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Complete destruction of deep-tissue buried tumors via combination of gene silencing and gold nanoechinus-mediated photodynamic therapy

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ABSTRACT

Cancer is one of the major diseases leading to human deaths. Complete destruction of deep tissue-buried tumors using non-invasive therapies is a grand challenge in clinical cancer treatments. Many therapeutic modalities were developed to tackle this problem, but only partial tumor suppression or delay growths were usually achieved. In this study, we report for the first time that complete destruction of deep tissue-buried tumors can be achieved by combination of gold nanoechinus (Au NEs)-mediated photodynamic therapy (PDT) and gene silencing under ultra-low doses of near infra-red (NIR) light irradiation (915 nm, 340 mW/cm^2; 1064 nm, 420 mW/cm^2) in the first and second biological windows. The average lifespan of the mice treated by the above combined therapy is beyond 40 days, which are ~2.6 times longer than that (15 days) observed from the anticancer drug doxorubicin-treated group. The current study points out a new direction for the therapeutic design to treat deeply seated tumors in future cancer treatments.

1. Introduction

Treating deep tissue-buried tumors with the existing medical technologies is one of the long-lasting challenges in the cancer therapy [1,2]. Clinically, it is sometimes impossible to treat the deep tissue-buried tumors (for example, lung and liver cancers), especially when a patient's physical condition cannot co-operate and tolerate with the invasive surgical treatments [3]. Therefore, there is a great demand for the development of less or non-invasive modalities that can treat the complicated deeply seated tumors. In the recent years, phototherapies were widely explored for treating various types of cancers [4,5]. The major advantages of phototherapies are (1) non-invasiveness, (2) superior tissue penetration, (3) reduced side effects, and (4) more cost-effective than conventional treatment modalities. Photodynamic therapy (PDT) was emerged where it involves the use of an organic photosensitizer (PS) molecule to absorb and transfer the photon energy to the normal tissue oxygen ($^3O_2$) to generate cytotoxic singlet oxygen ($^1O_2$), which is able to kill cancer cells [6,7]. Most of the clinical PDT treatments are restricted to surface tumors, because organic PS can be photochemically excited by either uv or visible light, which have very short tissue penetration depths. To treat deep tissue-buried tumors using PDT, one has to use NIR light as the light source since NIR light has large tissue penetration depths. However, organic PS able to be excited by NIR light is very rare. Therefore, it leaves a grand challenge to treat deep seated tumors with the existing photodynamic therapeutic drug molecules.

To overcome the absorption of NIR light and excitation of organic photosensitizers, upconversion nanoparticles (UCNPs) co-doped with organic PS were developed to absorb and convert the deeply penetrating NIR light to visible wavelengths, which was then used to photochemically excite the co-loaded organic PS to facilitate organic PS-mediated PDT in treating deep tissue buried tumors [8–10]. In such kind of nanomaterial design, partial tumor suppression or delay tumor growths were usually achieved. Many limitations are associated with the design of UCNPs-doped organic PS-mediated PDT, for example, (1) only a single choice of

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wavelength of 980 nm can be used to excite UCNPs; (2) high incident laser power (0.33–2.5 W/cm^2) [8–10] has to be used due to the very low extinction coefficient of UCNPs at 980 nm; (3) water has very strong absorption at 980 nm wavelength and thus creates the overheating problem; (4) very low singlet O_2 generation efficiency was obtained due to the multi-step processes (via UCNPs light absorption efficiency x upconversion emission quantum yield × efficiency of light absorption of organic photosensitizers x singlet O_2 sensitization efficiency of organic photosensitizers); and (5) only delay growth or partial suppressions, rather than complete destruction, of tumors was achieved [8–10]. Previously, gold nanorods-in-shell particles were reported to be able to absorb and convert 1064 nm light (NIR II) to heat and mediate photothermal therapy (PTT) effect for the treatment of surface tumors. However, only partial tumor suppression was achieved, albeit a very strong incident laser power of 3 W/cm^2 was used. The use of high laser power, beyond the skin tolerance threshold value (0.42 W/cm^2) prescribed by the American National Standards Institute (ANSI), severely limits its practical clinical applications [11]. Previously, we have reported a morphology of a gold nanostructure, called gold nanoechinus (Au NEs), which possesses ultra-high molar extinction coefficients (~10^{12} $M^{-1}cm^{-1}$) and can also sensitize formation of singlet oxygen in the NIR windows I and II to exert *in vivo* nanomaterial-mediated photodynamic and photothermal therapeutic (NmPDT & NmPTT) for the destruction of surface tumors in mice [12]. Due to its exceptionally high extinction coefficients in the NIR windows I and II, this unique nanomaterial might have great potential for treating deep tissue-buried tumors, which is not yet proven or demonstrated.

It is often highly difficult to achieve successful therapeutic destruction of deep tissue-buried tumors using a single treatment modality. Gene therapy involves knocking down or silencing of specific oncogenes, and plays a vital role in treating surface tumors [13,14]. Superoxide dismutase 1 (SOD1) is one of the very effective anti-apoptotic and self-defending genes, and can destroy the free radicals or reactive oxygen species in the human body. Knocking down of anti-apoptotic genes can trigger apoptosis and induce cellular deaths very efficiently [15]. Using gene silencing alone, partial suppression, rather than complete destruction, of tumor growth was usually observed [13–15]. Combining gene therapy and NmPDT for the treatment of deep tissue buried tumors might be a promising modality for treatment of solid tumors, and was not yet reported or demonstrated. To the best of our knowledge, the current work is the first demonstration that complete destruction of deep tissue buried tumors can be achieved with the ultra-low doses of NIR light by combining SOD1 gene silencing and Au NEs-mediated PDT in the second biological window (NIR II; 1064 nm).

2. Materials and methods

2.1. Preparation of lipid-coated Au NEs

Gold nanoechinus were synthesized according to the previously reported literature procedure [12]. For the preparation of lipid-coated Au NEs, the as-synthesized gold nanoechinus were washed using DI water for two times. Then, Au NEs were re-dispersed in a Lipofectamine 2000 (LP-2000) reagent (100 μL, Invitrogen, USA)-containing aqueous solution to promote the formation of lipid-bilayer coated Au NEs.

2.2. Detection of singlet oxygen phosphorescence emission

The lipid-coated Au NEs (1 mg/mL) dispersed in D_2O was used for the singlet oxygen phosphorescence measurements. The singlet O_2 phosphorescence emission was detected using a FLS 920 (Edinburgh) spectrometer, which uses a 450 W broadband Xe lamp as a light source. An 1100 nm long pass filter (Newport, LP1100) was placed in-between the detector and the sample to cutoff both the stray light and the scattering light having wavelengths shorter than 1100 nm.

2.3. Preparation of siRNA-lipid-coated Au NEs

A stock solution of 0.5 μM of superoxide dismutase 1 (*SOD1*) siRNA (siGENOME Human *SOD1* (6647), siRNA SMART pool, Thermo Scientific, USA) was diluted to 100 nM using RNAse free water. Then the stock solution of lipid-coated Au NEs (1 mg/mL) was added to the dilute siRNA solution and vortexed for few minutes and kept undisturbed for 15 min at room temperature to form a stable complex before use.

2.4. Particle size and zeta-potential measurements

The average particle size of lipid-coated Au NEs was measured using dynamic light scattering (DLS, W3180, Microtrac). The zeta-potential (Zetasizer, Malvern, USA) values were also measured for as-synthesized Au NEs, lipid-coated Au NEs and siRNA-lipid-coated Au NEs respectively in PBS buffer (pH ~ 7.4) solution.

2.5. Cell culture procedures

HeLa (human cervical cancer) and B16F0 (mouse skin cancer) cells were cultured in Dulbecco's Minimum Essential Medium (DMEM, Gibco, USA) containing 10% heat-inactivated fetal bovine serum (Invitrogen, USA), 100 μg/ml penicillin, 2 mM L-glutamine, and 100 U/mL streptomycin. The cells were grown under anaerobic conditions in an incubator maintained at 37 °C (95% humidity, 5% CO_2).

2.6. In vitro cellular uptake experiments using confocal laser scanning microscopy (CLSM)

HeLa cells (2.0 × 10^5 cells per well in 6-well plates) were cultured in a medium containing serum medium. Cells were treated siRNA-lipid-coated Au NEs nanoconjugates of different concentrations for 4 h. The cells were washed with a phosphate buffer solution (PBS, pH 7.4), further fixed onto a glass slide using paraformaldehyde solution (4%) in PBS and washed with PBST (5% Tween-20 in PBS) solution for one time. Finally, the HeLa cells nuclei were stained using DAPI dye and further subjected to confocal laser-scanning microscope (Leica, TCS SP5 X). The confocal optical microscope was equipped with a UV laser (λ_{ex} = 404 nm) and a white light laser (λ_{ex} = 470–670 nm).

2.7. Isolation of total RNA from siRNA-lipid-coated Au NEs internalized HeLa cells

HeLa cells were cultured in a 6-well plate with a cell number of 2.0 × 10^5 cells per well and incubated for 24 h. The cells were treated with siRNA-lipid-coated Au NEs nanoconjugates of different concentrations for 4 h. The cells were then washed with a phosphate buffer solution (PBS, pH 7.4) and incubated for an additional time of 24 h, so that the cells would be ~70% confluent. After 24 h, the cells were washed with PBS for one more time and trypsinized to make a cell pellet. Then 1 mL of TRIZOL (Sigma–Aldrich) reagent was added to the cell pellet and the contents were kept at −80 °C for 24 h. The contents were then centrifuged at 12,000 rpm for 10 min at 4 °C, and 200 μL of chloroform was added into it and vortexed for 20 s and then kept it stand by in ice for 10 min. The contents were centrifuged at 12,000 rpm for 20 min at 4 °C and RNA