# Effects of gold nanoparticles on cell death induced by Radiation and Ultrasound.

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## 研究分野 Research area

**放射線科学 (Radiological Sciences)**

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## 研究内容 Research content

Radiation (X-rays) and Ultrasound have been shown to induce apoptosis in cancer cells. Gold nanoparticles (Au-NPs) are used as promising sensitizers due to the high atomic number and greater biocompatibility than other metals. However, effects of Au-NPs (2 nm size) with physical modalities remain to be elusive. Therefore, this study is intended to investigate the effects of Au-NPs, on X-rays and ultrasound induced apoptosis in human lymphoma U937 cells. Effects of Au-NPs on X-rays and ultrasound-induced apoptosis were determined by observing the changes in intracellular reactive oxygen species (ROS) formation and apoptotic signaling pathways. Radiation-induced apoptosis was significantly inhibited in the cells pre-treated with Au-NPs. In contrast, Au-NPs showed enhancement of apoptosis in combination with ultrasound. ROS generation was increased in the combined treated cells with ultrasound and Au-NPs, which ultimately enhanced the DNA damage, while no such changes were observed following combination of radiation and Au-NPs. Our findings indicate the potential use of Au-NPs in combination treatment and would further clarify the role of Au-NPs in radiation and ultrasound-aided therapies.

## 研究のポイント Research point

Au-NPs suppress X-irradiation induced apoptosis via inhibition of extrinsic and intrinsic pathway without interference with ROS. The activity of caspase-8 and caspase-3 were decreased and does not allow to change the mitochondrial membrane potential which ultimately inhibit X-irradiation induced apoptosis.

However, Au-NPs enhance ultrasound induced apoptosis by increasing the caspase-8 and caspase-3 activity. In late hours Au-NPs in combination with ultrasound can switch the apoptotic cell death into ir-reversible non-apoptoic cell death. The exact mode of cell death is still unclear.

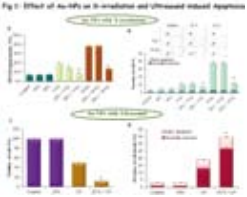
Taken together, these findings suggest that Au-NPs can produce differential effects in combination with physical modalities.

## 研究への取組、今後の展望

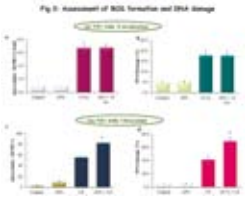
This study demonstrated that small size gold nanoparticles can induce differential effects depending on the physical stress applied, either protective as anti-oxidants or anti-cancer. It has been known that radiotherapy induce cell death in tumor tissue via generation of ROS but the effects of radiotherapy are limited due to the adverse reactions in the normal tissues surrounding the tumor. Therefore, it is very important to protect normal tissue in order to increase the therapeutic window by sensitizing more tumor cells with lesser side effects. These small size gold nanoparticles can be utilized as effective radio protector by targeted delivery to normal tissue during radio therapy.

In addition, the small sized gold nanoparticles enhanced cancer cell death under the influence of ultrasound-induced mechanical stress. These gold nanoparticles can be used in US-aided anti-cancer therapies.

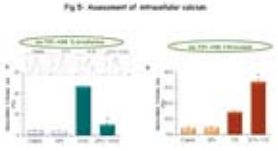
## 研 究 REPORT



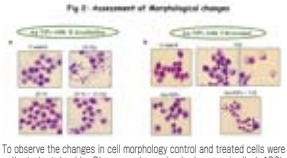
U937 cells were exposed to (10 Gy) resulted in a large percentage of apoptotic cell death as manifested by DNA fragmentation. This apoptotic percentage had decreased significantly when cells were pre-incubated with 20% Au-NPs for 3 h (1a). Similarly the flow cytometric analysis of the membrane changes indicative of different stages of apoptosis progression using annexin V-FITC and PI showed that the percentage of early apoptotic cells significantly suppress following X-irradiation treatment in the presence of 20% Au-NPs in U937 cells (1b). U937 cells with or without pre-treatment with 20% of Au-NPs for 3 h were sonicated. Cell viability was significantly decreased at 72 h in combine treated cells (c). Cells with or without pre-treatment with 20% of Au-NPs for 3 h were sonicated at intensity of 0.4 W/cm2 for 2 min and analysed at 18 h after treatment. The percentage of secondary necrotic cells significantly increased following US treatment in the presence of 20% Au-NPs (d). Data are presented as mean ± SEM. Asterisk (*) denotes statistical significance ($p < 0.05$) vs Gy and US alone.



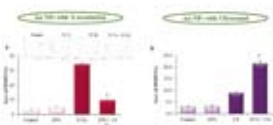
The percentages of cells with elevated species of ROS were analysed immediately after X-irradiation with flow cytometry using DCFH-DA. No change was observed in formation of ROS in combined treated cells as compare to alone treated cells (a). Similarly no such changes were seen in the extent of H2AX phosphorylation in cells pre-treated with Au-NPs (b). U937 cells were pre-treated with Au-NPs for 3 h before sonication; there was an increase in intercellular H2O2 formation observed immediately post sonication (c). The extent of H2AX phosphorylation was determined immediately after US was also significantly increased in combine treatment (d). Data are presented as mean ± SEM. Asterisk (*) denotes statistical significance ($p < 0.05$) vs Gy and US alone.



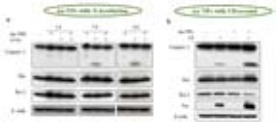
Pre-treatment of Au-NPs for 3 h can significantly suppressed X-irradiation induced intracellular [Ca2+]i level after 6 h of post-treatment (a). While pre-treatment of Au-NPs for 3 h can significantly increase US-induced intracellular [Ca2+]i level after 6 h of post-treatment.Data are presented as mean ± SEM. Asterisk (*) denotes statistical significance ($p < 0.05$) vs Gy and US alone.



To observe the changes in cell morphology control and treated cells were collected, stained by Giemsa and examined microscopically (x400). X-irradiation alone showed typical morphological changes such as chromatin condensation and nuclear fragmentation which was significantly suppressed in combined treatment. Cells showed the occurrence of apoptotic cell death after ultrasound application, while in presence of Au-NPs pre-treatment, cell death was still ensuing with atypical pyknotic features.



We found that the loss of MMP ($\triangle\Psi m$) which is the end point of apoptosis was significantly decreased in cells pre-treated with Au-NPs for 3 h as compare to the cells treated with X-irradiation alone (a). The loss of MMP ($\triangle\Psi m$) was significantly increased in cells pre-treated with 20% of Au-NPs for 3 h as compare to the cells treated with US alone (b). Data are presented as mean ± SEM. Asterisk (*) denotes statistical significance ($p < 0.05$) vs Gy and US alone.



Pre-treatment with Au-NPs for 3 h significantly decreased cleaved caspase-3 as compare to X-irradiation alone. No change was observed in Bcl-2 family proteins in combine treatment (a). Whereas, pre-treatment with Au-NPs for 3 h significantly increased cleaved caspase-3, and Fas as compare to US alone. In Bcl-2 family proteins Bax was significantly increased and Bcl-2 was significantly suppress in combine treatment (b).

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