mental toxicity, but does not have the same effect on carbon-ion irradiation-induced developmental toxicity.

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4 - 62 Apoptosis in Zebrafish (Danio rerio) Embryos Induced by ^{56}Fe Ion Radiation*

Si Jing, Zhou Rong and Zhang Hong

All living organisms and ecosystems are permanently exposed to ionizing radiation. Of all the types of ionizing radiation, heavy ions such as ^{56}Fe have the potential to cause the most severe biological effects. We therefore examined the effects and potential mechanisms of iron ion irradiation on the induction of apoptosis in zebrafish embryos. Numerous experiments have shown that high-LET heavy ions may cause more DNA breaks than low-LET radiation. When DNA damage is irreparable, cell death occurs. DNA fragmentation is a characteristic feature of apoptosis, which is considered to be one consequence of radiation, via the induction of programmed cell death. TUNEL assay is designed to detect apoptotic cells, which undergo extensive DNA degradation during the late stages of apoptosis. Because of the optical transparency of zebrafish embryos, fluorescent TUNEL assay could allow the detection of apoptosis in zebrafish embryos directly and visually.

Zebrafish embryos at 4 h post-fertilization (hpf) were divided into five groups: a control group; and four groups irradiated with 0.5, 1, 2, and 4 Gy radiation, respectively. The beam energy of Iron particles was 160 MeV/u. The dose rate was 0.5 Gy/min approximately. The extent of apoptosis was examined by performing TUNEL staining (the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling) assay using ApopTag®Fluorescein In Situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD, USA). The method is based on the ability of TdT to label blunt ends of double-stranded DNA breaks independent of a template[1]. The TUNEL intensity within the samples was examined under a fluorescence microscope (Olympus BX51, Tokyo, Japan). The fluorescence was measured and quantified using ImagePro Plus software (Media Cybernetics, Silver Springs, USA). As shown in Fig. 1, there were few TUNEL-positive cells in the control, 0.5, and 1 Gy irradiation groups, whereas the fluorescence signal in embryos was markedly increased following 2 or 4 Gy irradiation (Fig. 1(a)). The relative fluorescence results in zebrafish embryos at 24 hpf showed that cell apoptosis increased in a dose-dependent manner. Iron ion irradiation resulted in slight increases in apoptosis at 0.5 and 1 Gy irradiation, but more significant increases at 2 and 4 Gy irradiation, with an approximately 2.05-fold increase with 2 Gy and 2.87-fold increase with 4 Gy compared with the control group ($P < 0.01$) (Fig. 1(b)).

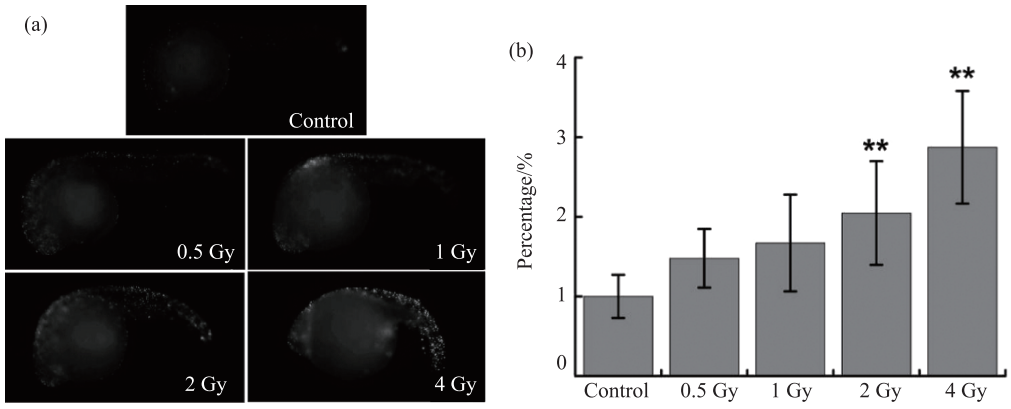

Fig. 1 Detection of iron ion radiation-induced changes in the cell apoptosis within live zebrafish embryos using the TUNEL assay. (a) Representative photographs of TUNEL labeling was emitted from cells undergoing cell apoptosis indicated by a green fluorescence signal on a black background. (b) Radiation dose–response curves of cell apoptosis measured as relative fluorescence within zebrafish embryos at 24 hpf. Each value is expressed as the mean ± SEM. Significant differences between the control and irradiated groups were determined using one-way ANOVA (* $P < 0.05$, ** $P < 0.01$), $N = 10$.

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* Foundation item: Key Program of National Natural Science Foundation of China (U1432248), National Natural Science Foundation of China (11305226)

4 - 63 Suppression of Radiation-induced Migration of Non-small Cell Lung Cancer through Inhibition of Nrf2-Notch Axis

Zhao Qiuyue and Zhang Hong

Nuclear factor E2 related factor 2 (Nrf2) is a transcription factor that is associated with tumor growth and resistance to radiation. The canonical Notch signaling pathway is also crucial for maintaining non-small cell lung cancer (NSCLC). Aberrant Nrf2 and Notch signaling has repeatedly been showed to facilitate metastasis of NSCLC. Here, we show that radiation induce Nrf2 and Notch1 expression in NSCLC. Knockdown of Nrf2 enhanced radiosensitivity of NSCLC and reduced epithelial-to-mesenchymal transition. Importantly, we found that knockdown of Nrf2 dramatically decreased radiation-induced NSCLC invasion(Fig. 1) and significantly increased E-cadherin, but reduced N-cadherin and matrix metalloproteinase (MMP)-2/9 expression. We found that Notch1 knockdown also upregulated E-cadherin and suppressed N-cadherin expression. Nrf2 contributes to NSCLC cell metastatic properties and this inhibition correlated with reduced Notch1 expression. These results establish that Nrf2 and Notch1 downregulation synergistically inhibit radiation-induced migratory and invasive properties of NSCLC cells.

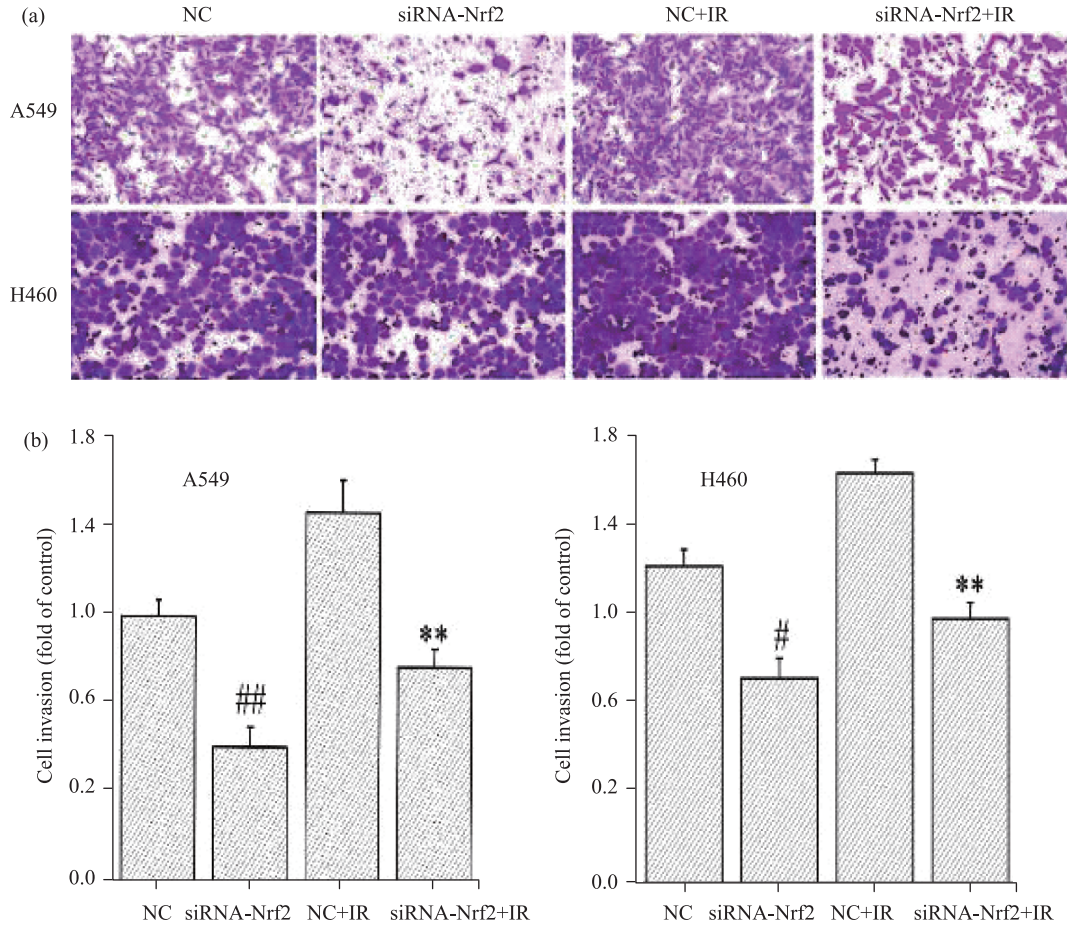

Fig. 1 (color online) Suppression of Nrf2 attenuated EMT in NSCLC cells. (a) and (b) Transwell invasion assay in A549 and H460 cells. The cells were then treated with or without the siNRA-Nrf2, or irradiation. The images (original magnification, ×200) were taken at 0 h and 24 h. The data are from triplicate experiments. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus NC group. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus irradiation-treated groups.