

RESEARCH COMMUNICATION

Gold Nanoparticles Induce Apoptosis in MCF-7 Human Breast Cancer Cells

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Abstract

Background: Gold nanoparticles have recently been investigated with respect to biocompatibility according to their interactions with cells. The purpose of this study was to examine cytotoxicity and apoptosis induction by well-characterized gold nanoparticles in human breast epithelial MCF-7 cells. **Methods:** Apoptosis was assessed by TUNEL, cytotoxicity by MTT assay and caspase 3, 9, p53, Bax and Bcl expression by real-time PCR assays. **Results:** Gold nanoparticles at up to 200 µg/mL for 24 hours exerted concentration-dependent cytotoxicity and significant upregulation of mRNA expression of p53, bax, caspase-3 & caspase-9, whereas expression of anti-apoptotic bcl-2 was down-regulated. **Conclusion:** To the best of our knowledge this is the first report showing that gold nanoparticles induce apoptosis in MCF-7 cells via p53, bax/bcl-2 and caspase pathways.

Keywords: Gold nanoparticles - MCF-7 - apoptosis - cancer

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Introduction

Nanoparticles are attractive for cancer applications as they can be engineered to have multifunctionality serving simultaneously as imaging contrast agents, therapeutic agents, and/or drug delivery vehicles. This multifunctionality opens the door to the possibility of new paradigms for cancer detection and treatment not previously considered possible. Nanoparticles have been increasingly used in biological and medical applications (Fox, 2000; Casey et al., 2001; Guntaka et al., 2003) including clinical diagnostics, therapeutics development and drug delivery, as well as advanced imaging. Meanwhile, effects of nanoparticles on health and safety have been also attracted more and more attention (Vasquez and Wilson, 1998; Guntaka et al., 2007). As one of the most abroad used nanoparticles, gold nanoparticles were recently investigated with regard to cytotoxicity and biocompatibility according to their interaction with cells (Hwu et al., 2004; Ceruti et al., 2005), which has been demonstrated that the gold nanoparticles was low endocytic and had inhibition to proliferation of some kinds of cells. Moreover, it was found that gold nanoparticles can interact selectively with heparin-binding glycoproteins resulting in inhibition of endothelial/fibroblast cell proliferation and angiogenesis (Fritsche et al., 1993).

Human breast cancer MCF-7 cells represent one of the most widely used experimental models for in vitro studies on breast carcinoma. We report herein for the first time that gold nanoparticles can enhance apoptosis of MCF-7 cells as disclosed by real-time PCR analysis.

Materials and Methods

Maintenance of cell cultures

MCF-7 cells were procured from ATCC. The cell lines were maintained and propagated in 90% Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were cultured as adherent monolayers (i.e., cultured at approximately 80% confluence) and maintained at 37°C in a humidified atmosphere of 5% CO₂. Cells were harvested after brief trypsinization. All chemicals used were of research grade.

MTT assays

MCF-7 cells were grown in DMEM at 37°C under 5% CO₂ in a humidified incubator. Cells were harvested, counted and transferred to 96-well plates and incubated for 24h prior to the addition of the gold nanoparticle. The gold nanoparticles were processed and applied in various concentrations, and the treated cells were incubated for 24h. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5mg) was dissolved in 1 mL of phosphate-buffered saline (PBS), and 25µL of the MTT solution was added to each of the 96 wells. The plates were wrapped in aluminium foil and incubated at 37°C for 4h. The solution in each well, containing media, unbound MTT and dead cells, was removed by suction, and 200µL of DMSO was added to each well. The plates were then shaken, and the optical density was measured using a microplate reader at 575 nm. Three independent experiments were performed for each study and all

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measurements were performed in triplicate. Results were expressed as the percentage proliferation with respect to vehicle-treated cells.

Real-time quantitative PCR analysis

Reverse transcription-PCR (RT-PCR; Applied Biosystems 7500 Fast, Foster City, CA) was used to analyse the expression of genes by using a real-time SYBR Green/ROX gene expression assay kit (QIAGEN, Germany). Fastlane® Cell cDNA kit (QIAGEN, Germany) was used to prepare cDNA directly from cultured cells, and the mRNA levels of p53, Bax, Bcl₂, Caspase-3 and Caspase-9 as well as the endogenous reference gene, GAPDH, were assayed using gene-specific SYBR Green-based QuantiTect® Primer assays (QIAGEN, Germany). Quantitative real-time RT-PCR was performed in a reaction volume of 25 μ L according to the manufacturer's instructions. Briefly, 12.5 μ L of master mix, 2.5 μ L of primer assay (10x) and 10 μ L of template cDNA (100ng) were added to each well. After a brief centrifugation, the PCR plate was subjected to 40 cycles of the following conditions: (i) PCR activation at 95°C for 5 minutes, (ii) denaturation at 95°C for 5 seconds and (iii) annealing/extension at 60°C for 10 seconds. All samples and controls were run in triplicates on an ABI 7500 Fast Real-time PCR system. The quantitative RT-PCR data were analyzed by a comparative threshold (Ct) method, and the fold inductions of samples were compared with the untreated samples. GAPDH was used as an internal reference gene to normalize the expression of the target genes.

Apoptotic assay

The apoptotic effects of gold nanoparticle on MCF-7 cells were examined by nuclear DNA staining assay. For nuclear DNA staining, gold nanoparticle-treated and untreated cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min, washed with PBS twice, stained with PI (Sigma) at 1 μ g/mL in PBS for 15 min. Stained cells were washed twice with PBS. The changes in nuclei were observed under ultraviolet fluorescent microscope (Carl Zeiss).

Statistical Analysis

Statistical analysis applied to real-time PCR data was performed by comparing ΔC_t values (cycle numbers at the threshold level of log-based fluorescence normalized to the GAPDH control gene) by Wilcoxon rank-sum test, with two-sided $P < 0.05$ indicating statistical significance. Mean differences in ΔC_t ($\Delta\Delta C_t$) were used to calculate fold differences in gene expression by the following formula: fold = $2^{-\Delta\Delta C_t}$.

Results

Nanoparticle-induced cytotoxicity

Human breast epithelial MCF-7 cells were exposed to gold nanoparticles at the concentrations of 0, 25, 50, 100 and 200 μ g/mL for 24 h and cytotoxicity was determined using MTT assays. MTT results have shown that as the concentration of nanoparticles increased to 25, 50, 100 and 200 μ g/mL cytotoxicity was observed in dose-dependent

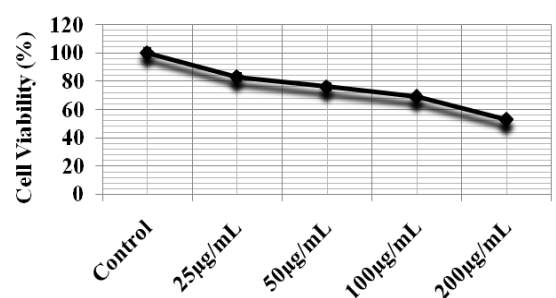


Figure 1. Cell Viability Determined by the MTT Assay. Cells were treated with various concentrations of gold nanoparticles and results are expressed as percentage of proliferation compared with untreated control (mean \pm SD, n=3)

fashion. In MTT assay cell viability was significantly reduced to 83%, 76% and 69% for the concentrations of 25, 50, 100 and 200 μ g/mL respectively ($p < 0.05$ for each) (Figure 1).

Nanoparticle- Regulated Gene Expression

In this study, quantitative real-time PCR was utilized to analyze the mRNA levels of apoptotic markers (e.g. p53, bax, bcl-2, caspase-3 and caspase-9) in MCF-7 cells exposed to gold nanoparticles at a concentration of 200 μ g/mL for 24 h. Results showed that the mRNA levels of these apoptotic markers were significantly altered in MCF-7 cells due to gold nanoparticles exposure. The mRNA level of tumor suppression gene p53 was 1.66 fold higher (Figure 2, $p < 0.05$) in treated cells as compared to the control. Furthermore, we examined the effect of gold nanoparticles on the mRNA expression of caspase-3 and caspase-9. The expression of caspase-3 was 1.38 fold and caspase-9 (Figure 2, $p < 0.05$) was 1.31 fold higher in treated cells in comparison with untreated control cells. In addition, we further found higher mRNA expression levels of pro-apoptotic gene bax (1.43 fold) and lower expression of antiapoptotic gene bcl-2 (1.41 fold) (Figure 3, $p < 0.05$) in exposed cells than those of untreated cells.

Microscopic signs of apoptosis in gold nanoparticle-treated MCF-7 cells

The ability of gold nanoparticle into cell and alter morphology (an indication of apoptosis) in the cancer cell lines were assessed using PI staining (Figure. 4). After incubation for 24 h with gold nanoparticle, the cells became round in appearance, exhibited nuclear condensation and significant nucleus fragmentation

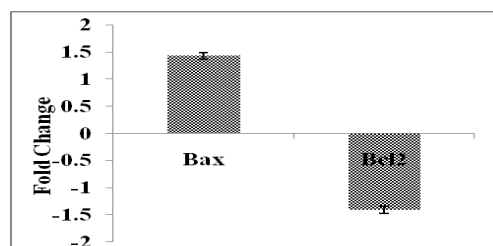


Figure 2. Effects of Gold Nanoparticles on the Levels of Bax and Bcl2 in MCF-7 Cells. Cells were cultured as described under Materials and methods. MCF-7 cells were treated with 200 μ g/mL for 24 h. Experiments were repeated three times with similar results.

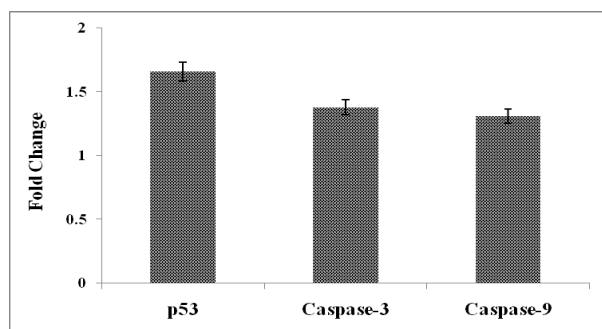


Figure 3. Effects of Gold Nanoparticles on the Levels of p53, Caspase-3 and Caspase-9 in MCF-7 cells. Cells were cultured as described under Materials and methods. MCF-7 cells were treated with 200 µg/mL for 24 h. Experiments were repeated three times with similar results

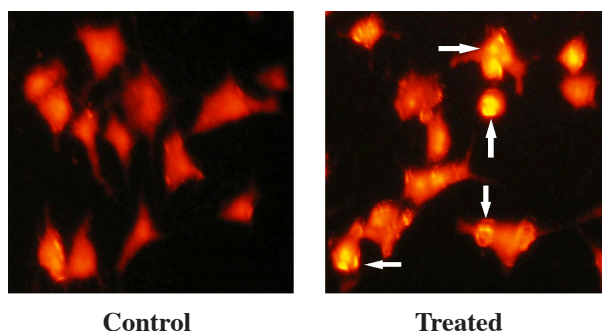


Figure 4. DNA Staining (Microscopic) after 24 Hours Incubation of MCF-7 Treated Against 200 µg/mL Gold Nanoparticles with Control. Red fluorescence is due to Propidium Iodide staining and observed under green filter. Observations done at 200x magnification

indicating apoptosis.

Discussion

Gold nanoparticles with different sizes and characteristic properties were developed and extensively analyzed in recent years for various biomedical applications. The stabilizing molecules, besides preventing the formation of aggregation, are also essential for controlling the chemical reactions and subsequently responsible for the well dispersed size and its uniformity.

In this study, toxic responses of gold nanoparticles to human breast epithelial MCF-7 cells were investigated. Our results demonstrated that exposure of gold nanoparticles to MCF-7 cells cause cytotoxicity. We have also observed apoptotic response of gold nanoparticles in MCF-7 cells. MTT assays revealed that the gold nanoparticles exert significant cytotoxicity to MCF-7 cells in dose-dependent fashion in the concentration range of 25-200 µg/mL.

We analyzed the mRNA expression levels of five genes; p53, bax, bcl-2, caspase-3 and caspase-9 in response to gold nanoparticles exposure in MCF-7 cells, because apoptosis is controlled through these pathways. Quantitative real-time PCR results showed that gold nanoparticles up-regulated mRNA level of cell cycle checkpoint protein p53 and pro-apoptotic bax. Expression of anti-apoptotic bcl-2 was down-regulated in cells exposed to gold nanoparticles. Furthermore, both the mRNA expression of apoptotic caspase-3 and

caspase-9 were higher in nanoparticles treated cells. Taken together, up-regulation of p53 leads to activation of pro-apoptotic members of bcl-2 family, such as bax induces permeabilization of the outer mitochondrial membrane, which releases soluble proteins from the intermembrane space into the cytosol, where they promote caspase activation (Lanvin et al., 2004; Youle and Strasser, 2008).

Recently, several groups have reported the use of gold nanoparticles for the successful delivery of drugs like doxorubicin for overcoming drug resistance in cancer (Nadeau et al, 2011) as well as peptide functionalized gold nanoparticles for targeting tumor (Arosio et al., 2011). An ideal therapeutic approach would be to deliver multiple drugs specifically to the primary tumors, as well as at the site of metastasis and its microenvironment while simultaneously monitoring the prognosis through non-invasive approaches. In cancer therapy, targeted delivery in a localized way is one of the key challenges. Gold nanoparticles have the potential to play a significant role to achieve such goals. It is anticipated that nanoparticle-mediated targeted delivery of drugs might significantly reduce the dosage of anti-cancer drugs with better specificity, enhanced efficacy and lower toxicities.

In conclusion, we have shown that gold nanoparticles produce significant cytotoxicity to MCF-7 cells in dose-dependent manner in the concentration range of 25-200 µg/mL. Furthermore, quantitative real-time PCR analysis displayed that mRNA levels involved in the apoptosis was altered by gold nanoparticles. Overall, our data suggesting that gold nanoparticles may induce apoptosis in MCF-7 cells via p53, bax/bcl-2 and caspase pathways. This in vitro study showed the induction of apoptosis by gold nanoparticles warrants further investigation to determine if *in vivo* exposure consequences may exist for gold nanoparticles application.

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