3 - 69 Effects of Carbon-ion Beam Irradiation on MSH2 Expression in HepG2 Cells*

Miao Guoying, Zhang Hong and Di Cuixia

Primary liver cancer (namely hepatocellular carcinoma, HCC) represents the third most frequent cause of cancer death. National Comprehensive Cancer Network guidelines suggest that radiotherapy (RT) can be considered as an important role for unresectable HCC[1]. Heavy ion radiotherapy (*e.g.* carbon ion) has showed a strong role for killing malignancies especially for radioresistant malignancies that are insensitive to conventional radiotherapy, such as X-ray.

The mismatch repair (MMR) is a highly conserved DNA repair pathway that recognizes and repairs base-pairing errors during DNA replication or recombination. When the genes (mainly including *MutS* and *MutL* families) that mediate MMR are mutated or epigenetically silenced, the predisposition to cancer is significantly increased. MMR deficiency can be inherited, as in the case of Lynch syndrome also known as hereditary non-polyposis colorectal carcinoma (HNPCC). MSH2 protein also can modulate the homologous recombination that is a prominent double-strand break (DSB) repair pathway.

Although the role of DSB repair pathways in the survival response to IR has been extensively studied, DNA mismatch repair (MMR) has received less attention. The role of this pathway has been largely studied in the DNA damage response to chemotherapeutic agents, but the activation of the MMR system after IR remains controversial. And so far little information is available on the relationship between $MSH2$ and high-LET radiation.

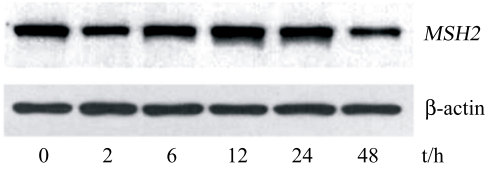

Fig. 1 (color online) Western blot detected $MSH2$ protein expression at different times of heavy ion irradiation.

We used western blot and RT-PCR assay to analyze $MSH2$ expression in human HepG2 carcinoma cells after low or high-LET radiations (Fig. 1). The $MSH2$ gene activated by carbon ion irradiation suggests that DNA mismatch repair gene $MSH2$ should be involved in DNA repair pathways.

Reference

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3 - 70 Capsazepine, a TRPV1 Antagonist, Enhances Radiation Sensitivity in Human Hepatocellular Carcinoma HepG2 Cells*

Han Lu, Di Cuixia and Zhang Hong

Transient receptor potential vanilloid 1(TRPVl) that is known as capsaicin receptor is a non-selective cation ion channel[1]. TRPV1 can regulate Ca^{2+} influx, participate in a variety of physiological and pathological process of tumor[2]. One such agent is Capsazepine (CPZ) that is now widely used as a selective vanilloid type 1 receptor (TRPV1) antagonist. CPZ can be directly actived on the capsaicin receptor, blocked its biological effects, and abolished osteosarcoma-induced hyperalgesia when administered subcutaneously at doses ranging from 3 to 10 mg/kg, blocked calcium channels[3]. However, the mechanisms underlying the anticancer effects of CPZ have not fully been understood. Whether CPZ can induce apoptosis of human hepatocellular carcinoma cell line HepG2 is not known. Therefore, the objective of the study reported here was to determine whether CPZ can enhance radiation sensitivity in HepG2 cell, and impact on cell proliferation.

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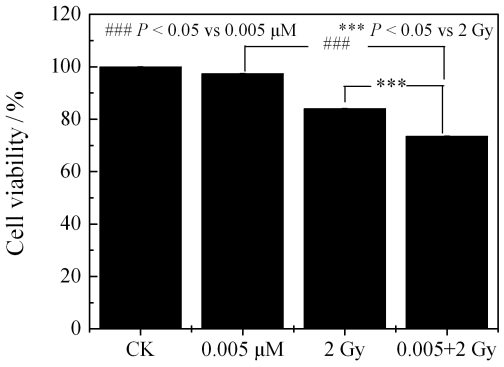

Fig. 1 CPZ significantly inhibited the proliferation of HepG2 cells exposed to carbon ion irradiation.

To investigate the effects of CPZ on cell proliferation, human hepatocellular carcinoma HepG2 cells were pretreated with 0.005, 0.01, 1, 25, 100 μmol/L CPZ for 24 h. The result suggested that CPZ could inhibit the proliferation of HepG2 cells in a dose dependent manner. Additionally, HepG2 cells were pretreated with 0.005 μmol/L CPZ for 6 h and irradiated with 2 Gy $^{12}C^{6+}$ ion beam irradiation. As shown in Fig. 1, the cell viability rate of IR+CPZ group was 73.46 ± 0.004% ($P < 0.05$), while the viability rate of IR group was 84.00 ± 0.001%. The result indicated that CPZ significantly inhibit the proliferation of HepG2 cells exposed to carbon ion irradiation. Therefore, these findings provide an opportunity to consider CPZ as a potential agent of radiation sensitivity.

References

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3 - 71 Researches on Radiation Related MicroRNAs and Risk Assessment of Heavy Ion Irradiation

Wang Jufang

The mechanisms of radiation induced bystander effects, functions of radiation related microRNAs and radiosensitivity of cancer and normal cells have been conducted by two- and three-dimensional cultured method during the past year in Department of Space Radiobiology. An international symposium on DNA repair and space radiobiology was successfully held in Institute of Modern Physics. There were more than 20 scientists from all over the world have taken part in the symposium. Moreover, Gansu Key Laboratory of Space Radiobiology has been in the cultivated period since July, 2014. Some of the achievements can be summarized as followings:

1) We demonstrated that miR-21 is involved in the radiation induced bystander effects (RIBE). It was found that exposure of normal human lung fibroblast MRC-5 cells to 150 MeV/u helium, 135 MeV/u carbon and 500 MeV/u iron ions could induce bystander effects through medium mediated way. Compared with the bystander cells treated with conditioned medium from non-irradiated cells, the bystander cells treated with conditioned medium from irradiated cells showed apparent increase in the frequency of micronuclei and 53BP1 foci, and dramatic decrease in survival fraction, suggesting that the RIBE could be induced by different types of charged particles. Significant upregulation of miR-21 in both directly irradiated cells and bystander cells were found by the expression levels of miR-21 precursor and its target genes. Transfection of miR-21 mimics into nonirradiated MRC-5 cells caused bystander-like effects. Elucidation of such a miRNA-mediated bystander effect is of utmost importance in understanding the biological processes related to ionizing radiation and cell-to-cell communication.

2) It was found that miR-454-3p modulated cellular radiosensitivity by regulating BTG1 which has long been recognized as a tumor suppressor gene. To investigate whether BTG1 responds to carbon ion exposure, we detected the mRNA levels and protein levels of BTG1 in renal carcinoma 786-O cells. The results implied that BTG1 response to 2.5 Gy of carbon beam significantly. To confirm that miR-454-3p participated in the process of DNA damage and repair through regulating BTG1, we transfected 786-O cells with miR-454-3p and treated the cells with 2.5 Gy of carbon beam. At 36 h after treatment, the genetic integrity of the cells was estimated by calculating the number of micronuclei in binucleated cells. As a result, the number of micronuclei increased markedly in upregulated miR-454-3p cells. However, miR-454-3p did not influence the genomic integrity of 786-O cells which were not exposed to carbon beam. Our results indicate that BTG1 contributes to maintain the genetic integrity and miR-454-3p regulates the cellular radiosensitivity after carbon ion irradiation by targeting BTG1. These findings may shed light on the potential application of microRNA in tumor radiotherapy.