

### 3 - 45 Radiosensitizing Effect of Functionalized Gold Nanoparticles on Human Hepatoma HepG2 Cells

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Radiotherapy along with surgery and chemotherapy are the major therapeutic strategies for cancer treatment. The central goal of radiotherapy is to deliver a therapeutic dose to the tumor as much as possible whilst sparing the surrounding normal tissues. Side effects are often observed in radiotherapy using conventional X-rays. Radiosensitizers, enhancing the radiation effects on tumors via increasing the dose specifically to the tumor tissues, can improve the modality of radiotherapy against tumors.

Since the experimental results concerning the radiation enhancement effect of gold nanoparticles (AuNPs) were presented by Hainfield et al<sup>[1]</sup> in 2004, evidence supporting the application of AuNPs as radiosensitizer has been provided from extensive *in vitro* investigations and a relatively limited number of *in vivo* studies using the radiations of X-,  $\gamma$ -rays and electron beams<sup>[2]</sup>. Recently we also studied the radiosensitizing effect of citrate-capped AuNPs on human cervical carcinoma HeLa cells<sup>[3-4]</sup> and found a very weak radiation enhancement of polyethyleneglycol-capped AuNPs (PEG-AuNPs) to human hepatoma HepG2 cells exposed to X-rays. Therefore, we wonder if it is possible to improve the radiosensitizing effects of PEG-AuNPs by surface functionalization although Gilles et al<sup>[5]</sup> thought the gold nanoparticles functionalization notably decreased radiosensitization. In this study, we compared the radiosensitizing effects of 20 nm AuNPs conjugated with/without tirapazamine (TPZ) moiety at the concentration of 10  $\mu\text{g}/\text{mL}$  in HepG2 cells irradiated with X-rays.

Using Fens' procedure, we obtained tirapazamine-functionalized AuNPs (TPZs-AuNPs) with an average size of 20 nm in diameter based on the measurement with TEM. Then, we evaluated hydroxyl radical production of TPZs-AuNPs solution on the X-ray radiation dose comparing to AuNPs using coumarin-3-carboxylic acid (3-CCA) as probes. After calculation, the enhancement factors were obtained as shown in Fig. 1(a). A significant increase in hydroxyl radical production was observed at the dose of 1.0 Gy, and the enhancement factor is beyond 3.0. The enhancement factors of TPZs-AuNPs were higher than those of AuNPs at the same irradiation dose although the enhancement factor decreased while increasing the radiation dose. The result demonstrated that the functionalizing of AuNPs by TPZ improved the hydroxyl radical production in aqueous solution. On the basis of this, we further investigated the hydroxyl radical production of TPZs-AuNPs and AuNPs in HepG2 cells under X-ray irradiation. The results showed that the addition of AuNPs did not obviously improve the hydroxyl radical production in HepG2 cells. In comparison, The hydroxyl radical production showed 25% enhancement in HepG2 under X-ray irradiation when 10  $\mu\text{g}/\text{mL}$  TPZs-AuNPs was co-cultured with HepG2 cells for 24 h.

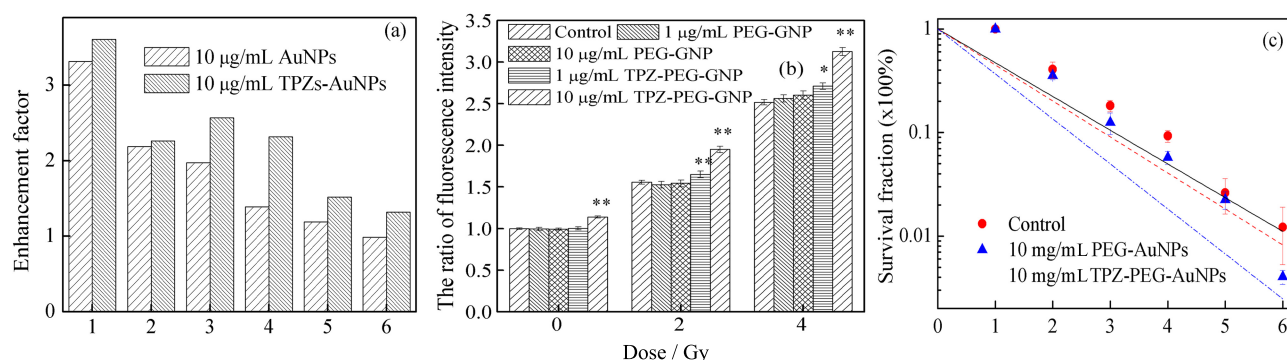


Fig. 1 (color online) (a) The dependences of enhancement factor on the X-ray dose in the presence of AuNPs and TPZs-AuNPs. (b) The dependence of hydroxyl radical production in HepG2 cells on the X-ray dose in the presence of AuNPs and TPZs-AuNPs. (c) The survival curves of HepG2 cells after exposure to 50 kVp X-ray.

Consequently, we investigated the clonogenic survival effect of HepG2 cells in the presence of AuNPs and TPZs-AuNPs upon X-ray irradiation. HepG2 cells were pretreated with AuNPs or TPZs-AuNPs at the concentrations of 10  $\mu\text{g}/\text{mL}$  for 24 h prior to irradiation. Fig. 1(c) presented the survival data of HepG2 cells TPZs-AuNPs as well as AuNPs after exposure to X-rays. There was a significant decrease in survival fraction for HepG2 cells irradiated with X-rays in the presence of TPZs-AuNPs. The radiation enhancement factor of TPZ-PEG-AuNPs at 10% survival level was 1.23 for HepG2 cells under the X-ray irradiation, which was larger than 1.05 using PEG-AuNP only.

The *in vitro* study showed that the introduction of tirapazamine moiety enhanced the radiosensitizing effect of gold compared to PEG-AuNPs only, which providing a way to improve the radiosensitizing effect of nanoparticles.

In conclusion, the addition of TPZs-AuNPs significantly improved the hydroxyl radical production produced by X-ray irradiation, which contributed to an increment of 23% in relative biological effect.

## References

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## 3 - 46 Autophagy Inhibition by Chloroquine Sensitizes Tumor to High-LET Carbon Ions

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Autophagy is the major regulation mechanism for degrading long lived proteins and the only known pathway for degrading organelles in cells. In this work, we investigated the impact of high linear energy transfer (LET) heavy ions combined with autophagy inhibitor chloroquine (CQ) on the radiosensitivity of tumor cells using tumor-bearing mouse experiment.

Survival and apoptosis of mouse sarcoma S180 cells were detected by means of colony-formation assay and flow cytometry, respectively. Tumor-bearing Kunming mice were randomly divided into groups of control, irradiation or CQ treatment alone, and irradiation combined with CQ pretreatment. The irradiations were conducted with carbon ions at a dose of 2 Gy. The mice were sacrificed at 3 or 15 d post-irradiation and tumor tissues were stripped for subsequent experiments.

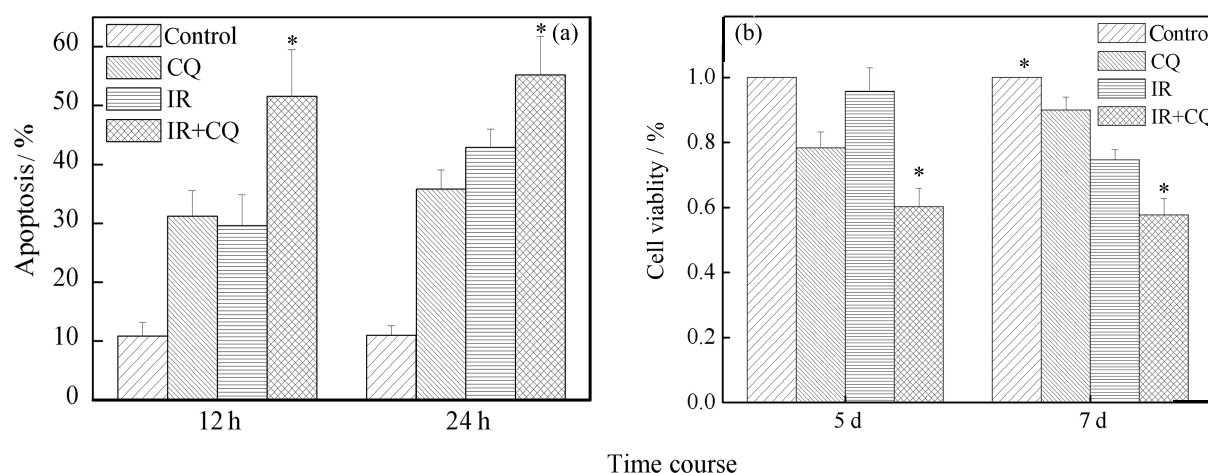


Fig. 1 Apoptosis and viability of cells were detected with flow cytometry. (a) Apoptosis, (b) Cell viability.

Carbon ion radiation combined with CQ pretreatment affected the morphology of the tumors. Compared with the control and single treatment groups, the co-treatment increased the apoptotic rate and decreased the viability of S180 cells (Fig. 1). Significant features of autophagy appeared in tumor tissues (Fig. 2), indicating high-LET carbon ions could effectively induce autophagy.

Carbon ion radiation combined with CQ treatment significantly increased the apoptotic rate of tumor cells. As shown in Fig. 3, only a few apoptotic cells (TUNEL-positive) were observed in the control group, and the irradiation alone caused only a slight increase in apoptotic rate. However, the combined treatment with CQ and carbon ions increased the apoptotic rate significantly.