3 - 67 Autophagy Induced by High-LET Carbon Ions in Tumor Cells at Morphological and Molecular Levels

Jin Xiaodong, Liu Yan, Li Qiang, Yoshitaka Matsumoto[1] and Yoshiya Furusawa[1]

Autophagy is a lysosome-based degradative pathway which plays an essential role in maintaining cellular homeostasis. Recently, autophagy as a novel important target to improve anticancer therapy attracts considerable attention.

In this study, the autophagic effect induced by high-LET carbon ions (75 keV/(m) in tumor cells at morphological and molecular levels was investigated. Monodansylcadaverine (MDC) dye was employed to assess levels of mature autophagic vesicle formation in human cervix adenocarcinoma HeLa cells following irradiation. Control cells did not show a punctate staining pattern while displaying diffusive MDC staining. In contrast, cells irradiated with the high-LET radiation demonstrated extensive bright blue punctate staining as shown in Fig. 1(a). Moreover, we examined the autophagic effect using acridine orange (AO) staining. As shown in Fig. 1(a), control HeLa cells displayed diffusive green fluorescence. In irradiated cells, numerous acidic vesicular organelles (AVOs), characterized by red fluorescence, accumulated in acidic compartments and forming dot-like structures, which were distributed within the cytoplasm and localized to perinuclear regions, are consistent with accumulation of MDC in acidic vesicles.

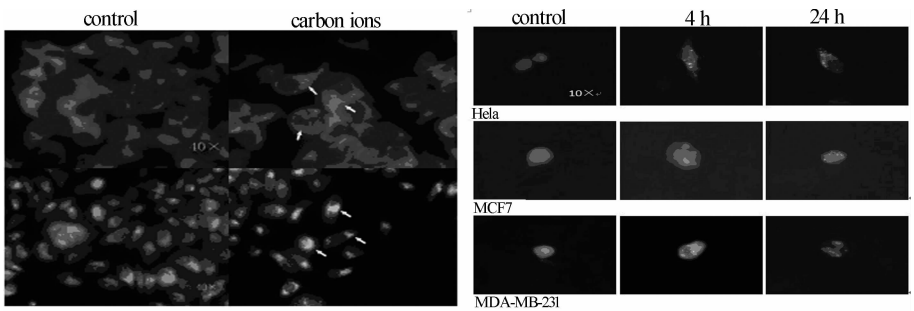

Fig. 1 Morphological analysis of the autophagy induced by high-LET carbon ions (LET=75 keV/(m, dose=2 Gy) in tumor cells. (a) HeLa cells were irradiated with the carbon ions and stained with MDC or AO 4 h post-irradiation. Cells were then observed using a fluorescence microscope. (b) GFP-LC3 staining patterns were analyzed by fluorescence microscopy.

To assess whether the vacuoles induced by the high-LET radiation were autophagic, we examined the autophagy inducing effect of the carbon ions using GFP-LC3 plasmid as well. Fig. 1(b) showed that the redistribution of GFP-LC3 from a diffusive cytosolic to a punctate autophagosome associated pattern was observed in HeLa cells and two breast cancer MCF7 and MDA-MB-231 cell lines following irradiation. In contrast, diffusive cytoplasmic localization of GFP-LC3 was founded in the control group. Taken together, the morphological results support that the high-LET carbon ions induced autophagy effectively in various tumor cells.

LC3 protein is a marker for autophagy. It exists in two cellular forms such as LC3-I and LC3-II. LC3-I is converted to LC3-II by conjugation to phosphatidylethanolamine, and the amount of LC3-II is closely correlated with the number of autophagosomes. To assess the effect of radiations on the autophagic pathway in HeLa cells, the results of LC3 conversion were analyzed at different time points after irradiation (0.5, 1, 4, 24 and 48 h). As shown in Fig. 2, control cells presented a low amount of LC3-II at any time after irradiation. However, the level of LC3-II protein expression in irradiated cells increased markedly with the time after irradaition, implying the process of autophagy in the cells. Similar results were also found for MCF7 and MDA-MB-231 cells as shown in Fig. 2. The analysis of LC3-II formation within the time period under investigation indicates that radiation-induced autophagy occurs primarily within a time period of up to 48 h. Based on these results, it can be concluded that high-LET radiations may be linked to autophagy induction, demonstrated by increase in LC3-II formation. For different radiation qualities, as shown in Fig. 2, LC3II expression level was different. The LC3II expression level induced by the high-LET radiation was obviously enhanced compared with X-rays at the same time points in HeLa cells.

1 National Institute of Radiological Sciences, Japan.

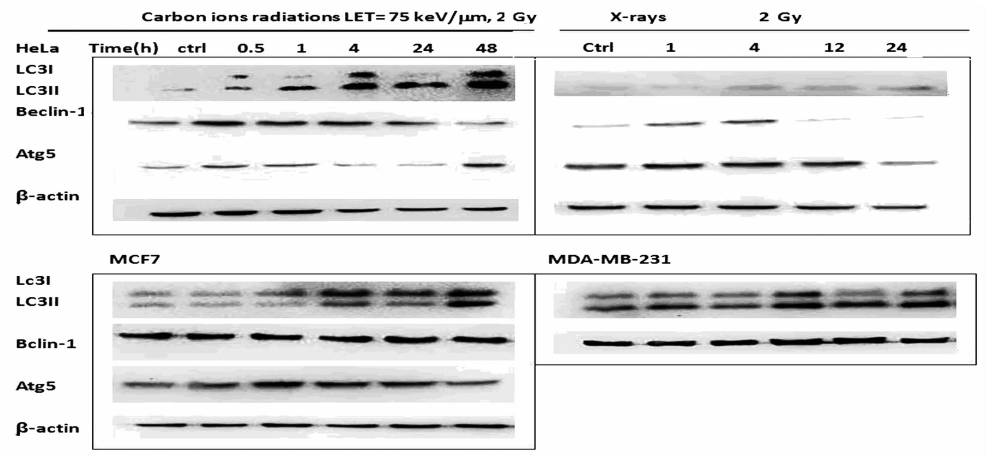

Fig. 2 LC3II conversion and expression of Beclin-1 and Atg-5 proteins in different cells after exposure to high-LET carbon ions or X-rays.

We also detected the expression of other key proteins related to autophagy under high-LET radiations stressor. We found that there was a diversity for the expression of LC3II and the others. For example, Atg5 expression gradually increased and reached a maximum, and then declined with time in HeLa and MCF7 cells. The beclin-1 expression was similar to the Atg5 expression in HeLa cells, but no change with time after irradiation in MCF7 cells.

3 - 68 Mutagentic Mechanisms of Ciliary Neurotrophic Factor in *E. coli*. Irradiated with Neon Ions

Jin Xiaodong, Li Qiang, Chen Yong [1], Lu Jian [1] and Jiang Lin [1]

Ciliary neurotrophic factor (CNTF) is a 22-26kD acid protein, which contacts with receptor located in thalamencephalon, decreases fat and loses weight in mice. In diet-induced obesity (DIO) mice model, CNTF has the same function. Noticeably, this model represents the real instance of human obesity. In our previous study, constructed *E. coli.*, which expresses recombinant CNTF (Fig. 1), as original strain was irradiated with high-LET neon ions and some new strains with enhanced CNTF protein expression were obtained. The mutagenic mechanisms of CNTF in *E. coli.* after exposure to heavy ions were investigated in this work.

The plasmids of the mutants, in which CNTF protein expression was higher or lower compared with control, were isolated and their genomic DNA was extracted. The CNTF gene and its upstream region were amplified with the primer of TCTTACTGTCCACTGAGACAGC-. Sequencing was performed by Sangon Biotech (Shanghai) Co., and the mutations were detected by using Bioedit software.

Sequencing analysis of the CNTF fragments amplified from the mutant lines shows that single nucleotide deletions were the principal mutation in each case as shown in Fig. 2 and the base substitutions and insertions occurred as well. The distribution of the deleted positions appeared to be dispersed around the promoter. These deleted positions also showed no homology to pET42a, and similar sequences in the host genome were therefore searched using the BLAST algorithm. We found that the most amount of deletions occurred in No. 60 strain. There were G deletion in 1685 position, A in 1707, A in 1719, G in 1727, C in 1832, A in 1838, G in 1855, G in 1858, respectively. Moreover, 1727 and 1855 were the hottest mutant positions and G had the highest frequency to be lost compared with the other positions and bases.

1 Lanzhou Institute of Biological Products.