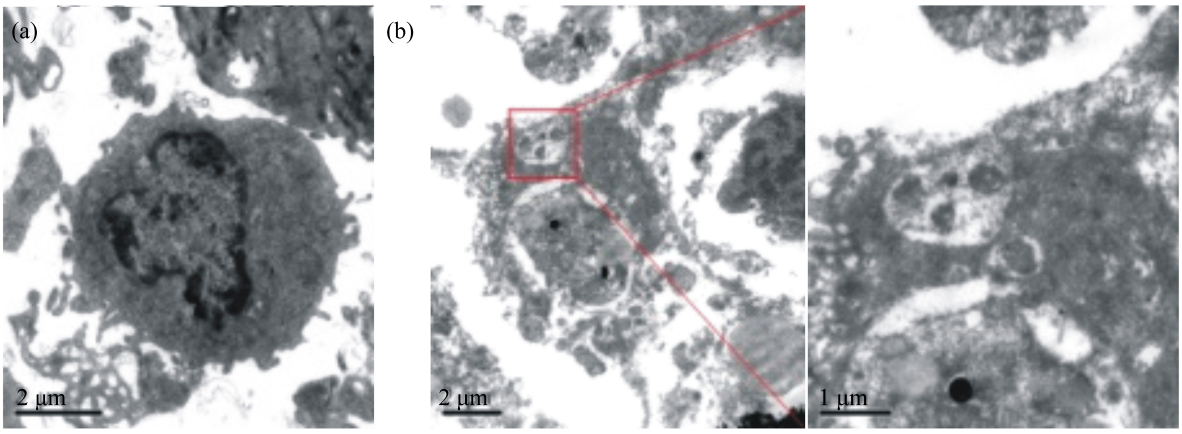

Fig. 2 Autophagy was detected with electron microscopy. (a) Control, (b) Irradiation.

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.

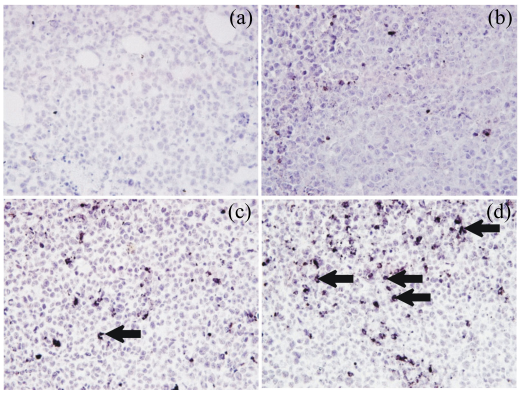

Fig. 3 (color online) Cell apoptosis was analyzed with the TUNEL assay. (a) Control, (b) CQ, (c)Irradiation, (d) Irradiation+CQ.

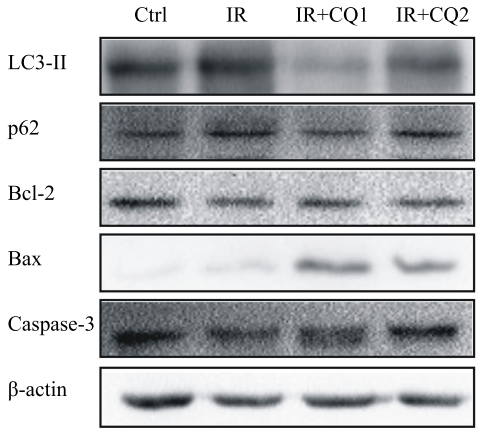

Fig. 4 (color online) Key autophagy related proteins were detected with western-blot analysis. CQ1: 3 d, one injection; CQ2: 15 d, continuous injection.

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

caused obvious decrease in the level of p-Akt proteins compared to X-rays. The activity of p-Akt in cells irradiated with X-rays was also depressed at 48 and 72 h after irradiation, but not to a much lower extent in cells exposed to high-LET carbon ions. Similar to p-Akt, the activities of p-mTOR and p-70S6 were depressed in SHG44 cells in a time- and LET-dependent manner (Fig. 1(a)). As shown in Fig. 1(b), high-LET carbon ions also depressed the activity of Akt/mTOR pathway in HeLa cells.

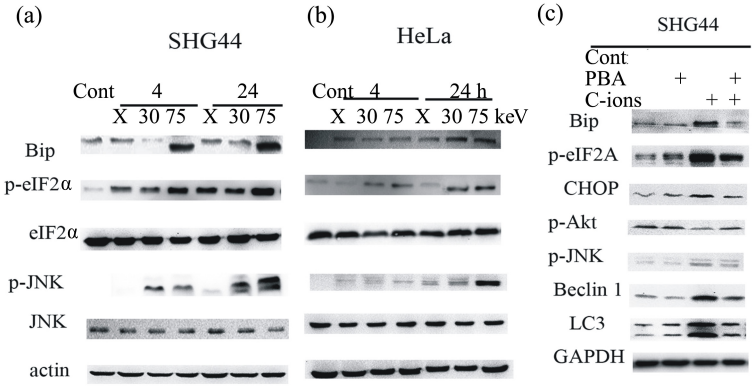

Fig. 1 PI3K/Akt signaling pathway was effectively depressed by X-rays or carbon ions with different LETs. (a) SHG44 cells irradiated at 2 Gy, (b) HeLa cells exposed to carbon ions with 75 keV/ m and 2 Gy (left) or different LETs with 2 Gy (right).

Subsequently, the UPR was detected as well. As shown in Figs. 2(a) and (b), the expression of Bip, a major indicator of UPR, was promoted by the radiations, indicating the occurrence of ER stress in tumor cells. It has been well known that eIF2α and JNK involve in autopahgy induction through UPR. The phosphorylated levels of these two proteins increased in a LET- and time-dependent manner, suggesting that carbon ion radiation activated UPR

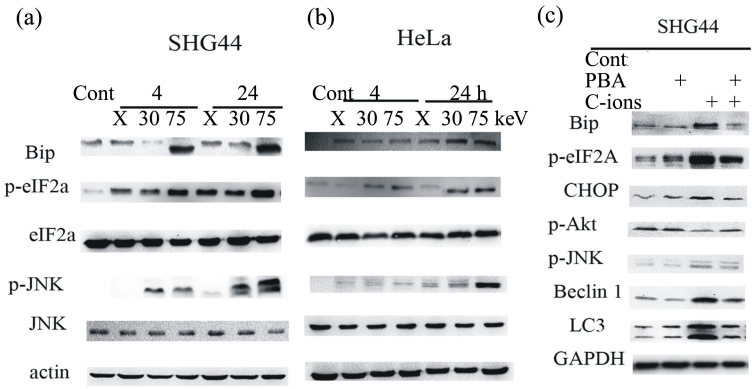

Fig. 2 Radiation induced autophagy via UPR. Bip and the key molecules of autophagy were upregulated by carbon ions with different LETs and 2 Gy in SHG44 (a) and HeLa (b). (c) PBA treatment prevented the activation of UPR and inhibited autophagy.

may involve in autopahgy induction in tumor cells. Then, we examined whether UPR inhibition influences the autophagy level in SHG44 cells. PBA acts as a chemical chaperone in the ER to prevent the activation of UPR and inhibits ER stress. In mock cells, PBA did not change the level of Bip, CHOP, p-Akt, p-JNK and Beclin 1, but slightly enhanced p-eIF2α and LC3II expressions (Fig. 2(c)). As shown in Fig. 2(c) (lines 3 and 4), PBA rescued carbon ion radiation induced UPR, as indicated by decreased Bip, CHOP expression and phosphorylation of Akt, JNK compared with radiation alone. PBA also decreased Beclin 1, p-eIF2α and LC3II expressions, but the levels were not reversed to the basal conditions.

In summary, the change of autophagy level in a LET-dependent manner is due to the fact that high-LET carbon ions suppress Akt/mTOR and activate UPR pathways more effectively compared with low-LET radiation.