82% with Glu-GNP treatments ($P < 0.01$) after 24 hours. However, there was no difference on cell growth between the TGS-GNPs and Glu-GNPs exposures *in vitro*.

FIGURE 3. Distribution of GNPs in DU-145 cells.

FIGURE 4. DU-145 cell growth was decreased by 13.52%, 17.82% and 14.72% after the treatment with TGS-GNPs, GNP-Glu, or 2 Gy X-ray at 24h, respectively. Cell growth was not different after exposure to TGS-GNPs, GNP-Glu or 2 Gy X-ray treatment *in vitro* ($P > 0.1$).

Effect of 2 Gy 200 kVp X-ray on DU-145 cell viability

The cytotoxic effects of X-ray on DU-145 cells were analyzed after 24, 48 and 72 hours of irradiation. Untreated control samples were arbitrarily assigned a value of 100% and the results of all treatments were normalized to 100% (i.e., % of control). The data in Fig. 4 shows that 2 Gy X-ray induced an inhibition of

FIGURE 5. Nanoparticles enhanced significantly radiosensitivity of DU-145 cells. Irradiation of 2Gy alone induced the inhibition rate of cell growth ($15.78 \pm 2.85\%$) after 24 hours and ($19.03 \pm 6.00\%$) after 48 hours. A. Irradiation of 2Gy+TGS-GNPs were ($30.57 \pm 3.32\%$) at 24 hours and ($32.18 \pm 2.12\%$) at 48 hours. B. Irradiation of 2Gy+Glu-GNPs were ($45.97 \pm 3.95\%$) at 24 hours and ($44.63 \pm 1.87\%$) at 48 hours. Both TGS-GNPs and Glu-GNPs can increase radiosensitivity of 2Gy ($P < 0.001$) X-ray on DU-145 cells after 24 and 48 hours.

cell growth by 15.78%, 19.03% and 9.22% at 24, 48 and 72 hours respectively.

Gold-particles enhance radiation cytotoxicity on DU-145 cell

To determine whether GNPs had enhanced radio sensitivity of DU 145 cells to 2 Gy X-ray, cells were treated with either a single dose of 2Gy X-ray or 2Gy X-ray and GNPs, whereas control group did not receive any treatment. Untreated control samples were arbitrarily assigned a value of 100% and the results of all treatments were normalized to 100% (i.e., % of control). Fig. 5A shows that either TGS-GNPs or X-ray induced an inhibition of cell growth by 13.52% or 15.88% at 24 h, individually. However, a combination of TGS-GNPs and X-ray induced an inhibition of cell growth of 30.57% ($P < 0.005$) (Fig. 5A). Similarly, the data in Fig. 5B shows that Glu-GNPs induced an inhibition of cell growth by 17.82% after 24 hours but the combination of Glu-GNPs plus X-ray induced an inhibition of cell growth by 45.97% ($P < 0.005$).

Enhancement of radiosensitivity by either TGS-GNPs or Glu-GNPs

To evaluate whether glucose will help the delivery of gold-nanoparticles to cancer cells, the cellular uptakes (Fig. 2) and radiosensitivity enhancement induced by Glu-GNPs were determined and compared to those induced by TGS-GNPs. Fig. 6 shows that the inhibition rate of 2Gy X-ray plus TGS-GNPs was ($30.57 \pm 3.32\%$) at 24 hours and ($32.18 \pm 2.12\%$) at 48 hours. The inhibition rate of 2Gy X-ray plus Glu-GNPs was ($45.97 \pm 3.95\%$) at 24 hours and ($44.63 \pm 1.87\%$) at 48 hours. Glu-GNPs increased radiosensitivity by 50.37% ($P < 0.001$) at 24 hours and 38.68% ($P < 0.005$) at 48 hours compared with TGS-GNPs that have no glucose bound.

Discussion

Radiation therapy combined with metallic nanoparticles is a new treatment approach in cancer therapy. The growth inhibition was most obvious in the Glu-GNP group, and was significantly more compared to the control, x-ray alone and TGS-GNP groups. These

FIGURE 6. Comparison of radiosensitivity enhancements of between DU-145 cells induced by Glu-GNPs and those induced by TGS-GNPs. The combination of Glu-GNP with X-ray induced an increased inhibition rate of 50.3% and 38.7% compared with GNPs only after 24h ($P<0.005$), and 48h ($P<0.001$) respectively.

results demonstrated that Glu-GNPs increased cellular uptake and enhanced cancer cell killing.

As a heavy metal, gold increases the f-factor and enhances radiosensitivity. In our previous *in vitro* study, the radiotherapy significantly killed more breast cancer cells that were treated with GNP compared to those without GNP.¹⁷ The use of GNPs to enhance radiotherapy has been demonstrated before in mice⁶. Mice with subcutaneous breast cancers were divided into three groups, one receiving GNP injection prior to 250 KVp x-ray radiotherapy. The second group received radiation only, and the last group received GNPs only. One year survival was 86% for the GNP and radiation group, versus 20% for radiation alone and 0% for gold alone. The authors of the study also postulated the increased radiosensitivity was due to high-Z radio-enhancement by gold particles. With the present experiments, we are the first group worldwide to demonstrate *in vitro* the increase of radiosensitivity in prostate cancer cells with the use of both GNPs and Glu-GNPs.

Fluorodeoxyglucose (FDG) has been widely used in clinical oncology as a tracer to bring isotopes to

cancer locations for positron emission tomography (PET)¹⁶. This unique metabolic characteristic of malignant cells can be used for targeted delivery of nanoparticles. In this study, we observed that in prostate cancer DU-145 cells, Glu-GNP uptake was 7.35 times more than GNPs without glucose binding. This result indicated that the glucose can help deliver GNPs into glucose-metabolizing cells to achieve the targeted delivery and localized GNPs in DU-145 cells. Our next step is to bind FDG with metallic nanoparticles to enable combined radiologic, diagnostic and radio-therapeutic effects on cancer.

In conclusion, both TGS-GNPs and Glu-GNPs demonstrated improved radiosensitivity on DU-145 prostate cancer cells *in vitro*. Cell uptakes of Glu-GNPs were significantly increased and the cell-killing effects were enhanced compared to GNPs without glucose binding when 200 kVp X-ray was applied. These results suggest the promising clinical applications of the nanoparticles in future cancer treatment, targeting high radiation doses to metabolically active tumour cells, but sparing adjacent normal tissues.

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References

1. Walsh PC, DeWeese TL, Eisenberger MA. Clinical practice. Localized prostate cancer. *N Engl J Med*. 2007; 357:2696-705.
2. Kuban DA, Potters L, Lawton CA et al. Prostate Cancer. In: Gunderson LL, Tepper JE, eds. *Clinical Radiation Oncology*. Philadelphia, PA: Elsevier Churchill Livingstone; 2007:1165-236.
3. Nguyen PL, Zietman AL. High-dose external beam radiation for localized prostate cancer: current status and future challenges. *Cancer J*. 2007;13:295-301.
4. Kuban DA, Tucker SL, Dong L, et al. Long-term results of the M. D. Anderson randomized dose-

- escalation trial for prostate cancer. *Int J Radiat Oncol Biol Phys.* 2008; 70:67-74.
5. Eade TN, Hanlon AL, Horwitz EM, et al. What Dose of External-Beam Radiation is High Enough for Prostate Cancer? *Int J Radiat Oncol Biol Phys.* 2007;68:682-9.
6. Hainfeld JF, Slatkin DN and Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. *Phys Med Biol* 2004; 49:N309-15.
7. Yih TC, Wei C. Nanomedicine in cancer treatment. *Nanomedicine: Nanotechnology, Biology and Medicine* 2005;1:191-2.
8. Kawasaki ES, Player A. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. *Nanomedicine: Nanotechnology, Biology and Medicine* 2005;1:101-9.
9. Hirsch LR, Stafford RJ, Sershen NJ et al. Nanoshell-assisted tumor ablation using near infrared light under magnetic resonance guidance. *Proc Natl Acad Sci* 2003;100:113549-54.
10. Loo C, Lowery A, Halas N et al. Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.* 2005;5:709-11.
11. Cho SH. Estimation of tumor dose enhancement due to gold nanoparticles during typical radiation treatments: a preliminary Monte Carlo study. *Phys Med Biol* 2005;50:N163-73.
12. Ellen E. Connor, Judith Mwamuka, Anand Gole, Catherine J. Murphy, Michael D. Wyatt. Gold Nanoparticles Are Taken Up by Human Cells but Do Not Cause Acute Cytotoxicity *Small.* 2005;1:325-7.
13. Andre Nel, Tian Xia, Litz Madler, Ning Li. Toxic Potential of Materials at the Nanolevel, *Science* 2006; 311:622-7.
14. Gallego O, Puentes V. What can nanotechnology do to fight cancer? *Clin Transl Oncol.* 2006;8:788-95.
15. Khan FM. *The Physics of Radiation Therapy.* Third Edition. Philadelphia, PA: Lippincott Williams and Wilkins; 2003:106-56.
16. Weir L. Worsley D. Bernstein V. The value of FDG positron emission tomography in the management of patients with breast cancer. *Breast Journal.* 2005; 11:204-9.
17. Kong, T; Zeng, J; Yang, J, Yao, Y; Wang, X; Roa, W; Xing, JZ; Chen, J. Surface Modifications of Gold-nanoparticles to Enhance Radiation Cytotoxicity. (**Best Paper Award**), IEEE/NIH workshop on life science systems and applications. Nov. 8-9 at the Lister Hill, National Institute of Health, USA. Nov 8-9, 2007
18. Xing, J.Z.; Zeng J.; Yang, J.; Tao, K.; Xu, T.; Roa, W.; Wang, X.P.; Chen, J. Gold-based nanoparticles for breast cancer diagnosis and treatment. *Proceedings of 2007 IEEE International Symposium of Circuits and Systems.* New Orleans, USA. May 27-30, 2007.
19. B. Devika Chithrani, Arezou A. Ghazani, and Warren C. W. Chan. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano Lett.* 2006; 6: 662-8.
20. Betancur-Galvis LA, Morales FE, Forero JE, Roldan J. Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Mem Inst Oswaldo Cruz,* 2002;97:541-6.
21. Whelan LC, Ryan MF. Ethanolic extracts of *Euphorbia* and other ethnobotanical species as inhibitors of human tumor cell growth. *Phytomedicine.* 2003;10:53-8.

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