4 - 64 Selective ATP Hydrolysis Inhibition in F1Fo ATP Synthase Enhances Radiosensitivity of Non-small-cell Lung Cancer Cells (A549)*

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F1Fo ATP synthase, or named ATPase (EC 3.6.1.3) (referred as the same enzyme hereafter) catalyzes the phosphorylation of ADP to ATP by exploiting a the transmembrane proton motive force (Δp) produced by the oxidation of nutrient substrates in mitochondrial respiration in all organisms. However, under peculiar physiological conditions when mitochondrial respiration is impaired which lead to the electrochemical gradient across the inner membrane collapse, the F1Fo ATP synthase switches its catalytic activity from ATP synthesis to ATP hydrolysis for maintaining the Δp.

A novel molecule BTB06584 (hereafter referred as BTB) developed by Fabrice Ivanes[1] *et al.* represent a valuable tool to selectively inhibit mitochondrial F1Fo-ATPase activity without compromising ATP synthase. Here we showed reduced cell proliferation, enhanced radiosensitivity after ionizing radiation with BTB preincubation, which indicates that the ATP hydrolysis activity of F1Fo ATPase was indeed responded to ionizing radiation (Fig. 1).

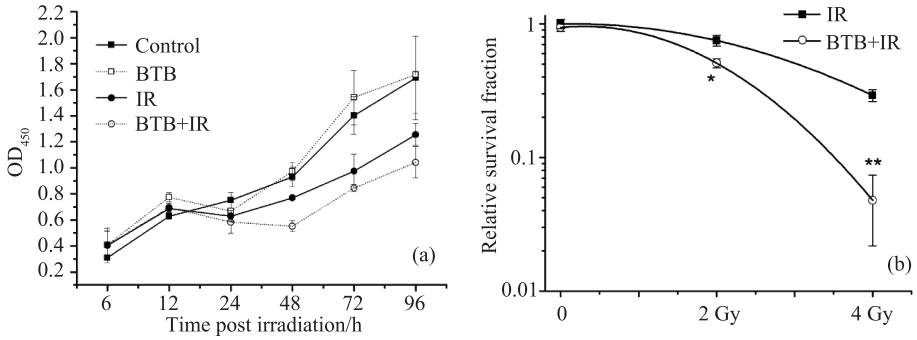

Fig. 1 Combination of BTB with ionizing radiation increased radiation sensitivity in NSCLC cells by inhibiting F1Fo ATPase. (a) Cell proliferation was analyzed by CCK8 assay. Growth curve was showed for each treatment at 0, 6, 12, 24, 48, 72 and 96 h. (b) Cell surviving fraction curve was performed with cell clonogenic survival assay. The relative surviving fractions were calculated as surviving fraction of treated cells divided by that of the control. P values were calculated with Student's t test. Multiple comparisons by one-way ANOVA with Dunnett post-hoc test confirmed the statistical significance. ($^*P \leq 0.05$, $^{**}P \leq 0.01$).

To determine whether BTB incubation can inhibit mitochondrial membrane potential recovery after IR, changes in Ψm was assessed by immunofluorescence using JC-1. As shown in Fig. 2, compared to control, A549 cells exposed

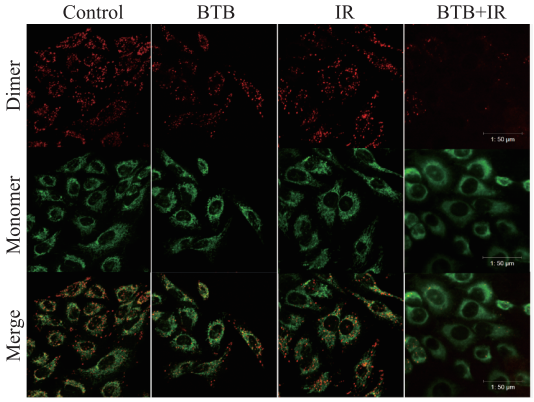

Fig. 2 (color online) Mitochondrial membrane potential (Ψm). Representative micrographs captured by confocal laser scanning microscope were evaluated for Ψm by staining with JC-1 at 24 h post ionizing radiation. Red dimers indicated normal mitochondrial function and green monomers indicated collapse of Ψm with cytoplasmic fluorescence. Scale bar = 50 μm.

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to 4 Gy ionizing radiation combined with BTB showed a marked reduction in red /green fluorescence intensity ratio at 24 h post radiation, suggesting a loss of Ψm, which is important to sensitize the cancer cells. The finding of the present study providing a novel inhibition target for enhancing radiosensitivity and BTB could be a potential radiosensitizer in radiation therapy.

Reference

[1] Fabrice Ivanes, Stefania Cocco, Jemma Gatliff, et al., British Journal of Pharmacology, 171(2014)4193.

4 - 65 Researches on Assessment, Protection and Mechanisms of Ionizing Radiation in Department of Space Radiobiology

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Ionizing radiation existing in heavy ion cancer therapy, nuclear device, spaceflight or terror attack is one of the major threats to human health and public security. During the past year, the main research task of Department of Space Radiobiology focus on the assessment, protection and underlying mechanisims of ionizing radiation both in vitro and in vivo.

Noninvasive biomarkers that can rapidly assess the exposure degree in the early stage of ionizing event are urgently needed for optimal medical treatments when an unexpected accident happens. Serum microRNAs (miRNAs) are ideal biomarkers because they are stable in responding to the changes of environments, conservative in different species and easy for collection. To identify serum miRNAs for the assessment of exposure degree, 8-weeks-old Kunming mice were whole-body exposed to 0.5 and 2 Gy of X-rays, carbon ions and iron ions.

The miRNA PCR array was performed to analyze the expression profiles of the serum miRNAs at 24 h after exposure. A specific signature with 12 radio-sensitive miRNAs was identified for further validation. After 0.1∼2.0 Gy of iron ion, X-ray or carbon ion irradiation, $miR-183-5p$, $miR-9-3p$, $miR-200b-5p$, $miR-342-3p$ and $miR-574-5p$ were selected as universal radio-sensitive biomarkers because they responded to all three kinds of ionizing radiation significantly. Finally, we developed a model including those five miRNAs by multiple logistic regression analysis from all three kinds of radiation data to assess the exposure risk scores (ERS):

$$\mathrm{ERS} = 0.300 + 0.125(X_{miR-183}) + 0.112(X_{miR-9}) + 0.154(X_{miR-200b})$$

$$-0.083(X_{miR-342}) - 0.062(X_{miR-574})$$

The receiver operating characteristic (ROC) analysis showed the model could predict the exposure degree with high specificity and sensitivity. The ERS values obtained from the model had significant difference for the assessment of exposure degree (ED): 0 for dose of 0 Gy, 1 for dose around 0.1 Gy and 2 for dose between 1∼2 Gy.

The radiation protective effects of the GANRA nanoparticles against X-ray radiation in lymphoblast cells were observed. Cell cytotoxicity, proliferation and cytokinesis-block micronucleus assay were conducted to evaluate the toxicity and radio-protective effects of GANRA nanoparticles. The results indicated that GANRA-nanoparticles exhibited low toxicity, while providing high radio-protective effects for lymphoblasts against X-ray radiation. It was also found that GANRA-nanoparticles acted as free radical scavenger, suggesting that GANRA-nanoparticles had the potential to be used as a safe and efficient radio-protectant.

Nuclear Receptor-binding SET domain2 (NSD2) is a histone methyltransferase that is abnormally expressed in Wolf-Hirschhorn syndrome(WHS) and many kinds of carcinomas including melanoma. We used p53 wild-type human melanoma cell line 92-1 as a cell model to study the funtion of NSD2 in radiation respons. Firstly, 92-1 cells were exposed to four different kinds of DNA damage inducer to prove that NSD2 was downregulated at protein levels which was related to upregulation of p53 and its downstream p21 after severe DNA damage. Then, it was verified that p21 mediated the degradation of NSD2 by premature activation of the APC/Ccdh1 in G2/M arrest by the RNA interference technology after induction of DNA damage. Finally, the DNA damage responses of 92-1 cells exposed to X-rays or hydroxyurea were detected. It was found that the functions of NSD2 in DNA damage respons which was induced by X-rays or hydroxyurea were different. It was p53, rather than p21 which directly repressed the transcription of NSD2, and the repression depended on a certain post-translational modification of p53 which was obviously different between the two different treatments.

In addition, it was found that radiation altered $miR-142-3p$ and $Bod1$ expression and thus contributed to early stages of radiation-induced genomic instability in carcinoma cells. An International Symposium on Bone Science