3 - 69 Radiosensitization of HeLa Cells to Carbon Ions through Inhibition of Autophagy

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In cancer therapy, the role of autophagy is paradoxical. In some reports, autophagy appears to function as a protective mechanism against cellular stress. However, the induction of autophagy still plays a pivotal role in cell death induced by radiations or reagents in other reports. Therefore, whether autophagy helps to kill cancer cells or to sustain their survival under stressful conditions remains controversial.

To assess whether autophagy contributes to the resistance or sensitivity of tumor cells to high-LET carbon ions (75 keV/μm), human cervix adenocarcinoma HeLa cells wre used in our experiment. We depleted two key regulators of autophagy, Beclin 1 and Atg5 with short interfering RNA (siRNA). Beclin 1 and Atg5 are required at vesicle nucleation and elongation steps, respectively. These genes were knocked down separately and together in HeLa cells. The levels of knowdown (KD) achieved for each gene, were greater than 50% at 24 and 48 h(Fig. 1). Twenty-four hours after transfection, cells were irradiated with the carbon ions. The inhibitory effects of siRNA on the proportion of AVO-positive cells were analyzed with flow cytometry. As shown in Fig. 2, the KD of Beclin-1 together with Atg5 signi? cantly attenuated the high-LET radiation-induced AVOs formation. Beclin-1 or Atg5 KD alone had only marginal effects.

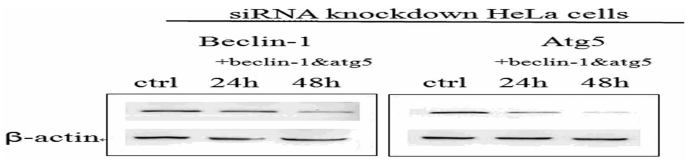

Fig. 1 Western blot analysis of Beclin-1 and Atg5 levels in double knockdown HeLa cells. The protein levels of Beclin-1 and Atg-5 were reduced at 24 and 48h after transfection.

Clonogenic survival experiments showed that the Beclin-1 KD alone or together with Atg5 sensitizes HeLa cells to the carbon ion irradiation. Shown in Fig. 3 are the survival curves for HeLa cells under the four conditions (irradiation, irradiation+siRNA Beclin-1, irradiation+siRNA Atg5 and irradiation+siRNA Beclin-1&Atg5). The survival fractions (SF) for the group of siRNA both genes were significantly lower than those for other three groups ($p<0.05$ at the various doses); for example, the survival fraction at 2 Gy (SF2) for the group of siRNA both genes was 1.2%, which was much lower than the SF2 of the untreated (30%) groups by a factor of 25. There was no significant difference in cell survival for irradiation alone and siRNA Beclin-1 or Atg5 alone ($p>0.05$). Clearly, the radiosensitivity of HeLa cells to the carbon ions was enhanced remarkably by the co-treatment of Beclin-1 together with Atg5 siRNA through inhibiting autophagy.

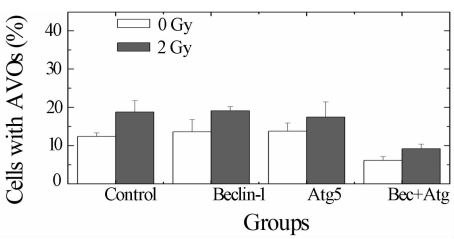

Fig. 2 Inhibition of siRNA on autophagy by carbon ions after 24 h, knockdown (KD) of Beclin-1 together with Atg5 significantly attenuated the high-LET radiation induced AVOs formation. Beclin-1 or Atg5 KD alone had only marginal effects.

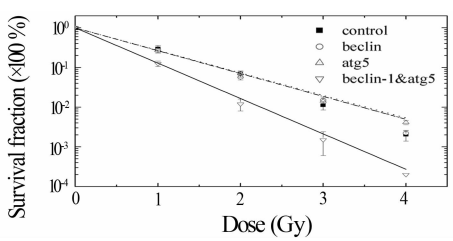

Fig. 3 The effect of inhibition autophagy on the clonogenic survival of HeLa cells. Untreated cells served as a control. Twenty four hours after transfection, the cells were irradiated with the indicated doses. After incubation for 14 d, colonies with cells greater than 50 were counted.

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