

Ag nanoparticles sensitize IR-induced killing of cancer cells

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Dear Editor,

Nanosized particulate systems combining better cancer diagnosis with therapeutic effect are being designed based on the merging of nanotechnology with cellular and molecular techniques. The surface of these nanoscale carriers is often functionalized with biological molecules for stabilization and targeted delivery. The combinations of nano-core and associated functional molecules can cross the cell membrane [1], and the surface of nanomaterials (including coating and associated functional molecules) plays a critical role in determining the outcome of their interactions with cells [2, 3]. Studying the potential effects of nanomaterials in biological systems often requires the administration of nanoparticles into a cell culture system or into living organisms *in vivo*. It should be noted, however, that under such conditions nanoparticles are known to adsorb proteins from the biological system, and the resulting heterogeneity in surface proteins may in turn affect the biological effects of nanomaterials [4, 5]. To minimize such non-specific effect, in this study we have modified the surface of our nanomaterials with proteins from fetal bovine serum (FBS), a supplement normally used for cell culture. Interestingly, we have recently found that for silane-coated monodisperse magnetite nanoparticles (MNPs), additional surface modification by FBS appears to facilitate particle uptake by cancer cells.

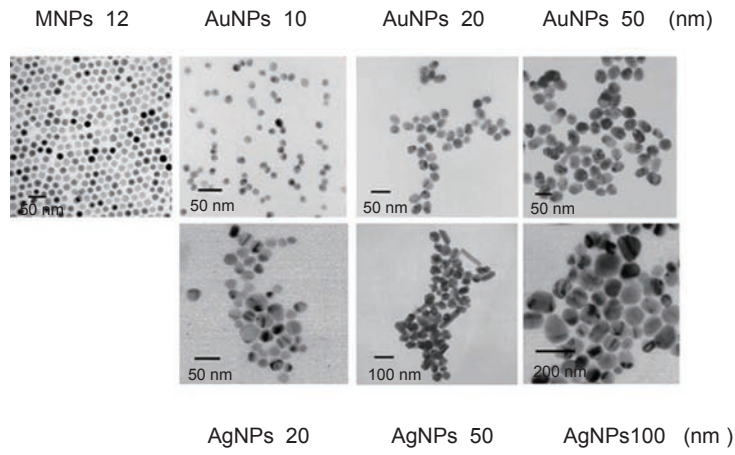
A number of previous studies have examined cytotoxicity elicited by nanoparticles after their uptake into cells under regular cell culture conditions [6-8]. However, the property of intracellular nanoparticles and their effect on cellular processes under more complex scenarios such as when cells are treated with ionizing radiation (IR) remain largely unclear. Recently, it was reported that certain gold nanoparticles (coated with polyethylene glycol (PEG) and amino acids) could enhance radiation-induced cytotoxicity in mice colorectal adenocarcinoma and breast cancer cells [9, 10]. However, there has been a lack of systematic analyses on how different nanomaterials generally affect cellular radiation sensitivity, as well

as the respective contributions by nanoparticles and their coatings. To address this issue, we have examined in this study three different nanomaterials, magnetite, gold and silver nanoparticles, with either distinct surface coatings (for magnetite particles) or distinct size ranges (for gold and silver particles) (Supplementary information, Figure S1), for their potential effects on radiation-induced killing of glioma cells (experimental details in Supplementary information, Data S1). We chose glioma cells as a model system because most of glioblastoma multiform (GBM) is insensitive or even resistant to IR therapy, which is also reflected in the established glioma cell lines. Thus, enhancing the effect of IR-induced killing of glioma cells may offer therapeutic benefit for patients with GBM.

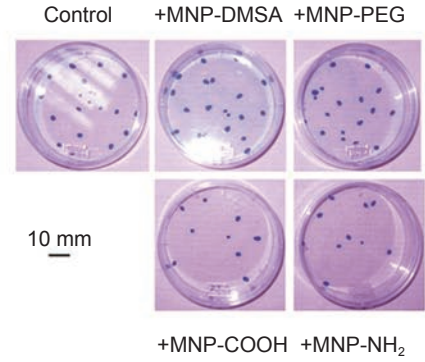
We prepared 12-nm monodisperse MNPs (Supplementary information, Figure S2), and coated them with one of the following four surface molecules: meso-2, 3-dimercaptosuccinic acid (DMSA), and silanes bearing various functional groups including amino group (NH₂), short-chain PEG, and carboxylic group (COOH) (Figure 1A). We also prepared gold nanoparticles (AuNPs) of diameter 10, 20 and 40 nm, and silver nanoparticles (AgNPs) of diameter 20, 50 and 100 nm (Figure 1A). All the particles were modified with FBS followed by radiation-mediated sterilization (see Methods for details and Supplementary information, Figure S3, S4). We then examined the effects of these prepared nanoparticles on cell survival and cell radiation sensitivity in three different glioma cell lines, namely C6 (originated from mouse), U251 and SHG-44 cells (originated from human GBM).

We treated C6 glioma cells with a fixed concentration of MNPs (0.1 mg/ml), and compared the effects of different coatings. We found that DMSA- and PEG-silane-coated MNPs had little effect on glioma cell survival or cell sensitivity to radiation (Figure 1B). In contrast, treatment with COOH- and NH₂-silane-MNPs led to a decrease of cell survival as indicated by the clonogenic assay (Figure 1B). Treatment with 6 Gy (gray, J/kg) of IR killed a significant portion of glioma cells, and the com-

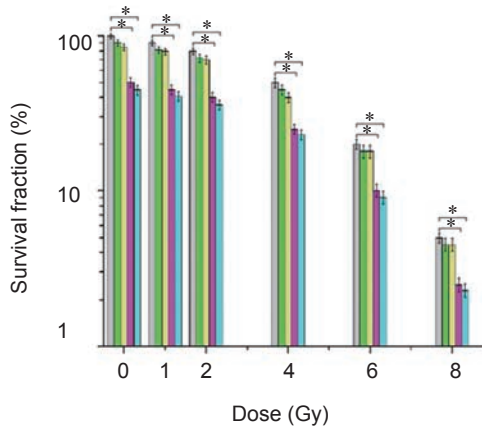
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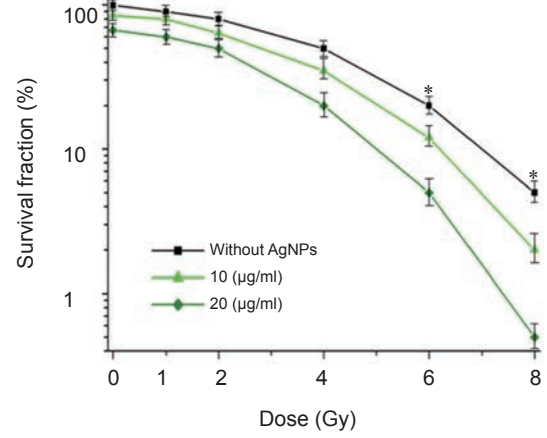
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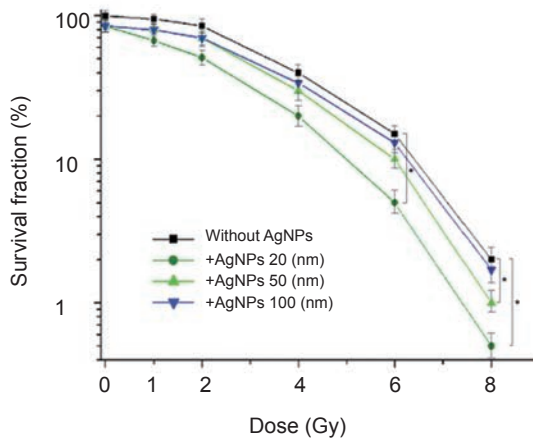
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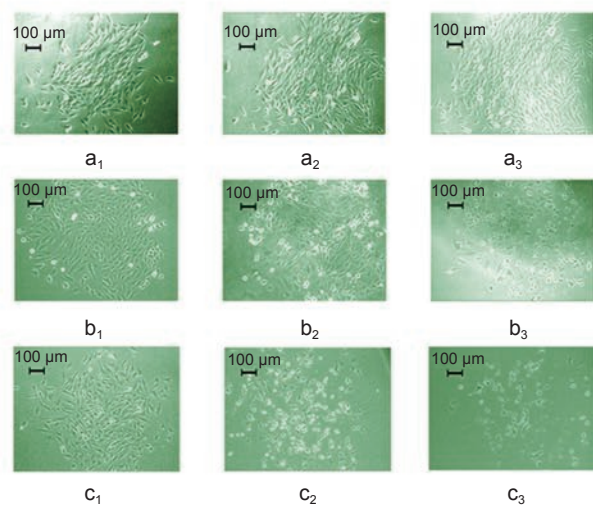
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bination of IR and COOH- or NH₂-silane-MNPs treatment further reduced cell survival (Figure 1C). However, the inspection of the quantified results across different doses of IR indicated that COOH- or NH₂-silane-MNPs caused a similar level of reduction of cell survival with or without IR (Figure 1C). Thus, these nanoparticles did not appear to increase cell radiation sensitivity *per se*; rather, they likely further increased cell killing by IR as a result of their general cytotoxicity toward these cells. Moreover, since MNPs with different surface coatings showed distinct effects on cell survival, we conclude that surface coating molecule could critically affect MNP-cell interactions and consequently influence cellular outcomes. We also performed similar experiments in U251 and SHG-44 cells and found similar results to C6 cells (data not shown).

We next treated SHG-44 glioma cells with our FBS-modified AuNPs, and found that our AuNPs had little overall effect on cell survival, nor did they affect cell survival across different doses of IR. Similar results were obtained with C6 and U251 cells. Thus, these AuNPs, ranging from 10 to 40 nm, do not appear to influence cell radiation sensitivity, at least under our experimental conditions. Our results are in contrast with previous reports showing that certain AuNPs coated with PEG or amino acids could enhance radiation-induced cytotoxicity in mice colorectal adenocarcinoma and breast cancer cells [9, 10]. While the reason for this discrepancy is currently unknown, it raises an interesting possibility that different coatings of the AuNPs used in these studies may be responsible for the different outcomes observed.

Treating glioma cells with AgNPs led to dose-dependent cytotoxicity, with the smaller size particles (20 and 50 nm) being most cytotoxic (Supplementary information, Figure S5); and these results are consistent with previous studies using other cell lines [2, 7]. To determine whether AgNPs affect cell radiation sensitivity, for each size of AgNPs, we chose a dose that when present alone only minimally affects cell survival, which is at about

1/10 to 1/5 of the concentration required to kill 50% of the cells. Interestingly, we found that at such relatively harmless doses, both 20 and 50 nm AgNPs significantly enhanced radiation sensitivity of U251 cells, with 20 nm particles performing better than 50 nm ones (Figure 1D and 1E), while the effect of 100 nm AgNPs was much weaker. A similar particle size-dependent radiation sensitization effect was also observed for C6 and SHG-44 cells. Taken together, our results demonstrate that 20 nm and 50 nm AgNPs could function to enhance radiation-induced killing of glioma cells.

We hypothesize that the mechanism of radio-sensitization by AgNPs might be related to the release of Ag⁺ cation from the silver nanostructures inside cells (Supplementary information, Figure S6). Ag⁺ cation has the ability to capture electron and thus functions as an oxidative agent, which could further reduce ATP content of the cell and increase production of reactive oxygen species [8]. Indeed, treating cells of an established colony with Ag⁺ cation led to rapid cytotoxicity (Figure 1F), and further IR completely killed the cells within the colony. Consistent with our radio-sensitization results, treating cell colonies with 20 nm AgNPs led to some cytotoxicity on its own while it greatly enhanced further cell killing by IR (Figure 1F). The fact that the radio-sensitization effect of AgNPs decreases upon the increase of particle size is consistent with the Ag⁺ cation release model, as smaller size AgNPs have a better ability to release silver ion.

Based on our results, we suggest that cellular effects of nanoparticles could be mediated by two distinct mechanisms that are contributed by either the particle itself or the nature of its surface coating. Among the MNPs, those coated with DMSA- and PEG-silane are relatively non-toxic, while MNPs coated by COOH- and NH₂-silane show obvious toxicity to cells. As a result, combination of the cytotoxic MNPs with IR led to more cell killing than IR alone, but the effect is largely additive and these MNPs do not appear to increase cell radiosensitivity *per se*. While AuNPs showed little effect in our experiments,

Figure 1 Two types of nanoscale systems designed to investigate the effect on IR. **(A)** Monodisperse MNPs at the diameter of 12 nm coated with DMSA, and silanes bearing various functional groups including NH₂, short-chain PEG and COOH. AuNPs at 10, 20 and 40 nm. AgNPs at 20, 50 and 100 nm. **(B)** MNPs coated with DMSA and PEG-silane (0.1 mg/ml) showed little toxicity on C6 cells and did not affect cell killing by IR, while MNPs coated with COOH-silane and NH₂-silane (0.1 mg/ml) reduced cell survival. **(C)** At every dose of X ray, from left to right the data of control, MNPs-DMSA, -PEG, -COOH and -NH₂ groups. **(D and E)** Dose- and size-dependent effects of AgNPs upon IR. The data represent the mean ± SD of three independent experiments. **P* < 0.05 was considered statistically significant. **(D)** The survival curves of U251 cells treated with 10 and 20 µg/ml 20 nm AgNPs. **(E)** The survival curve of U251 cells treated with 20 nm (20 µg/ml), 50 nm (50 µg/ml) and 100 nm (100 µg/ml) AgNPs. **(F)** (a₁), (b₁) and (c₁) were photos of C6 colonies; (b₂) was colony (b₁) after 24 h of 20 nm AgNPs (20 µg/ml), (c₂) was (c₁) after 24 h of Ag⁺ (0.1 µg/ml), and (a₂) was (a₁) with mock treatment. Colonies in (a₂), (b₂) and (c₂) then received 3 Gy of IR and were photographed after another 24 h, shown in (a₃), (b₃) and (c₃).

AgNPs of particular sizes (20 nm and 50 nm) significantly sensitized glioma cells to IR. The radio-sensitization effect of AgNPs suggests that intracellular nanostructures may interact with the cellular DNA repair system, and has important implications in the design of nanotechnology-based radiosensitizers for improving the outcomes of cancer radiotherapy.

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(Supplementary information is linked to the online version of the paper on the *Cell Research* website.)