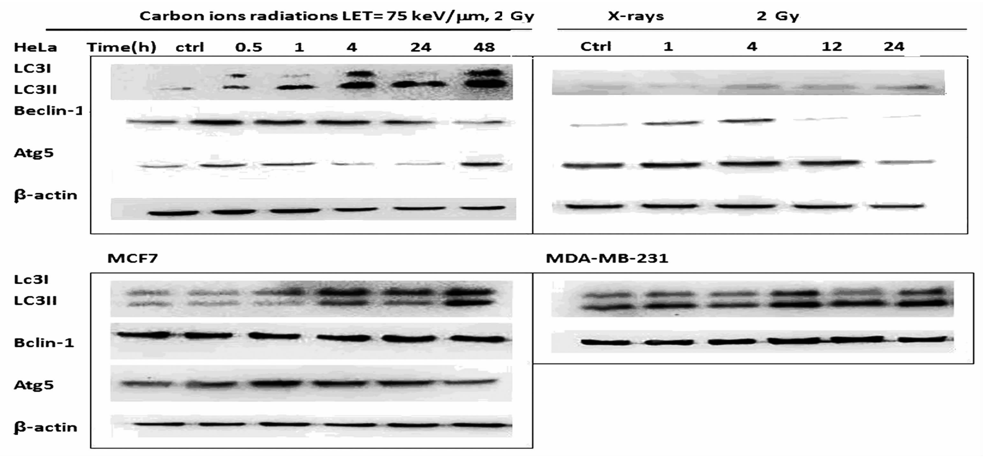

Fig. 2 LC3II conversion and expression of Beclin-1 and Atg-5 proteins in different cells after exposure to high-LET carbon ions or X-rays.

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

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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.

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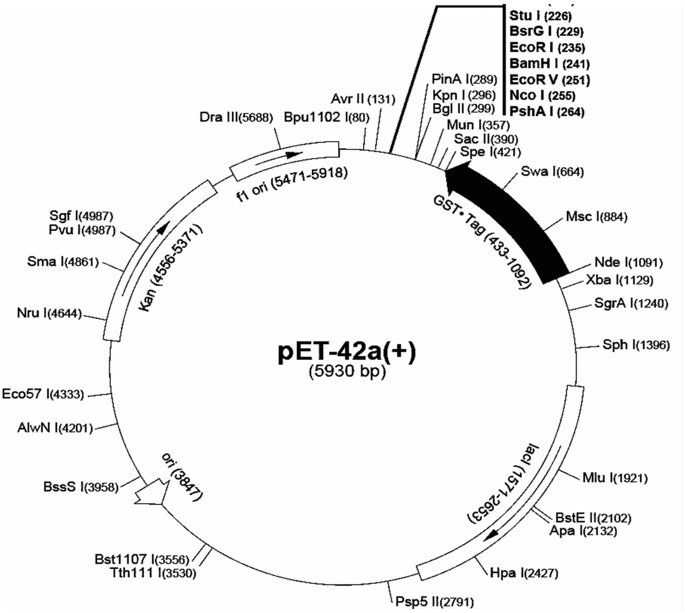

Fig. 1 Recombinant CNTF sequences map.

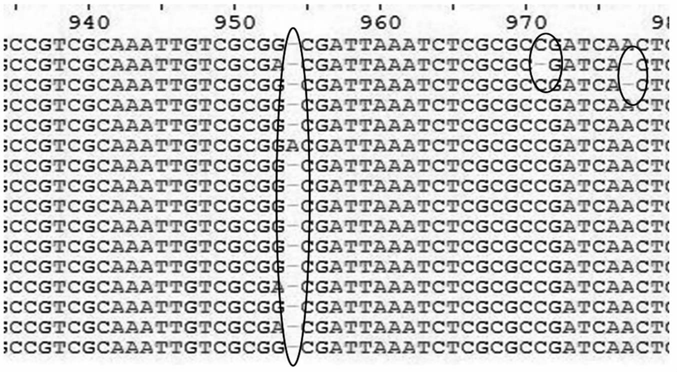

Fig. 2 Single nucleotide deletion in mutants.