Selective Targeting of Gold Nanorods at the Mitochondria of Cancer Cells: Implications for Cancer Therapy

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Supporting Information

ABSTRACT: We have observed that Au nanorods (NRs) have distinct effects on cell viability via killing cancer cells while posing negligible impact on normal cells and mesenchymal stem cells. Obvious differences in cellular uptake, intracellular trafficking, and susceptibility of lysosome to Au NRs by different types of cells resulted in selective accumulation of Au NRs in the mitochondria of cancer cells. Their long-term retention decreased mitochondrial membrane potential and increased reactive oxygen species level that enhances the likelihood of cell death. These findings thus provide guidance for the design of organelle-targeted nanomaterials in tumor therapy.

KEYWORDS: Gold nanorods, serum proteins, uptake and removal, intracellular localization, mitochondrion-targeted nanomaterials, tumor therapy

Gold nanorods (Au NRs) are promising nanomaterials for applications in biomedicine such as near-infrared imaging,1 X-ray computed tomography,2 photoacoustic imaging,3 biosensing,4,5 drug/gene delivery,6 and thermal therapy of tumor,7 due to their unique physicochemical and optical properties. Physicochemical properties of Au NRs such as their surface groups and modifications, charge, size, shape and aspect ratio, play key roles in their uptake, removal, and toxicity.8–12 However, some central issues concerning their use, including their intracellular localization, uptake and removal, and cell viability and metabolism, require further study. Recently, several studies have evaluated the potential effects of gold nanoparticles (Au NPs) on biological systems that are key in determining their applications in biomedicine, including their biodistribution and removal,13–16 intracellular localization,17,18 cytotoxicity and biocompatibility,18–20 and interactions between Au NPs and biological molecules.21–24 Our previous work also proved that surface chemistry and aspect ratio can mediate cellular uptake of Au NRs.12 Furthermore, after tail vein injection into rats, Au NRs were characterized in vivo using integrated analytical techniques and were proved to be stable and biocompatible.25

Among various potential applications, tumor therapy has attracted most attention owing to the ability of live imaging and treatment (drug delivery and hyperthermia). Due to strong absorption in the near-infrared spectral region, Au NRs are especially attractive for tumor thermal therapy.7 In order to achieve destructive effects from thermal absorption, their long-term retention in target cells at a high dosage is essential. At the same time, they should have a negligible impact on normal cells, especially for adult stem cells. Bone marrow mesenchymal stem cells (MSC cells) are derived from mesenchymal tissues. They are responsible for self-renewal of multiple tissues and organs since they readily proliferate and differentiate into multilineages.26,27 Maintenance of metabolism and cell viability is essential for their physiological functions.

In the present study, we investigated the effects of serum protein-coated Au NRs on carcinoma cells (A549 cells), normal bronchial epithelial cells (16HBE cells), and primary adult stem cells (MSC cells). Surprisingly, at concentrations between 25 and

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100 μmol/L, Au NRs killed A549 cells but caused little damage to 16HBE and MSC cells. We investigated why the same nanorods behaved so differently in these three cells by examining their uptake mechanism and intracellular localization. Our results demonstrate that intracellular localization is a key factor in determining both the final fates of the Au NRs (retention or exclusion) and the cells (viability and morphology due to changed metabolism, integrity of lysosomal membranes, and mitochondrial membrane potentials). Selective targeting of the Au NRs at the mitochondria of cancer cells led to their death. Native differences between cancer, normal, and stem cells probably resulted in the intrinsic selectivity.

Surface properties of Au NRs before internalization. Au NRs with a mean length of 55.6 ± 7.8 nm and width of 13.3 ± 1.8 nm were prepared according to previous publications and characterized by transmission electron microscopy (TEM) (see Figure S1A, Supporting Information). The surface of Au NRs is capped with a cetyltrimethylammonium bromide (CTAB) bilayer (Figure S1B, Supporting Information), thus exposing hydrophilic ammonium cation heads. Free CTAB in the final dispersion solution is very hard to eliminate during the manufacturing process. The CTAB-capped Au NRs were washed at least three times, and the residual free CTAB molecules were removed from dispersion solution as much as possible. Bovine serum proteins with negative charges are readily adsorbed onto the surface of the Au NRs when incubated at pH 7.4 as seen by a 6 nm thick gray shell around the rod surface in the TEM image (Figure S1C, Supporting Information). During incubation with 10% serum, the longitudinal surface plasmon resonance (SPR) of the Au NRs also changed as seen in visible and near-infrared absorption spectra: adsorption of serum ingredients induced a 20 nm red shift of the longitudinal SPR peak (Figure S1D, Supporting Information) and a decrease in peak intensity. Furthermore, adsorption of proteins by Au NRs was confirmed by SDS-PAGE, which showed that the protein contents in 0.1% and 0.2% serum solutions decreased greatly after incubation with Au NRs for 30 min (Figure S1E, Supporting Information). Serum protein-coated Au NRs maintained their stability, as determined by their uniform...
size and good dispersibility (Table S1, Supporting Information). Their zeta potentials changed notably from $+29.3 \pm 0.7$ to $-14.5 \pm 0.6$ mV and their hydrodynamic diameter increased significantly after incubation in a complete medium plus 10% fetal bovine serum for 10 min (Table S1, Supporting Information). Serum components are very complicated and consist of proteins, peptides, amine acids, and so on. They can be bonded to the surface of Au NPs via nonspecific adsorption by electrostatic attraction, hydrophobic interaction, or covalent linkage.\textsuperscript{24} According to quantitative analysis of adsorbed serum proteins by fluorescence assay (Table S1, Supporting Information), our results were in agreement with this report and indicated that Au NRs were serum protein-coated before internalization.

**Selectively Killing Tumor Cells by Au NRs.** Cell viability was evaluated by the CCK-8 assay. Our data indicate that continuous exposure to 50 or 100 $\mu$M Au NRs for 72 h strongly inhibited the viability of A549 cells, reducing cell survival to around 27% and 10%, respectively (Figure 1A). However, they are slightly toxic to 16HBE and hardly toxic to MSC cells (Figure 1B,C) even at a concentration as high as 100 $\mu$M for 72 h. Furthermore, cytotoxicity of Au NRs on A549 cells was dose- and time-dependent. Au NRs at these doses also showed a strong ability to kill other human cancer cell lines, including hepatocellular carcinoma cells (HepG2), cervical cancer cells (HeLa), and prostatic cancer cells (LNCaP) (Figure S2, Supporting Information).

The cytotoxicity of Au NRs also led to morphological changes in A549 cells. After a 24 h treatment with 50 $\mu$M Au NRs, A549 cells changed from polygonal to shrunken or fusiform shapes (Figure S3A, Supporting Information). In contrast, 16HBE cells maintained their polygon shapes and MSC cells kept their fibroblast-like shapes (Figure S3B and Figure S3C, Supporting Information). Specific staining of the actin cytoskeleton with rhodamine-labeled phalloidin was used to show these shape changes in detail. The actin filaments of normal A549 cells are arranged in parallel structures with thick stress fibers. After treatment with Au NRs, they aggregated and formed dotlike structures (Figure 1E). However, the order of actin filaments in nanorod-exposed MSC cells was preserved, implying that cell shape was intact (Figure 1E). The same was true for 16HBE cells. These results indicate that the impaired structure of actin cytoskeleton caused by Au NRs affected the shape of A549 cells.

Thus, our experiments have revealed interesting and very different effects of serum protein-coated Au NRs on cell viability in tumor cells, normal cells, and stem cells.

**Uptake Pathways of Au NRs in Three Cells Were Similar.** The uptake pathway for the internalization of nanoparticles has an impact on their bioactivity. Cellular uptake is a complex process and refers to the interaction between Au NRs and the plasma membrane. In order to understand whether the differences in cytotoxicity were dependent on amount of Au NRs internalized, we first investigated the process of uptake quantitatively using inductively coupled plasma mass spectrometry (ICP-MS). Cell volume is a key parameter, and we compared cellular uptake and removal of Au NRs by normalizing cell volume. According to flow cytometry, the mean volumes of A549, 16HBE, and MSC cells were $3156.7 \pm 280.0$, $1878.6 \pm 53.9$, and $4806.8 \pm 296.1 \mu$m$^3$, respectively. Here, we describe the capacity of internalization as the mean numbers of Au NRs per cell (Figure S4, Supporting Information) and per cell volume ($\mu$m$^3$) (Figure 2A). Both sets of data show that the internalization of Au NRs is time-dependent. Comparably, 16HBE cells have a slower rate and fewer numbers to internalize nanorods and reach equilibrium time at about 48 h. However, MSC cells have a more rapid rate of uptake and a shorter equilibrium time. Each MSC cell was also able to internalize twice as many Au NRs as one A549 cell. At 72 h, the numbers of internalized Au NRs in each A549 cell and 16HBE cell are calculated to be $12783 \pm 1787$ and $2080 \pm 345$, respectively (Figure S4, Supporting Information).

The process of removal of Au NRs was quite different for the three types of cells (Figure 2C). Both A549 and 16HBE cells were unable to exclude Au NRs when placed in fresh medium for 72 h. In contrast, in MSC cells, few Au NRs were excluded in the first 24 h period, and effective exclusion took place thereafter. After removing Au NRs from the culture medium, we observed that the intracellular nanorods continued to inhibit the viability of A549 cells, while no such effect occurred in 16HBE and MSC cells (Figure 1D).

To understand the uptake pathway in detail, different inhibitors were used to study the mechanism of Au NR internalization. Treatment with sodium azide and 2-deoxy-D-glucose (DOG) at different times after the removal of Au NRs from the culture medium, respectively, (B) Uptake pathways for Au NRs in two types of cells using specific endocytosis inhibitors. Mean values $\pm$ standard deviation, $N = 3$.

![Figure 2](dx.doi.org/10.1021/nl103992v/NanoLett.2011,11,772-780)
or at low temperature (4 °C) markedly reduced Au NR uptake (by >80%) and ATP synthesis, demonstrating that the internalization process is energy-dependent endocytosis. The three main modes of endocytosis are clathrin-mediated, caveola-lipid raft-mediated, and macropinocytosis/phagocytosis. Since three cells are known not to be phagocytic, we focused on our attention on the other two pathways (Figure 2B). Chlorpromazine and hypertonic challenge with sucrose are specific inhibitors of clathrin-mediated endocytosis, and they strongly constrained endocytosis in three cells by more than 75% and 80%, respectively. It demonstrates that the main pathway is clathrin-mediated. Specific treatment like cholesterol depletion from membrane structures can also be employed to study lipid raft-mediated endocytosis. Here, nystatin and methyl-β-cyclo-dextrin (MβCD) inhibited endocytosis by at least 54% and 48%, showing that lipid raft-mediated endocytosis is a secondary pathway for internalization of Au NRs. Dynamin, a GTP-dependent enzyme, forms a coil-like structure around the neck of invaginations on the plasma membrane and takes part in the hydrolysis of GTP and the process of pinching off vesicles. Dynasore, a specific inhibitor of dynamin-mediated lipid-raft endocytosis, strongly decreased internalization of Au NRs by 42%, indicating that the dynamin-mediated pathway represents the major mechanism for lipid raft-dependent endocytosis in three cells. Therefore, both clathrin- and lipid raft-dependent (dynamin-mediated) endocytosis pathways are involved in the internalization of Au NRs in A549, 16HBE, and MSC cells.

Selective Accumulation of the Au NRs in A549 Mitochondria. Since no obvious differences were observed in the uptake pathways of the three cells, we conducted further detailed investigations of Au NR ultrastructure and intracellular trafficking after internalization. TEM provides spatial and temporal information about the endocytosis and removal of Au NRs. In accord with ICP-MS results, TEM images show that internalization of the Au NRs occurred by a time-dependent process. Au NRs were first enclosed by the plasma membrane of A549 cells and internalized at 1.5 h (Figure S5B, Supporting Information). After continuous incubation for 72 h, more Au NRs entered the cell and were located in subcellular structures including the cytoplasm, endosomes/lysosomes and mitochondria (Figure 3A–D).
Figure S5B–H in Supporting Information). Consistent with other reports, Au NRs were not detected in the nucleus, thus ruling out the possibility of genotoxicity. The amount of Au NRs internalized and their distribution were time-dependent. Au NRs were first located close to the plasma membrane (at 1.5 h) and then trafficked to endosomes and lysosomes (at 3 h). During 24 h, more Au NRs entered and filled the endosomes/lysosomes. After the first 6 h, they were released into the cytoplasm and then trafficked to the mitochondria where they resided in an aggregated state (Figure 3B–D). At 12 h, some Au NRs appeared in the cytoplasm. After 24 h, the number of Au NRs in lysosomes did not increase markedly because internalization nearly reached a state of equilibrium (Figure 2A, Figure 3C, and Figure 3D). The number of Au NRs in mitochondria increased gradually (from 3 to 24 h) and the mitochondria became somewhat swollen (3 and 6 h, Figure 3, panels A and B) and round (after 6 h) due to the presence of Au NRs (6 h, Figure 3B). After the entry of the Au NRs, mitochondrial cristae partially disappeared and some mitochondria became vacuolus (at 12 h, Figure S5E in Supporting Information). Au NRs were positioned at the edges of the mitochondria or around their inner membranes (after 12 h, Figure 3C). Thus, Au NRs in A549 cells trafficked from endosomes/lysosomes to cytoplasm and then to mitochondria as internalization proceeded. Nevertheless, most A549 cells did not become obvious apoptotic during the 48 h incubation (Figure S5B–G, Supporting Information). Thereafter, cells changed their shape significantly (Figure S3A, Supporting Information) and became somewhat apoptotic. This implies that the time-dependent cytotoxicity of Au NRs is due to intracellular distribution. This conclusion is well supported by results from exclusion experiments (Figure 1D, Figure 3G, and Figure 3H). After these cells were placed into fresh medium without Au NRs, intracellular Au NRs were not excluded; most Au NRs were observed in the mitochondria and a few remained within lysosomes in A549 cells. Moreover, the lamellar cristae became irregular and disordered and mitochondrial structures became vacuolus, suggesting that the storage of Au NRs within mitochondria results in damage to these organelles (Figure 4A). In conclusion, after entering into A549 cells, Au NRs are transported to lysosomes and then translocated to mitochondria. Their mitochondrial storage impairs mitochondrial structures and further influences their ability to be excluded.
In contrast, intracellular trafficking in 16HBE and MSC cells were quite different from A549 cells. For 16HBE cells, a few Au NRs entered cells at 3 h and are mainly distributed in endosomes/lysosomes and very few in the cytoplasm (Figure S5J, Supporting Information). Of course, their release in cytoplasm decreased greatly 48 and 72 h after nanorod removal (Figure 3, panels I and J). Meanwhile, MSC cells maintained normal subcellular structures such as intact mitochondria (Figure 4C). Taken together, our results show that cellular trafficking of Au NRs in 16HBE and MSC cells is similar. Au NRs are translocated to endosomes/lysosomes, they remain there for some time for 16HBE, and about 80% are excluded for MSC cells.

The intracellular colocalization of Au NRs and organelles further verifies the above results. The locations of Au NRs can be imaged with SPR-enhanced two photon fluorescence. In this experiment, the excitation wavelength was fixed at 820 nm, and green and red luminescence signals were collected. Mito Tracker Red and Lyso Tracker Red are specific probes, giving red fluorescence for intracellular mitochondria and lysosomes, respectively. Combinations of green (Au NRs) and red signals (organelle probes) clearly indicate their intracellular location. Results indicated that both lysosomes and mitochondria are the main target organelles in A549 cells at 24 h (Figure S6, panels A and B, Supporting Information). Most Au NRs are preferentially located in the lysosomes of MSC cells (Figure S6, panels C and D, Supporting Information).

Leakage of Au NRs from A549 Lysosomes Due to the Damage in Lysosomal Membrane Integrity. Previous studies indicated that intracellular locations of Au NPs are normally in endosomes/lysosomes in the perinuclear region. In the case of A549 cells, translocation of Au NRs, however, does not stop at lysosomes. The integrity of the lysosomal membrane structure is critical for its physiological functions. An impaired membrane may result in the release of digestive enzymes and exogenous materials from the lysosome. Could damage to the lysosomal membrane lead to further translocation of the Au NRs to other organelles? Acidine orange (AO) was used as a probe to study the integrity of the lysosomal membrane. Our results show that there are significant changes in lysosomal membrane permeation (LMP) after internalization of Au NRs into lysosomes in A549 cells, but not in 16HBE and MSC cells (Figure 4A−D), which provide a chance for Au NRs to be released into cytoplasm.

This study shows that lysosomal membranes probably have different capacities to tolerate the CTAB molecules on the surface of Au NRs in three types of cells. As we know, the acidic environment of lysosome is below pH 5.0, where many proteins will be denatured and be broken down by a wide variety of lysosomal hydrolytic enzymes (acid hydrolases). The CTAB bilayer can be exposed in lysosomes because of the dissociation of serum proteins from nanorods. Simulated experiments using FBS-coated Au NRs (FBS/Au NRs) in lysosomes were therefore conducted by studying dynamic sizes of FBS/Au NRs in artificial lysosomal fluid (ALF, pH at 4.5) (Figure S1F, Supporting Information). In PBS (pH 7.2), FBS/Au NR complexes kept the same size of 30 nm during 60 min. However, in ALF, the mean size of the nanorods changed dramatically. At first, it dropped from 30 ± 1 to 23 ± 1 nm due to the decomposition of coated proteins, and then it recovered and rose to 118 ± 4 nm as a result of aggregation of CTAB-capped Au NRs.

In addition, as a quaternary ammonium cation, CTAB can destroy lysosomal membrane and be further released into cytoplasm in cancer cells. It has been reported that polycationic compounds can cause the disruption of lipid bilayers due to electrostatic attraction and quaternary ammonium cations adsorbed onto membrane that can form nanoscale holes within the membrane and increase its permeability. However, we found that the cytotoxicity and lysosomal membrane of three cells responded to CTAB molecules differently (Figure S7, Supporting Information). When evaluating the toxicity of CTAB on three types of cells (Figure S7, Supporting Information), we have found that CTAB do not affect the cell viability of 16HBE and MSC within 10 μM and show quite toxic at 20 μM. However, CTAB causes strong toxicity on A549 even at 1 μM. Therefore, the toxicity of CTAB is highly dependent on concentration and cell types, while cancer cells are sensitive to CTAB. At a concentration of 1 μM, CTAB resulted in significant reduction on the integrity of lysosomal membrane on A549 cells and negligible effects on 16HBE and MSC cells, respectively (Figure 4D). We roughly estimated that there were about 5700−12900 CTAB molecules on the surface of each Au nanorod by comparing the cytotoxicity of CTAB in the dispersion solution and that after complete replacement with HS-PEG-COOH (data not shown). It can be concluded that A549 cells are less tolerant to CTAB especially local concentrated CTAB on Au NRs, which largely increases LMP after internalization of Au NRs. Furthermore, normal cells have a strong resistance to CTAB and enclose most nanorods in lysosomes when exposed to 50 μM Au NRs. We also observed that some Au NRs could traffic to mitochondria in 16HBE cells when they were treated with a much higher concentration of Au NRs of 200 μM. From the point of view above, it is easy to understand that more internalized Au NRs would increase local CTAB amount and then rupture endosomal/lysosomal membrane, which finally caused the release of nanorods and their target at mitochondria (Figure S5T, Supporting Information). Of course, their release in cytoplasm decreased cell viability (Figure 1B). Thus, the resistance of endosomal/lysosomal membrane to Au NRs is probably a crucial factor for understanding the distinct fates of Au NRs in cells.

Mitochondrial Targeting of Au NRs in A549 Cells. Our present data suggest that residual CTAB on nanorods can also mediate the cytotoxic Au NRs targeting to the mitochondria in cancer cells. The idea can be proved by using CTAB-capped Au NRs to treat A549 cells in serum-free medium. After 6 h of incubation, some Au NRs are released into the cytosol and partly translocated to mitochondria according to TEM results (Figure S8, Supporting Information), which proves the roles of CTAB in mitochondrial target. Therefore, the exposure of the residual
CTAB on nanorod surface in acidic lysosomal microenvironment actually facilitates the trafficking of Au NRs into mitochondria. CTAB is a group of quaternary ammonium compounds belonging to delocalized lipophilic cations and has a smart structure with a positively charged polar head and a nonpolar hydrophobic tail. The nonpolar hydrophobic tail of CTAB takes part in the process of insertion, fusion, and permeation in membrane structures. Moreover, the delocalized positive charge around the head is responsible for mitochondrial target in response to negative inside transmembrane potentials ($\Delta \Psi_M$). Therefore, CTAB can easily overcome hydrophobic barriers of the lipid bilayer, enter into cells, and target organelles like mitochondria with obvious potential difference between outer and inner membranes. It is possible that the bilayer of CTAB leads to the directional movement and accumulation of Au NRs in mitochondria.

**A549 Cell Death Is Caused by Au NR-Induced Mitochondrial Damage.** It is well-known that the mitochondrion takes part in oxidative phosphorylation and functions as the energy factory of the cell. What kind of effects will Au NRs have on mitochondria, mitochondrial membrane potentials, and/or production of cytoplasmic reactive oxygen species (ROS)? The mitochondrion-specific dye JC-1 was used to detect changes in mitochondrial membrane potential. Apoptotic cells mainly show green fluorescence, while healthy cells show red and green fluorescence, making JC-1 suitable for the detection of mitochondrial damage. After a 24 h exposure of cells to Au NRs, JC-1 results showed that Au NRs induce mitochondrial damage in A549 cells but not in 16HBE and MSC cells (Figure 4A–C). A549 cells showed a decreased ratio of red to green fluorescence (Figure 4A) and exhibited impaired mitochondrial membranes that might disrupt cellular metabolism, release reactive oxygen species from the mitochondria, and even cause apoptosis. In contrast, 16HBE and MSC cells stained bright red and orange due to a high ratio of red to green, which implies that both cells maintained intact mitochondrial membranes (Figure 4, panels B and C). Thus, Au NRs in A549 cells have indeed significantly influenced cellular metabolism via the mitochondrial pathway. The reason why A549 cells were less viable after exposure to Au NRs for 24 h or longer is that the CTAB-capped Au NRs are located in the mitochondria and then destroy mitochondrial structures, disrupting the production of ATP energy molecules and then increasing cytoplasmic oxidative stress. Mitochondria are known to be the main source of reactive oxygen species (ROS) such as superoxide and peroxide anions. High doses of ROS are harmful and destructive as they exert strong oxidative stress and thus decrease cell viability. Our flow cytometry data show that the level of ROS in A549 cells increased after 24 h of exposure to Au NRs, while those in 16HBE and MSC cells were not markedly affected (Figure 4E).

**Different Fates for Au NRs: Residence or Exclusion.** Three kinds of endocytosis pathways are involved in regulating the process of internalization of Au NRs. However, the final fates of Au NRs were markedly different in A549, 16HBE, and MSC cells (Figure 2C and Figure 4G–L). Other reports have demonstrated that nanoparticles such as citric acid-stabilized and protein-coated Au NPs, carbon nanotubes, and poly (D,L-lactide-co-glycolide; PLGA) nanoparticles can be quickly removed by cells. Transferrin or citric acid-stabilized Au NPs can be internalized by receptor-mediated endocytosis, and their uptake reaches a climax at 6 h. After fusion with lysosomes, the endosomes which trap Au NRs are further digested. Inert and hard to be degraded by digestive enzymes in lysosomes, the best way to protect the cell is to remove Au NRs by way of lysosomal secretion. It has been reported that the exclusion process is quick and dependent on recycling endosomes reaching equilibrium within 5 h. Here, we assume that a similar process for removing Au NRs takes place in 16HBE and MSC cells: endosome-trapped Au NRs are transported to lysosomes for further digestion. Inert and hard to be degraded by digestive enzymes in lysosomes, the best way to protect the cell is to remove Au NRs by way of lysosomal secretion. It has been reported that the exclusion process is quick and dependent on recycling endosomes reaching equilibrium within 5 h. Here, we found that most Au NRs in stem cells were resident in lysosomes and could be finally cleared from MSC cells after a time lag, resulting in the maintenance of normal metabolism. In contrast, large numbers of Au NRs coated by CTAB in

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**Figure 5.** Different fates and effects of Au NRs in cancer cell, normal cell, and stem cell due to distinct pathways for cellular trafficking.
A549 cells were difficult to exclude due to their translocation from endosomes/lysosomes to mitochondria, which caused cell death. This provides good evidence to explain why Au NRs are toxic in A549 cells and biocompatible in 16HBE and MSC cells. We have that Au NRs have distinct effects in vivo. These effects in vitro provide good evidence to explain why Au NRs are toxic in A549 cells and biocompatible in 16HBE and MSC cells. Death. This provides good evidence to explain why Au NRs are toxic in A549 cells and biocompatible in 16HBE and MSC cells. Our study demonstrates that intracellular localization, not uptake pathway, determines the final fate of both Au NRs and cells. Due to the enhanced permeation of the lysosomal membrane after Au NR uptake, Au NRs are released into the cytoplasm of A549 cells and translocated from endosomes/lysosomes to mitochondria, inducing decreased mitochondrial membrane potentials, increased oxidation stress and finally reduced cell viability. However, Au NRs show almost no toxicity in 16HBE and MSC cells since their lysosomal membranes remain more intact and Au NRs are localized in lysosomes. These differences in intracellular trafficking for three kinds of cells are shown schematically in Figure 5.

The results of this study will deepen our understanding of the mechanism of cell-specific cytotoxicity of Au NRs. They will be helpful for designing organelle-targeted nanomaterials used in tumor therapy, live imaging, pharmaceutical carriers, and regenerative medicine. Mitochondrion-targeted Au NRs would be an ideal carrier for releasing mitochondrion-specific drugs to kill tumor cells. In contrast, clearance of Au NRs from stem cells is advantageous for the safe and efficient delivery of therapeutic genes without affecting functions of normal cell and producing adverse effects in vivo. These findings thus provide guidance for the design of organelle-targeted nanomaterials in tumor therapy, for example, using Au NRs as drug/gene delivery and laser photo-thermal therapy.

ASSOCIATED CONTENT

Supporting Information. Methods and experimental procedures and several methods to characterize CTAB-capped and FBS-coated Au NRs, including TEM images, zeta potential, and size distribution, changes of cell viability and shape after exposure to Au NRs, intracellular localization of Au NRs by two-photon laser scanning confocal microscope and TEM, and quantitation of Au NRs during uptake and removal by ICP-MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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