Cytotoxicity and Genotoxicity Induced by Photothermal Effects of Colloidal Gold Nanorods

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Gold nanorods (Au NRs) that absorb near-infrared (NIR) light have great potential in the field of nanomedicine. Photothermal therapy (PTT), a very attractive cancer therapy in nanomedicine, combines nanomaterials and light. The aim of this study was to elucidate the molecular mechanism involved in Au NR-mediated cytotoxic, genotoxic, and other biological responses, in the presence or absence of NIR irradiation. Specifically, cell death mode, generation of reactive oxygen species, DNA damage, apoptotic gene expression, and cell morphological changes induced by Au NRs under NIR irradiation were evaluated in cancer cells. In human lung adenocarcinoma epithelial cells (A549 cells), mild necrosis via DNA damage was induced by NIR responsive Au NRs. Unlike in the cancer cells, cell viability of normal human lymphocyte was not affected by the combined treatment of Au NRs and NIR irradiation. This study delineates differential cytotoxic and genotoxic susceptibility of cancer and normal cells during photothermal treatment of Au NRs. In conclusion, our results suggest that the photothermal cyto-/genotoxic activity of Au NRs is an effective method for cancer therapy in human lung cancer cells.

Keywords: Cytotoxicity, Genotoxicity, Gold Nanorods, Near-Infrared Light, Apoptosis, Necrosis, Photothermal Therapy.

1. INTRODUCTION

Nanotechnology, nanomedicine, and nanotoxicology are fields of nanoscience that aim to improve the quality of human life.1 Numerous types of nanomaterials have been developed for use in cell-based bioapplications, including semiconductor nanomaterials, mesoporous materials, and carbon-based nanomaterials.2–6 Nanomedicine is defined as the medical application of nanoparticles (NPs) to diagnose and cure disorders such as cancer and infectious diseases, and has become one of the most promising and active fields of nanotechnology.7 Additional avenues in nanomedicine can be explored when light is combined with NPs. Near-infrared light (NIR) radiation is able to penetrate the skin without significantly damaging normal tissues, and thus can be used to treat specific cells targeted by nanomaterials. Several nanomaterials that absorb NIR radiation, including gold nanoshells, gold nanorods (Au NRs), and single-walled carbon nanotubes, have potential therapeutic applications.7–9 Among these nanomaterials, Au NRs, which are rod-shaped gold nanoparticles, are especially attractive candidates for applications in photothermal therapy (PTT) because they have unique photophysical properties that can make use of broad NIR frequency absorption. Au NR-mediated PTT under NIR irradiation is considered promising for selective killing of cancer cells. The radiation that is absorbed by Au NRs can be efficiently converted into heat, causing cell destruction on a picosecond time scale. PTT, unlike conventional cancer therapies (e.g., surgery, radiotherapy, and chemotherapy), is a minimally invasive cancer treatment strategy. The PTT process converts light into heat to kill cells via hyperthermia.10 The effectiveness of PTT has been
demonstrated in cultured mammalian cells, tissues, and the mouse xenograft tumor model.\textsuperscript{11}

Gold nanoparticles (Au NPs), which have a spherical shape, have been well explored due to their unique physicochemical properties, exhibiting tremendous potential for biomedical applications.\textsuperscript{12} The toxicity of spherical Au NPs controversial due to their various sizes and use of a variety of surface modifiers.\textsuperscript{13, 14} For example, recent studies showed that Au NPs with a variety of surface modifiers were not toxic to human cells.\textsuperscript{15, 16} In contrast, it was reported that Au NPs was toxic to cells and the degree of cytotoxicity was dependent upon size (0.8–15 nm) in HeLa cells.\textsuperscript{17}

Differently from spherical Au NPs, Au NRs, which are rod-shaped, have large absorption cross section in NIR region. The absorption behavior is characterized by two plasmon resonant peaks, longitudinal and transverse plasmon peaks in the spectra.\textsuperscript{12} The Au NRs’ strong NIR absorption can cause sufficient heating to kill cells for cancer therapeutic application. The surfactant hexadecyltrimethyl-ammonium bromide (CTAB) is used during the synthesis of Au NRs in order to control their size and shape. In many cases, it is not so much the toxicity of Au NR itself as that of CTAB molecule that is responsible for the Au NR-mediated cytotoxicity.\textsuperscript{18} Thus, many studies have been performed to remove CTAB from Au NRs or encapsulate them in order to reduce the toxicity.\textsuperscript{19} However, the cyto-/genotoxicity and the detailed mechanism induced by CTAB free gold nanorods in human cells remains to be settled. Moreover, many researches about the photothermal effect of Au NRs have been focused only on the development of efficient PTT system;\textsuperscript{20, 21} however, there have been little studies regarding the cyto-/genotoxic effects induced by Au NRs in human cells. Given the importance of Au NRs in nanomedicine, it is necessary to scrutinize the Au NRs’ cyto-/genotoxicity in biological system. Although several studies have applied PTT to lung cancer using gold nanoshells, Au NPs, and Au NRs under NIR irradiation,\textsuperscript{22–24} no such studies has been performed to examine differential effects of Au NRs between normal and cancerous cells under NIR irradiating PTT conditions.

Here, we investigated the cyto-/genotoxic effects induced by Au NR treatment with NIR irradiation in normal human blood lymphocytes and lung cancer cells. This is the first study to demonstrate genotoxic effect as well as cytotoxic effect induced by Au NRs under NIR irradiation in A549 cancer cells.

2. MATERIALS AND METHODS

2.1. Cell Culture

Adenocarcinomic human alveolar basal epithelial cells (A549 cells) were obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured in RPMI 1640 medium supplemented with filtered 10% fetal bovine serum (FBS), along with penicillin and streptomycin (100 U/mL of each) in a humidified atmosphere at 37 °C and 5% CO\textsubscript{2}.

Freshly drawn heparinized blood from a donor was used to isolate lymphocytes by Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) density gradient centrifugation at 400 × g. After the lymphocytes were extracted from the gradient interface, they were washed twice with phosphate buffered saline (PBS) and resuspended in RPMI 1640 medium (Gibco, Invitrogen, CA, USA) containing 10% FBS and 100 U/mL each of penicillin and streptomycin. Lymphocytes were stimulated with 1% phytohemagglutinin (PHA; Gibco, Invitrogen, CA, USA) and cultured in a humidified atmosphere at 37 °C and 5% CO\textsubscript{2}.

2.2. Preparation and Characterization of Au NRs

Au NRs solubilized with 1% cetyl trimethyl ammonium bromide (CTAB) were obtained from Nanopartz Inc. (peak LSPR wave, 808 nm; axial diameter, 10 nm; long size, 41 nm; Loveland, CO, USA). To remove excess CTAB, Au NRs solutions stabilized with CTAB were centrifuged at 10,000 rpm for 15 min\textsuperscript{25} and resuspended in RPMI 1640 medium. This procedure was repeated twice. Surface plasmon resonance (SPR) peaks were measured by UV-vis spectrophotometry (Jasco V-650 UV/vis spectrophotometer; Tokyo, Japan). Dynamic light scattering (DLS) is an indirect method to measure particle size. Briefly, scattered light intensity fluctuations caused by Brownian motion of the particles in suspension are measured. DLS was performed at room temperature with an ALV/CGS-3 Compact Gonimeter System equipped with a He-Ne laser operating at 632.8 nm (Hessen, Germany). The scattering angle was 90°. The Au NR size distribution was determined using a constrained regularization method in RPMI 1640 medium. The Au NR surface charge was measured in distilled water using a Zeta potential analyzer (Zeta plus; Brookhaven Instruments Corporation, NY, USA). Au NRs cellular uptake was observed by transmission electron microscopy (TEM). A549 cells were exposed to Au NRs (10 µg/mL) for 4 h, and then cells were trypsinized and washed with PBS buffer to remove non-internalized Au NRs. The pellet was fixed overnight with cold 2.5% cacodylate buffered glutaraldehyde (Sigma-Aldrich, St. Louis, Mo, USA). Cells were centrifuged at 1,100 rpm for 5 min and sectioned at 500–650 nm thickness and examined with a JEOL 100CX electron microscope (Jeol-LTD, Tokyo, Japan).

2.3. Cytotoxicity and Photothermal Effects Induced by Au NRs

Au NRs with NIR-induced cytotoxic effects and photothermal effects were evaluated using the water soluble tetrazolium salt (WST) assay (Enhanced Cell Viability
2.4. Single Cell Gel Electrophoresis Assay

The subsequent steps of the single cell gel electrophoresis assay were performed as previously described by Singh et al. A549 cells were treated with Au NRs for 3 h, followed by NIR irradiation for 10 min. After being washed twice with PBS, the cells were trypsinized, resuspended in RPMI 1640 medium, and then maintained at 4 °C to prevent DNA repair. Slides were prepared according to the method of Singh. Briefly, images of 60 randomly selected cells were analyzed from each sample using the Komet 5.5 image analysis system (Kinetic Imaging Limited, Nottingham, UK). The olive tail moment (OTM) of each cell was measured under a fluorescence microscope (Nikon, Tokyo, Japan) equipped with a 515–560 nm excitation filter and a 590 nm barrier filter.

2.5. Cytokinesis-Block Micronucleus Assay

The cytokinesis-block micronucleus (CBMN) assay was performed as described by Fenech. A549 cells were cultured in RPMI 1640 medium supplemented with 10% FBS. Cells were then irradiated by a NIR laser after 3 h of Au NRs treatment. Cytochalasin B (4 μg/mL; Sigma-Aldrich) was added 20 h after the start of the culture and incubation continued for an additional 28 h.

Cells were harvested after 48 h total incubation. Briefly, they were treated with 0.075 M KCl hypotonic solution for 1 min and washed twice with fixative solution (a 3:1 mixture of methanol and acetic acid). Air-dried cell preparations were stained with Giemsa solution (5%). Slides with a total of 1,000 binucleated cells with well-preserved cytoplasm were scored according to standard criteria.

2.6. Measurement of Apoptosis and Necrosis

Apoptotic cells induced by Au NRs treatment with or without NIR were assessed using an apoptosis assay kit (Cell Death Detection ELISAPLUS kit; Roche Applied Science, Mannheim, Germany) according to the manufacturer’s protocol. A549 cells were treated with Au NRs in the presence or absence of NIR irradiation and then incubated in lysis buffer. After lysis, the supernatant was transferred to the streptavidin-coated well of a microplate and incubated for 2 h at room temperature to bind nucleosomes in the supernatant to the antibodies. Then, the immobilized antibody-histone complexes were washed three times to remove cell components that were not immunoreactive and followed by incubation with peroxidase substrate to spectrophotometrically determine the amount of colored product. Finally, the absorbance was measured using an ELISA reader at 405 nm wavelength (Tecan, Männedorf, Switzerland).

Necrosis was determined using a lactate dehydrogenase (LDH) assay (Cytotoxicity Detection KitPLUS; Roche Applied Science, Mannheim, Germany), which measures the LDH release from necrotic cells into the medium caused by plasma membrane rupture. Cell culture was performed under the same conditions as the apoptosis assay. Absorbance at a wavelength of 490 nm was measured by a microplate reader (Tecan, Männedorf, Switzerland).

2.7. Measurement of ROS

The level of intracellular ROS generation was determined using 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (DCFDA; Molecular Probes, Eugene, OR, USA). DCFDA penetrates the cells, which is hydrolyzed by esterase and converted into the fluorescent DCF in the presence of oxidative species. A549 cells were treated with Au NRs for 3 h, and then were irradiated with NIR for 10 min. After 1 h, samples were centrifuged and new medium containing 20 μM DCFDA was added in the dark. The cells were then incubated for 20 min at 37 °C and monitored using a fluorescence spectrophotometer at 535 nm emission with an excitation wavelength of 485 nm (Tecan, Männedorf, Switzerland).

2.8. RNA Extraction and Quantitative Real Time Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from A549 cells after Au NRs treatment in the presence or absence of NIR irradiation using the AxyPrep Multisource Total RNA Miniprep Kit (Axxygen Biosciences Union City, CA, USA) according to the manufacturer’s instructions. cDNA was synthesized using an AccuPower RT PCR Premix kit (Bioneer, Daejeon, Korea). Real-time polymerase chain reaction (PCR) of each sample was performed using the Express Start SYBR Green qPCR Supermix (Applied Biosystems, Carlsbad, CA, USA) under the following thermal cycling conditions: incubation at 94 °C for 5 min, cycled 40 times
Photothermal Cyto-/Genotoxicity Induced by Gold Nanorods

Choi et al.

Table I. Primer sequences used for quantitative real-time PCR.

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Sequence (5′-3′)</th>
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<tbody>
<tr>
<td>ATM</td>
<td>–CGAGGTGACGCCGATCACAA–; –TTGGCCCACAGCAACCTT–</td>
</tr>
<tr>
<td>p53</td>
<td>–GTGACGCCTTCGGAGATGTC–; –ATGCAGGAGGTAGATGAC–</td>
</tr>
<tr>
<td>bax</td>
<td>–CAAACTGTGCTCAAGGCC–; –GCACCCCGCCACAAAGAT–</td>
</tr>
<tr>
<td>GAPDH (endogenous control)</td>
<td>–GGAAGGTGAAGGTCGGAGTCA–; –GTCATTGATGGCAACAATATCCACT–</td>
</tr>
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</table>

at 94 °C for 15 sec, 55 °C for 30 sec, and 72 °C for 30 sec. Each assay was performed in triplicate, using the Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). GAPDH was used as an endogenous control to normalize expression. The sequences of the synthesized primers of the PCR products are shown in Table I. The data are expressed as the ratio of ATM, p53, and bax relative to a reference using the comparative threshold cycle method \(2^{-\text{ΔΔCt}}\). \(\Delta\text{ΔCt}\) refers to the difference in the values of \(\text{ΔCt}\) between the test group and the reference.

2.9. Statistical Analysis

The effects of Au NRs with/without NIR irradiation on DNA damage was tested using the non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) test and Mann-Whitney test. One-way ANOVAs followed by Dunnett’s or Tukey’s post hoc tests were performed to examine cell viability, ROS generation, induction of apoptosis and necrosis, and mRNA gene expression using SPSS statistics, v.12 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Physicochemical Characterization and Cellular Internalization of Au NRs

To remove excess CTAB from Au NRs, the Au NR solution was centrifuged at 10,000 rpm for 15 min and resuspended in RPMI 1640 medium. These CTAB-free Au NRs dispersed in RPMI 1640 media were used for all experiments in this study. The average Au NR size was 119±4.24 nm, as determined by DLS. Although Au NR size slightly increased due to aggregation following CTAB removal, the average size was still in the low nanometer range. The Zeta (ζ) potential of the Au NRs was +25.76±3.39 mV, which confirmed the colloidal stability of the Au NR suspension. From the Au NRs UV-vis spectra in Figure 1(A), the absorption maximum (\(\lambda_{\text{max}}\)) was approximately 808 nm, which confirmed that the Au NRs’ photophysical properties were not changed after CTAB removal and solvent exchange. The shape and size of Au NRs were determined in TEM images. The Au NRs aspect ratio (longitudinal axis/short axis) was 4.27±1.08 (a total of 100 particles were counted) [Fig. 1(B)]. After the treatment of Au NR for 4 h, localization of Au NRs within the intracellular compartment could be clearly observed [Fig. 2].

3.2. Combined Effect of Au NR and NIR Irradiation on Cell Morphology

A549 cells exhibited changes in cell morphology after exposure to NIR irradiation for 10 min and the following 4 h of incubation in the presence of Au NRs. In contrast, cells irradiated with NIR in the absence of Au NRs maintained normal morphologies [Fig. 3(B)]. Likewise, cells treated with Au NRs alone did not show...
any morphological changes [Fig. 3(C)]. Conversely, microscopic phase contrast images showed that the cells treated with Au NRs (20 μg/mL) in combination with NIR exhibited morphological abnormalities compared to other groups [Figs. 3(E); (F)]. The majority of A549 cells in this group became rounded, which is likely resulted from hyperthermic cellular toxicity (Fig. 3).

3.3. Cytotoxicity of Normal Lymphocytes and A549 Cells Induced by Au NRs Under NIR Irradiation Condition

To assess the potential photothermal cytotoxicity induced by Au NRs with/without NIR, cell proliferation assays were performed in normal lymphocytes and A549 human lung adenocarcinoma cells using WST assays. The results revealed that while the Au NRs only treatment had a mild effect on cell viability (110 ± 5.4 for 5 μg/mL Au NRs; 90.9 ± 19.1% for 10 μg/mL Au NRs; 68.14 ± 16.7% for 20 μg/mL Au NRs), cell viability was significantly decreased (39.0 ± 7.1%) when the cells were treated with 20 μg/mL Au NRs in the combination with NIR irradiation in A549 cells [Fig. 4(A)]. In contrast, the cell viability of normal lymphocytes was not discernibly decreased in a dose dependent manner in regardless of Au NRs treatment with/without NIR irradiation [Fig. 4(B)].

3.4. Assessment of Apoptotic and Necrotic Effects

We next performed an apoptosis assay to investigate the mode of Au NRs/NIR induced cell death. We used a one-step sandwich ELISA assay, which is a colorimetric method to quantify the relative level of apoptosis. This assay uses mouse monoclonal antibodies directed against DNA and histones, which allows the accurate quantification of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. The results showed that the groups treated with Au NRs in combination with NIR irradiation showed a moderate increase in apoptosis as compared to control groups [Fig. 5(A)].

The LDH assay, a sensitive marker of cytotoxicity, was used to evaluate the induction of necrosis. The amount of LDH released increased in a statistically significant manner in the group of cells treated with both Au NRs (20 μg/mL) and NIR [Fig. 5(B)].

3.5. Measurement of ROS

The level of ROS generated in A549 cells treated with Au NRs ± NIR irradiation was analyzed using DCFDA.
Fig. 4. Cell viability assay for Au NRs in the presence or absence of NIR. Cells were incubated on a 96-well microplate for 24 h, treated with Au NRs ± NIR. Cell viabilities of A549 cells (A) and normal human lymphocytes (B) were determined by WST-1 assay. Data are shown as percentage of control and error bars represent standard errors from duplicate experiments. Results were statistically analyzed with one-way ANOVA test. * = p < 0.05 compared with control.

Intracellular ROS content slightly increased in the Au NRs treatment groups with/without NIR, but the levels were not statistically significant in either group (Fig. 6).

3.6. Gene Expression Profiles Involved in Apoptotic Signaling

A549 cells were exposed to 10 μg/mL Au NRs for 4 h and then irradiated with NIR for 10 min. After 24 h, mRNA expression levels were determined by RT-PCR. The mRNA expressions of ATM, p53, and bax genes were quantified using the 2^−ΔΔCt method. Quantitative RT-PCR primer sequences used for gene expression analysis are presented in Table I.

To address the question of whether ROS induced by Au NRs/NIR could activate apoptosis signaling pathways, we evaluated the mRNA levels of ATM, p53 and bax, which are important apoptosis signaling pathway markers (Table II). GAPDH was used as an endogenous control to normalize relative gene expression levels. In the NIR irradiation only group, the differential mRNA expression levels of ATM, p53, and bax were similar to that of the control group (cells without any treatment). Likewise,

<table>
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<tr>
<th>Concentrations (μg/mL)</th>
<th>ATM ratio relative to control</th>
<th>p53 ratio relative to control</th>
<th>Bax ratio relative to control</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Control + NIR</td>
<td>1.19 ± 0.49</td>
<td>1.45 ± 0.35</td>
<td>1.57 ± 0.54</td>
</tr>
<tr>
<td>Au NRs 10</td>
<td>1.35 ± 0.26</td>
<td>2.67 ± 0.85</td>
<td>1.82 ± 0.55</td>
</tr>
<tr>
<td>Au NRs 10 + NIR</td>
<td><strong>1.46 ± 0.12</strong></td>
<td><strong>5.59 ± 3.1</strong></td>
<td><strong>1.71 ± 0.11</strong></td>
</tr>
</tbody>
</table>

Notes: Results were analyzed using one-way ANOVAs, but no significant differences were found in either group (p > 0.05). Values represent the means of triplicate determinations ± standard errors from independent experiments.
Choi et al. Photothermal Cyto-/Genotoxicity Induced by Gold Nanorods

Fig. 6. Induction of reactive oxygen species of Au NRs under NIR in 549 cells. Bars represent standard errors. Results were statistically analyzed with one-way ANOVA, no significant differences were found in either group ($p > 0.05$).

in Au NRs-treated cells (10 [$\mu$g/mL]), the ratios of gene expression levels relative to control levels for ATM, p53, and bax were 1.35 ± 0.26, 2.67 ± 0.85, and 1.82 ± 0.55, respectively. Au NRs treatment (10 [$\mu$g/mL]) in combination with NIR irradiation resulted in a slight increase—but not statistically significant—in the mRNA expression of ATM and p53, in which the fold changes were 1.46 ± 0.12 and 5.59 ± 3.1, respectively.

3.7. DNA Damage and Genotoxicity as Evidenced by Comet Assay and Micronucleus Assay

We evaluated genotoxicity induced by Au NRs with/without NIR irradiation using the comet assay. The extent of DNA migration is represented as olive tail moments (OTMs). Significant DNA damage was observed in A549 cells treated with Au NRs (20 [$\mu$g/mL]) and NIR irradiation as compared to Au NRs-only treated cells or control cells (Fig. 7(A)).

We next performed micronucleus (MN) assay to detect genotoxic effects induced by Au NRs with/without NIR irradiation. Figure 7(B) shows the MN assay results, in which MN formation frequencies in A549 cells were 30 ± 3.54, 31 ± 1.41, 32 ± 4.95, 32 ± 2.83, 32 ± 0.71, and 47 ± 0.71 for the control, control with NIR, 10 [$\mu$g/mL] Au NRs, 10 [$\mu$g/mL] Au NRs with NIR, 20 [$\mu$g/mL] Au NRs, and 20 [$\mu$g/mL] Au NRs with NIR, respectively. The MN formation frequency was significantly increased in 20 [$\mu$g/mL] Au NRs with NIR irradiation as compared to the 20 [$\mu$g/mL] Au NRs treatment alone, indicating that the Au NRs photothermal effect could induce genotoxic effect on the cancer cells.

4. DISCUSSION

Au NRs are promising nanomaterials that have desirable physicochemical, electronic, and optical features for potential applications in medical diagnosis and therapeutic treatments. Although Au NRs have been applied in a variety of research areas, such as photothermal therapy, biosensing, and gene delivery, the surfactant CTAB can cause significant problems in Au NRs’ use in nanomedicine. CTAB is indispensable in Au NR synthesis, but it is toxic. In the current study, the cell viability was found to be less than 20% in A549 cells following 24 h treatment with Au NRs containing 1% CTAB (data not shown). Therefore, we removed CTAB by repeated centrifugation process, resuspended Au NRs in cell culture media, and made it sure that Au NRs’ physicochemical properties did not change in a condition similar to cell treatment. To remove excess CTAB, Au NRs containing CTAB were centrifuged twice at 10,000 rpm for 15 min. Although repeated centrifugation of Au NRs could result in irreversible aggregation of the particles, we confirmed that the Au NRs did not lose their physicochemical and morphological integrity as nanorods, even after double centrifugation process (Fig. 1). Au NRs (longitudinal axis:short axis = 4:1) were characterized by two spectral plasmon resonance peaks at around 808 nm and 520 nm, which revealed that their photophysical properties were well preserved even after repeated centrifugation and resuspension processes [Fig. 1(A)].

Au NRs have been used in cancer treatment for the purpose of developing effective therapeutics to treat cancer cells; however, little is known regarding their comparative and comprehensive cytotoxic effects with/without NIR irradiation on normal human lymphocytes and cancer cells. So far, most PTT studies of Au NRs with NIR irradiation have been performed to assess the efficiency of photothermal effect; however, there are limited mechanistic studies of cytotoxic and genotoxic data available as of now. Herein, we assessed differential cytotoxic effects of Au NRs in two intrinsically different cell types (normal vs. cancer cells) and examined the mechanistic pathways leading to cancer cell death by Au NRs with/without NIR using variety of toxicological assessment methods.

We first examined morphological changes in A549 cancer cells. Neither Au NRs treatment alone nor NIR irradiation alone caused discernible morphological transformation (Fig. 3). The changes in cell morphologies following combined Au NRs/NIR treatment indicate hyperthermia and result of cellular toxicity induced by PTT.

The WST assay, which is similar to MTT assay without any solubilization or washing process, results revealed a slight decrease in viability of A549 cells following 24 h treatment with both Au NRs (10 and 20 [$\mu$g/mL]) and NIR irradiation, compared to the control group. In contrast to A549 cells, a cancer cell line, the viability of normal human lymphocytes was hardly affected in any condition tested (i.e., Au NRs only, NIR only, or Au NRs plus NIR...
For the first time, Au NRs-induced genotoxicity was investigated by using comet and micronuclei assays in A549 cells. We chose these tests due to their wide applicability for evaluating genotoxicity in various cell types. OTMs were significantly increased in a dose-dependent manner and were augmented by NIR irradiation [Fig. 7(A)]. Conversely, the micronuclei formation frequencies significantly increased only in cells treated with combined 20 μg/mL Au NRs and NIR [Fig. 7(B)]. When unrepair single-strand breaks are converted into double-strand breaks, micronuclei are generated. Damages such as oxidative stress, single-strand and double-strand DNA breaks may lead to formation of comet cells and micronuclei. The DNA double-strand breaks induced by simultaneous Au NRs treatment and NIR irradiation could eventually lead to cell death via the apoptosis and/or necrosis pathways.

To further explore the involvement of apoptotic pathways, we analyzed mRNA expression of genes involved in the induction of apoptosis. Our results showed that the expression levels of ATM and p53 increased when cells were treated with Au NRs and NIR simultaneously, but it was not statistically significant (Table II). A recent study reported that Au NRs’ photothermal effects could induce apoptosis in macrophages by damaging mitochondria. It was also reported that the photothermal effect of Au NRs by a laser operating at lower energy levels could induce apoptosis in cancer cells, whereas sufficiently high energy fluence can burn the cell membrane, leading to rapid cell death similar to necrosis. The results from our study show that when the Au NRs are irradiated with a NIR laser, they induce DNA damage, resulting in necrotic cell death. The amount of LDH released was significantly increased only in the group of cells treated both with Au NRs (20 μg/mL) and NIR irradiation when compared to the control group. Unrepairable DNA damage caused by Au NRs after NIR irradiation would elicit necrotic cell death rather than apoptosis.

This study clearly demonstrated that simultaneous treatment with Au NRs and NIR irradiation can exert photothermal cytotoxic and genotoxic effects on A549 cells, which has not been reported previously. The results of the cell viability study showed that cancer cells were more susceptible to hyperthermia when compared to normal human lymphocytes. Taken together, our findings imply that targeted cancer treatment with Au NRs-mediated photothermal therapy holds great promise for nanomedicine, exerting the cytotoxic and genotoxic effects exclusively on cancer cells.

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Conflicts of Interest

The authors declare that there are no conflicts of interest. We are responsible for the content and writing of this paper.

References and Notes


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