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

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

Zheng Xiaogang, Jin Xiaodong and Li Qiang

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 gourps 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.

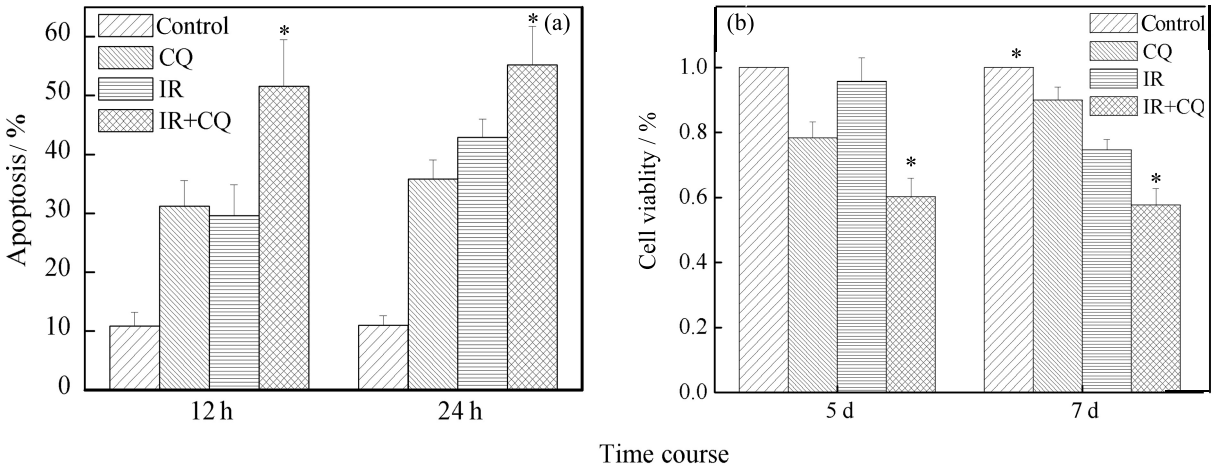

Fig. 1 Apoptosis and viability of cells were detected with flow cytometry. (a) Apoptosis, (b) Cell viability.

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.

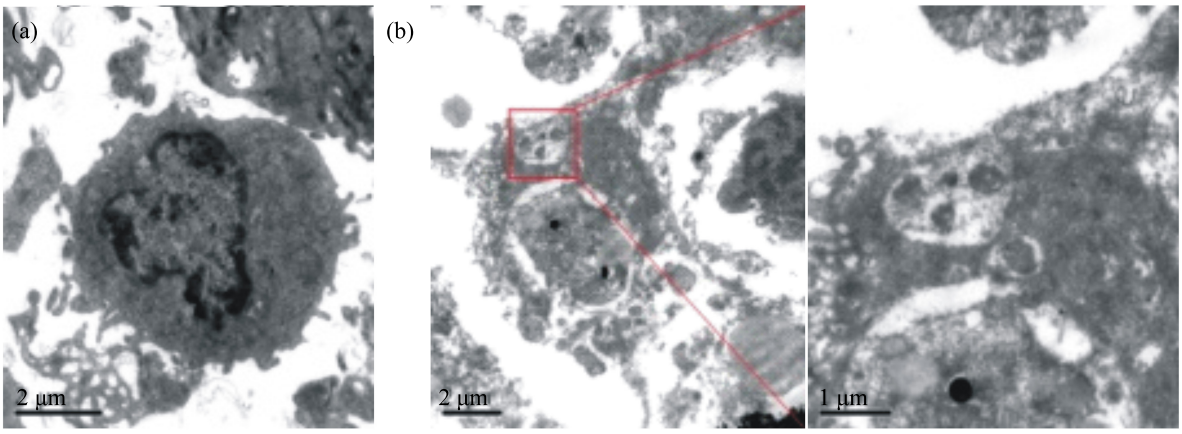

Fig. 2 Autophagy was detected with electron microscopy. (a) Control, (b) Irradiation.

Compared with the control group, the co-treatment with carbon ion and CQ decreased the expression of early protein LC3-II while increasing the expression of late protein p62 during autophagy flux (Fig. 4). The expression levels of pro-apoptotic protein Bax and the key apoptosis executioner molecule caspase-3 in the co-treatment group were higher than that in the group of irradiation alone or control. The expression level of Bcl-2 was not significantly affected by the irradiation. All the results reveal that inhibition of autophagy with CQ enhanced heavy ion radiation induced apoptosis.

In summary, for high-LET heavy ions, inhibition of autophagy could enhance tumor cell apoptosis, leading to an increase in radiosensitivity. Our finding might shed new light on promoting the efficiency of heavy ion radiotherapy.

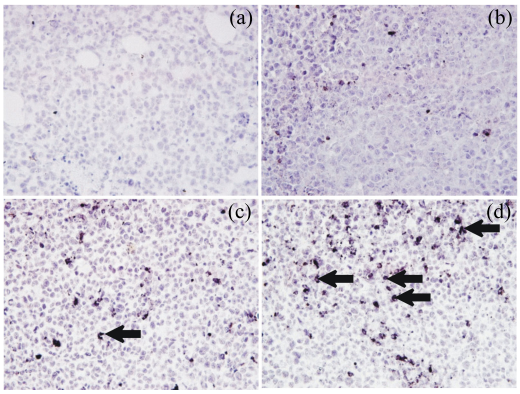

Fig. 3 (color online) Cell apoptosis was analyzed with the TUNEL assay. (a) Control, (b) CQ, (c)Irradiation, (d) Irradiation+CQ.

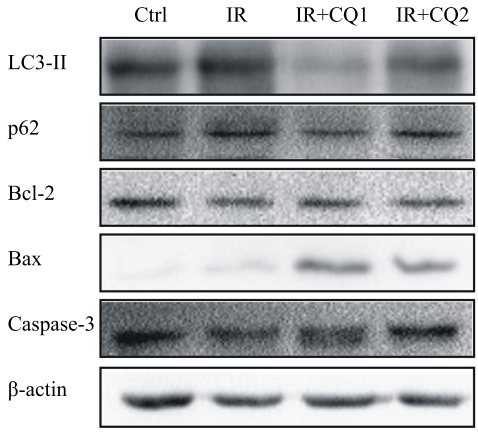

Fig. 4 (color online) Key autophagy related proteins were detected with western-blot analysis. CQ1: 3 d, one injection; CQ2: 15 d, continuous injection.

3 - 47 Carbon Ions Induce Autophagy Through Akt/mTOR and Unfolded Protein Response Pathways

Jin Xiaodong, Li Feifei and Li Qiang

In previous studies, we found that high-LET carbon ions induced autophagy in a LET-dependent manner in tumor cells. However, the mechanisms underlying are still poorly understood. In this study, we explored the activation of two important pathways such as Akt/mTOR and unfolded protein response (UPR), which are involved in the occurrence of autophagy.

First, we detected the levels of phospho-Akt (p-Akt), phosphor-mTOR (p-mTOR) and phospho-p70S6 (p-p70S6), the latter being MTORC1 substrates, in human glioblastoma SHG44 and cervical cancer HeLa cell lines exposed to X-rays and carbon ions of different LETs. For SHG44 cells, the activity of p-Akt was not obviously changed at 4 h post-irradiation in response to the various radiation qualities. At 24 h after irradiation, the high-LET carbon ions