

ADVANCES IN CANCER RESEARCH USING GOLD NANOPARTICLES MEDIATED PHOTOTHERMAL ABLATION

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Abstract

Recent research suggests that nanotechnologies may lead to the development of novel cancer treatment. Gold nanoparticles with their unique physical and chemical properties hold great hopes for the development of thermal-based therapies against human malignancies. This review will focus on various strategies that have been developed to use gold nanoparticles as photothermal agents against human cancers.

Keywords: gold nanoparticles, photothermal ablation, cancer

Introduction

The gold standard approaches for cancer treatment today are surgery, radiation and chemotherapy. Even if these conventional methods have been improved, there are still requirements for novel treatments that could destroy cancer cells without harming the healthy tissue [1-5]. Nanooncology represent a rapidly developing research field with great potential in fighting against a complex disease like cancer. Although nanoparticles are small in size, nanometric drug delivery carriers and bio-active nanomaterials are large enough to attach multiple functional moieties to the cell surface, allowing significant enhancement of their properties and distributions in living systems. Generally, the safety/toxicity of the nanoparticles depends on shape, surface coating and their electrical charge. It has been stated that the attachment of a targeting moiety, may optimize these nano-biosystems for a specific biomedical application and help prevent unwanted side effects [6-9].

Gold nanoparticles mediated thermal ablation

of tumors following exposure to NIR light is superior to conventional techniques, since is minimally invasive and easy to apply, with great capacity to destroy malignant lesions in vital regions where surgical resection is not feasible. Radiofrequency techniques or other thermally destructive methods cannot differentiate between tumor and surrounding normal tissue, often affecting the healthy tissue as well [10-12]. Photothermal therapy employing gold nanoparticles (irradiated with near-infrared laser that excites electrons of different atomic levels and sub-levels to ground state by emitting the energy in the form heat) can destroy cancerous cells and is able to produce selective necrosis at cellular levels [13-18].

With reference to the recent research data, we address here the current role of gold nanoparticles as photothermal anticancer agents.

Use of gold nanoparticles as photothermal agents against human cancers: recent developments

Zhang and co-workers used PEG-coated gold nanoparticles to enhance the in vitro and in vivo radiation response of gold NPs. They used multiple size nanoparticles such as 4.8, 12.1, 27.3, and 46.6 nm PEG-coated gold NPs in HeLa cells [19]. The toxicities of the PEG-coated gold

NPs with different sizes were investigated 24 and 48 hours after treatment. Apoptosis measurements were performed to evaluate the enhancement of radiation effects. Their conclusion was that PEG-coated gold nanoparticles can cause a significant decrease in cancer cell survival after gamma radiation. 12.1 and 27.3 nm PEG-coated gold nanoparticles proved to be highly dispersive in the cells and displayed stronger sensitization effects than 4.8 and 46.6 nm particles by both cell apoptosis and necrosis. The authors also performed *in vivo* by evaluating tumor size and weight after 5 Gy gamma radiations radiosensitization effects on 4.8, 12.1, 27.3, and 46.6 nm PEG-coated gold particles showing that 12.1 and 27.3 nm PEG-coated gold nanoparticles show high radiosensitivity and may be used for cancer photothermal ablation [19].

In one study performed by Kuo et al. 35 nm gold nanorods (Au NRs) and 50 nm gold nanoparticles (Au NPs) were used to test two different approaches of cancer thermal therapy: photodynamic therapy (PDT) and photothermal therapy (PTT). In this Au NRs and Au NPs were functionalized with a hydrophilic photosensitizer, indocyanine green (ICG). The authors showed that combination of PTT and PDT proved to be efficient in killing cancer cells as compared to PTT or PDT treatment alone because its stability [20].

A novel, 4-component bio-nanosystem composed of a phthalocyanine photosensitizer drug and a primary antibody (Anti-HER2) specific to cell surface receptors attached on a 4 nm gold nanoparticle was used to enhance drug targeting of breast cancer cells that overexpress the HER2 receptor on the cellular surface. The authors showed that the targeting capability of the 4-component nanoparticle conjugates enhances the efficacy of PDT cell death when tumor-associated antigens are present on the cytoplasmic membrane of the malignant cells [21].

Multifunctional doxorubicin (DOX)-loaded hollow gold nanospheres (DOX@HAuNS) against EphB4 receptors were administered in EphB4-positive tumors both *in vitro* and *in vivo*. *In vivo* release of DOX from DOX@HAuNS, triggered by NIR laser, was assessed by dual-radiotracer technique suggesting significantly decreased tumor growth when compared with treatments with nontargeted DOX@HAuNS plus laser or HAuNS plus laser. The authors obtained encouraging results may have promise as a new anticancer therapy: tumors in 6 of the 8 mice treated with T-DOX@HAuNS plus laser regressed completely with only residual scar tissue by 22 days following injection, and none of the treatment groups experienced a loss in body weight [22].

A nanoparticle-based probe (composed of 10 nm spherical gold nanoparticle (NP) with pH-responsive ligands and Raman probes on the surface) was tested as 'turn-on' theragnostic agent for simultaneous Raman imaging/diagnosis and photothermal therapy. The system was developed to provide the surface with both positive

and negative charges upon mildly acidic condition, which subsequently results in rapid aggregations of the gold NPs that provides hot spots for the SERS probe. Another advantage of this rapid aggregation was the accumulation mainly in B16 F10 mouse melanoma cells. As the result, both Raman imaging and photothermal efficacy were enhanced in treated malign cells [23].

In a study conducted by Puvanakrishnan et al. the effect of multiple dosing of GNPs and nanoparticle type to improve tumor-targeting efficiency was analyzed. Large pegylated gold nanoshells (GNSs) and small pegylated gold nanorods (GNRs) were used to compare the effect of the size of these particles on tumor targeting efficiency. Specifically, they compared the effect of single and multiple doses of GNRs and GNSs on *in vivo* tumor targeting. (Four- to six-week old nude mice (Swiss nu/nu) were inoculated with the A431 cells). Neutron activation analysis (NAA) was used to determine the amount of GNPs accumulated for the different doses and the percent accumulation of GNRs and GNSs in a squamous cell carcinoma tumor model. The authors showed a straight relationship between the efficacy of multiple dosing with increased accumulation of GNPs in tumors with low toxicity following administration [24].

Iron-oxide core/gold-shell nanoparticles 30 nm diameter were used to demonstrate that gold nanoparticles are phagocytosed by pancreatic cancer cells, thus permitting magnetic resonance imaging (MRI) of sensitizer delivery and photothermal ablation [25].

Using PANC-1 cells followed by photothermal ablation, the cell proliferation percentage changed from 100% to 71.3% and 47.0% for cells treated with 25 and 50 µg/mL GoldMag. According to the authors, photothermal ablation of PANC-1 cells demonstrated an effective treatment response, specifically a reduction to only 61%, 21.9%, and 2.3% cell proliferation for cells treated with 0, 25, and 50 µg/mL GoldMag. MRI was able to display GoldMag internalization inside PANC-1 cells. The conclusion of the study is that GoldMag nanoparticles could serve as photothermal sensitizers, and MRI is feasible to quantify intracellular internalization inside pancreatic malign cells [25].

In another study 15 nm AuNPs conjugated to covalently coupled to anti-EGFr antibodies were taken up by A431 cells in culture that catalytically aggregate them (by enzyme degradation of antibodies and pH effects), shifting their absorption into the NIR region, thus amplifying their photonic absorption. Treatment of human squamous cell carcinoma A431 (positive for epidermal growth factor receptor EGFr) murine xenografts with anti-EGFr antibodies conjugated to AuNPs and NIR resulted in complete tumor ablation in most cases with no adverse effects on the surrounding healthy tissue [26].

Several authors developed an aptamer switch probe (ASP) linking chlorin e6 (Ce6), to the surface of gold nanorods (AuNRs) to target a leukemia cancer cell line for photodynamic therapy (PDT) and photothermal therapy

(PTT) applications. In the presence of target cancer cells, the ASP changes conformation to drive Ce6 away from the gold surface, thereby producing singlet oxygen for PDT upon light irradiation. In addition, absorption of radiation by the gold nanorods enables further cell destruction by the photothermal effect. The authors claim that their approach offers a remarkably improved and synergistic therapeutic effect compared to PTT or PDT alone, providing high specificity and therapeutic efficiency [27].

Several authors [28] developed a bionanosystem composed of polydopamine polymerized onto gold nanorods, and EGF receptor antibodies (anti-EGFR) immobilized onto the layer. Antibody-functionalized NRs were significantly more toxic to cancer cells (OSCC15, MDA-MB-231 and MCF7) *in vitro* compared with untargeted NRs when irradiated with a broadband light source. PD-mediated surface modification is suggested to be a useful strategy for the conjugation of cancer-specific ligands to nanoparticle surfaces, enabling the formation of biofunctional diagnostic and therapeutic metal nanoparticles. Polydopamine-enabled surface functionalization of gold nanorods provided a potent light-activated photothermal therapeutic response, producing significant photoinduced toxicity of breast and oral cancer cells following NIR irradiation ((480–850 nm), 60 W/cm² for 5, 10 or 15 min [28]).

In another study, gold nanoshells were encapsulated in silica epilayers doped with iron oxide and further labeled with antibodies targeting NGAL in AsPC-1-derived xenografts in nude mice. The authors showed that AntiNGAL-conjugated TGNS specifically targeted pancreatic cancer cells-AsPC-1 (human, pancreas, adenocarcinoma, derived from metastatic ascites) *in vitro* and *in vivo* providing contrast for both NIR fluorescence (808 nm for 10 min at a power density of 5.81 W/cm²) and T2 weighted MR imaging with higher tumor contrast than can be obtained using long-circulating but non-targeted PEGylated nanoparticles. The nanocomplexes also enabled highly specific cancer cell death via NIR photothermal therapy *in vitro* [29].

In a very recent paper several authors report the construct of a biodegradable plasmon resonant liposome gold nanoparticles system (LiposAu NPs) with high thermal response in NIR irradiation. The pharmacokinetic study of LiposAu NPs were conducted on a small animal model showing that degradation of this nanoparticles occurs in hepatocytes and are excreted through the hepato-biliary and renal route. The authors next studied the therapeutic potential of LiposAu NPs on a mouse tumor xenograft model using NIR laser (750nm) illumination. The authors claim that resulting destruction of tumor mass was obtained combined with the prolonging of disease-free interval. Their ability to generate massive amount of γ H2A.X foci is a strong indicator of the mode of tumor ablation by DNA double strand breaks. Applicability of such novel biodegradable hybrid nanoparticle system holds great promise in cancer

nanotherapeutics [30].

In another study, a combined of photothermal and pharmacological effect in nanoparticle-drug conjugates was exploited. The authors used a thermosensitizing cytokine-tumor necrosis factor- α conjugated to 30 nm gold nanospheres (Au-TNF) to treat murine SCK and 4T1-GFP breast carcinomas and 2H11 lymphatic endothelial cells that were next heated by laser pulses (at various wave lengths). To enhance photothermal efficiency in near-infrared window of tissue transparency the authors analyzed slightly ellipsoidal nanoparticles, their clustering, and laser-induced nonlinear dynamic phenomena leading to amplification and spectral sharpening of photothermal and photoacoustic resonances red-shifted relatively to linear plasmonic resonances. Using a murine carcinoma model, they obtained higher therapy efficacy of Au-TNF conjugates compared to laser and Au-TNF alone or laser with TNF-free gold nanospheres [31].

We have recently showed that LASER photoexcited gold nanoparticles interfere with the mitochondrial electron transfer chain through interaction of the activated electrons from the surface of pre-GNP with the electrons within the mitochondrial membrane in pancreatic cancer cells [32]. Following this interaction, PANC-1 cells exhibit increased mitochondrial permeability transition (MPT) that generates immediate dissipation of the mitochondrial membrane potential and osmotic mitochondrial lysis. In addition we have used quantitative proteomic techniques to prove that treatment with activated gold nanoparticles leads to inactivation of Bcl-2 anti-apoptotic proteins causing a decrease of mitochondrial transmembrane potential (MTP) followed by cellular necrosis. We also identified an upregulation of the COX 5b factor following the treatment with photoexcited gold nanoparticles strongly suggesting that mitochondria enter a hypoxic state when treated with GNPs having electrons in a photoexcited state. The observation was correlated with the downregulation of a subunit of complex V (ATP5H) which is known to be involved in oxidative phosphorylation (since it generates an electrochemical gradient of protons across the inner membrane) leading to defective mitochondrial oxidative phosphorylation (OXPHOS). Since cancer cells exhibit increased metabolic activity, their mitochondria are fundamentally different compared with normal cells and exhibit an extensive metabolic reprogramming. This represents the underlying mechanism that renders malign cells more susceptible to mitochondrial dysfunction than benign cells [32].

Conclusion

We may assert that photothermal therapy using functionalized gold nanoparticles represents a feasible therapeutic method against human malignancies. However further studies are required in order to assess the interactions of nanoparticles with biological systems.

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