X-rays irradiation, Carbon-ion irradiation can induce more DNA damages in GSCs, the repair rate of DNA damage generated by carbon-ion was lower than that generated by X-rays in GSCs (Fig. 1) and compared with X-rays, heavy-ion could significantly reduce GSCs viability (Fig. 2). In the *in vivo* experiment, compared with X-rays, carbon-ion could significantly (P=0.0004) kill the glioma stem cells in the mice brains (Fig. 3).

Taken together, carbonion irradiation could induce lethality in the GSCs more efficiently than X-rays. Carbon-ion irradiation provides a significant performance over X-rays in targeting and inducing lethality in GSCs.

A. 53BP1 foci per cell of the #3 GSCs after irradiated with 2 Gy X-rays or carbon ions. B. 53BP1 foci (DSBs) repair rates of the #3 GSCs after irradiated with 2 Gy X-rays or carbon ions. C. XRCC1 foci per cell of the #3 GSCs after irradiated with 2 Gy X-rays or carbon ions. D. XRCC1 foci (SSBs) repair rates of the #3 GSCs after irradiated with 2 Gy X-rays or carbon ions.

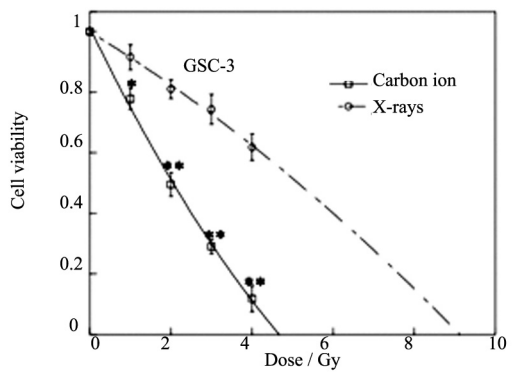

Fig. 2 Cell viability of #3 GSCs irradiated by 0 ∼ 4 Gy carbon-ion and X-rays.

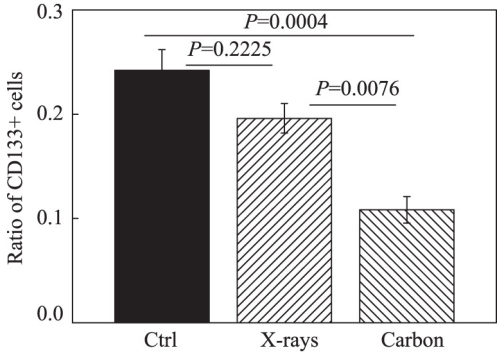

Fig. 3 Compared with X-rays, carbon-ion could significantly kill the glioma stem cells in the mice brains.

4 - 67 *miR*-300 Modulates the Cellular Radiosensitivity Through Targeting *p*53 in Human Non-small Cell Lung Cancer A549 Cells

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microRNAs (miRNAs) perform crucial roles in mediation of the cellular radiosensitivity by influencing DNA damage repair, cell cycle checkpoint, apoptosis, radio-related signal transduction pathway and microenvironment[1−3]. Our previous study have suggested that $miR-300$, whose expression is correlated positively with the cellular resistance to chemotherapy drug cis-platin in human ovarian cancer cells[4], is involved in the cellular response to the DNA damages induced by ionizing radiation[5]. However, the underlying molecular mechanisms remain unclear.

In the present study, the effects of $miR-300$ on the cellular DNA damage repair, cell cycle arrest and apoptosis were investigated in human non-small cell lung cancer cell line A549 cells. The results showed that ectopic expression of $miR-300$ by transfection with pre-$miR-300$ in A549 cells not only substantially enhanced the cellular DNA damage repair ability (Fig. 1), but greatly reduced the G2 cell cycle arrest and apoptosis induced by ionizing radiation. Bioinformatics analysis indicated that $p53$ is a putative target gene of $miR-300$, and the luciferase reporter assay demonstrated that miR-300 directly bind to the 3'-UTR of p53 mRNA (Fig. 2). Furthermore, overexpression of $miR-300$ significantly suppressed the p53 protein expression levels in A549 cells, indicating $p53$ is a direct target gene of $miR-300$. Of note, $miR-300$ could enhance the cellular radioresistance by inhibition of p53-related apoptosis and senescence signal pathways.

In summary, these data suggest that *miR*-300 functions as an important regulator for ionizing radiation induced DNA damage responses by targeting p53 and may lead to new potential strategies for enhancement of tumor radiosensitivity.

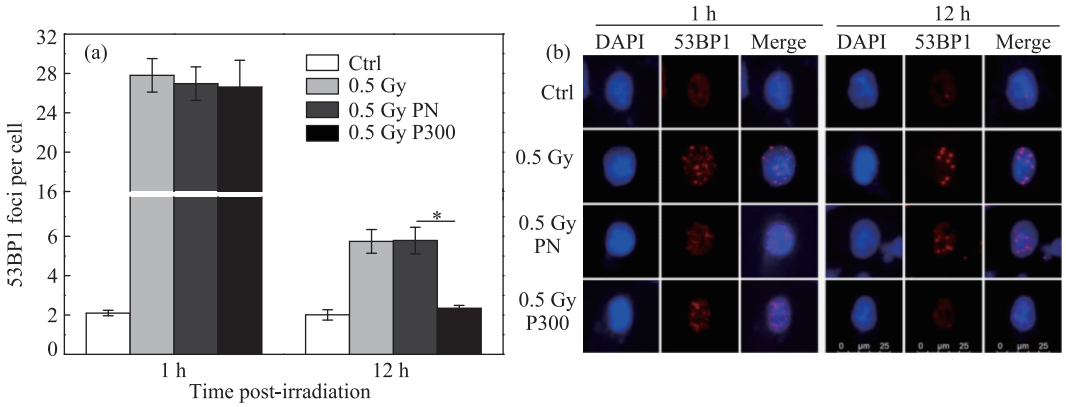

Fig. 1 (color online) *miR*-300 facilitates the double-strand DNA damage repair. Data (a) and images (b) of 53BP1 foci in A549 cells transfected with PN or P300 1 h or 12 h post 0.5 Gy of X-rays irraidation. Data are shown as mean ± SD. The experiments were repeated three times independently. $^*P < 0.05$. A two tailed Student's t-test was used to determine the P values. Ctrl, cells without transfection and irradiation treatments; PN, cells transfected with pre-negative-control; P300, cells transfected with pre-$miR-300$. Scal bar, 25 μm.

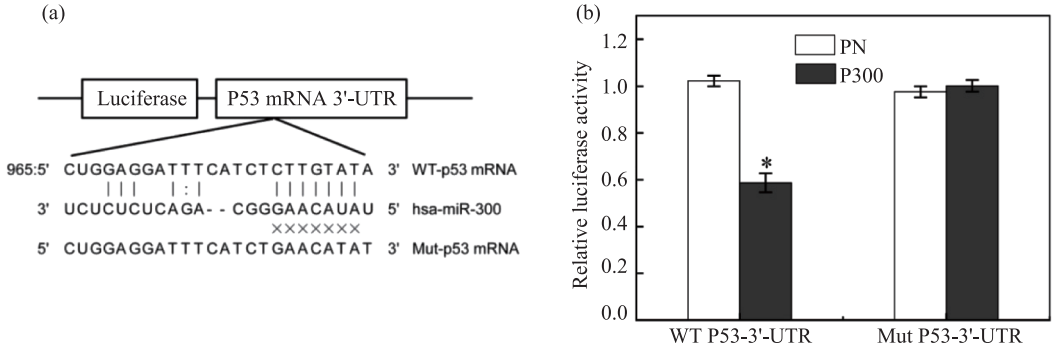

Fig. 2 (color online) p53 is a target of miR-300. (b) A schematic diagram illustrating the luciferase reporters with wild type (WT) or the mutated (Mut) binding sites of $p53$ mRNA 3'-UTR. (b) The effect of $miR-300$ mimic (P300) or the negative control (PN) on the luciferase activity of WT $p53$-3'-UTR or Mut $p53$-3'-UTR reporter in A549 cells. Data are shown as mean ± SD from three independent experiments. $^*P < 0.05$. All P values based on Student's t-test.

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