Nuclear Science and Techniques **20** (2009) 146–151

Effect of p53 on lung carcinoma cells irradiated by carbon ions or X-rays

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Abstract The study is to investigate the feasibility and advantages of heavy ion beams on radiotherapy. The cellular cycle and apoptosis, cell reproductive death and p53 expression evaluated with flow cytometry, clonogenic survival assays and Western blot analysis were examined in lung carcinoma cells after exposure to 89.63 MeV/u carbon ion and 6 MV X-ray irradiations, respectively. The results showed that the number colonyforming assay of A549 was higher than that of H1299 cells in two radiation groups; A549 cellular cycle was arrested in G_2/M in 12 h and the percentage of apoptosis ascended at each time point of carbon ion radiation with doses, the expression of p53 upregulated with doses exposed to X-ray or carbon ion. The cell number in G_2/M of H1299 and apoptosis were increasing at all time points with doses in $^{12}C^{6+}$ ion irradiation group. The results suggested that the effects of carbon ions or X rays irradiation on lung carcinoma cells were different, $^{12}C^{6+}$ ion irradiation could have more effect on upregulating the expression of p53 than X-ray, and the upregulated expression of p53 might produce the cellular cycle G_2/M arrested, apoptosis increasing; and p53 gene might affect the lung cancer cells radiosensitivity.

Key words $^{12}C^{6+}$ ion irradiation, Expression of p53, Cellular cycle and apoptosis, Survival of cells, X-ray

1 Introduction

Radiotherapy is an important treatment on cancers. Ionizing radiations kill tumour cells by inducing DNA damages, Non- or incorrectly repaired of DNA result in lethal chromosomal aberrations and finally cause the loss of proliferative capacity[1, 2]. Heavy ions may be better than X- or γ-rays in radiotherapy, because of their high linear energy transfer (LET), the Bragg peak of dose distribution and high relative biological effectiveness (RBE). However, different types of cell lines show various radiosensitivities, even for tumors from the same histopathological origin[3]. Thus, the clinical value of a radiation sensitivity predictive assay is important for radiation therapy, and it is essential to develop a predictive assay for tumor radiosensitivity in medical practice.

The remarkable functional dichotomy of wide-type p53, which can either promote cell survival or commit cells to a suicidal path, has been recognized and manifested in normal development and in the response of different types of embryonic and homeostatic tissues to DNA damage [4-6]. The palette of survival-promoting activities of wide-type p53 is much broader than what had been thought, covering a broad range of cellular responses including regulation of the cell survival, cycle and apoptosis. In this commentary we will discuss the possibility that wide-type p53 in some types of cancer cells may contribute to their overall survival potential, cycle and apoptosis, and therefore investigate the feasibility and advantages of heavy ion beams on radiotherapy. The data were expected to be available for tumor radiosensitivity in medical practice.

Supported by the National Natural Science Foundation of China (10675151 and 10835011), the Key Scientific Technology Research Projects of Gansu Province (0702NKDA045 and 0801NKDA001) and Science and technology brainstorm project of Lanzhou (07-2-07)

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Received date: 2009-01-20

2 Methods

2.1 Cell culture

Human lung cancer cell lines A549 (wild-type p53, bronchial carcinoma cells), H1299 (p53 null, human non-small cell lung cancer cell) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were grown in RPMI 1640 medium with 10% of fetal bovine serum and 1% penicillin/streptomycin in an incubator (37°C, 5% of CO_2).

2.2 Irradiation

Cells exposures were conducted at the Heavy Ion Research Facility in Lanzhou (HIRFL) at Institute of Modern Physics (IMP) on a vertical beam line of the therapy terminal by 100 MeV/u $^{12}C^{6+}$ ion beams. Due to energy losses in the vacuum window, air gap, Petri dish cover and medium, the ions impinging on the cell samples was estimated at 89.63 MeV/u, corresponding to an LET of 28.3 keV/μm. The dose rate was adjusted at about 0.5 Gy/min.

Low-LET irradiations were performed using SIEMENS Primus linac operated at 6 MV at a source-to-surface distance of 100 cm, and the dose rate was approximately 0.5 Gy/min. The doses for both the $^{12}C^{6+}$ ion or X-ray irradiation were 0, 0.25, 0.5, 0.75, 1, 1.5 or 2 Gy. The irradiations were performed at room temperature. A dose point in each group had three parallel samples.

2.3 Clonogenic assay

Cell survival was determined by the standard colony-forming assay. Briefly, cells were re-plated at a density of about 100 surviving cells into 60 mm Petri dishes supplemented with RPMI 1640 medium including 10% fetal calf serum after irradiation. After incubation for 7 days, the cells were fixed and stained with 5% Giemsa solution. Colonies with over 50 cells were counted as survivors, SF(%) = (Sr/Sc)×100%, where SF is the survival fraction, Sr is colony fraction of cells by X-ray or carbon ion irradiation, and Sc is colony fraction of control

2.4 Cycle and apoptosis of cells

In 0.5, 4 and 12 h after exposure, cells suspension were fixed by 75% cold ethanol solution, and were filtered through a mesh of 60 μm pores (Becton Dickinson) to remove debris or clusters of cells. The cells were stained for 30 min with 200 μL propidium iodide (PI, P 4170, Sangon, China), 20 μg/mL containing RNase (R 5503, Sangon, China) 0.2 mg/mL[7], and then 1×10^4 cells were analyzed with flow cytometry (Becton Dickinson) using an argon laser with 570 nm (PI) band pass filters. The data were acquired using FAC Scan software (Becton Dickinson, USA) and analyzed using the ModFit LT 3.0 software (Becton Dickinson, USA).

2.5 Western blot analysis

At 12 h after radiation, cells were digested with lysis buffer which contained 250 mmol/L NaCl; 5 mmol/L EDTA; 1% Igepal; 5 mmol/L dithiothreitol (DTT); 1 mmol/L phenylmethylsulfonyl fluoride (PMSF); and 1% protease inhibitor cocktail (Sigma, USA). Protein concentrations were evaluated with the Bio-Rad protein assay (Bio-Rad, USA). 40 μg of total protein was loaded onto NuPAGE^{TM} 10% Bis-Tris Gel (Invitrogen, USA). Protein on the gels was electro-transferred onto nitrocellulose membranes and blocked with blocking buffer (5% of non-fat milk, 500 mmol/L of NaCl, 20 mmol/L Tris and 0.1% Tween-20). The membranes were incubated with primary antibodies (p53 antibody polyclonal antibody and anti-β-actin polyclonal antibody, Cell Signaling Technology Inc., USA) at 4°C overnight. After washing with TBS-T (blocking buffer without milk) four times, 10 min each, the membranes were incubated with anti-rabbit Ig horseradish peroxidase linked to whole secondary antibodies (Amersham Pharmacia Biotech, USA) at room temperature for 1 h. After washing five times, 10 min each, a chemiluminescent detection system (ECL Western blotting detection reagents, Amersham Pharmacia Biotech, USA) was used to detect the secondary antibody. Finally, the membranes were exposed to X-ray films. The protein bands were analyzed by FluorChem^{TM}8900 (Alpha Innotech Corp., USA).

3 Results and discussion

Fig. 1 is the survival curves of lung carcinoma cells exposed to the $^{12}C^{6+}$ ions or X-rays. In the colony-forming assay the high number of A549 colonyform-

ing indicated that the H1299 lung carcinoma cellls have more radiosensitivity than A549. A549 cellular cycle was arrested in G_2/M in 12 h and the apoptosis ascended at each time point with doses in carbon ion group (Table 1), the expression of p53 up-regulated with doses in the two irradiation groups (Fig. 2). And there were significant increases in G_2/M of H1299 cell cycle and apoptosis exposed to $^{12}C^{6+}$ ion at all time points with doses (Table 2).

The mechanisms underlying this phenomenon could be complex. It is probably related to p53 gene. The tumor suppressor protein p53 is a key regulator of cell cycle control, apoptosis and genomic stability in response to various cellular stresses[8-13]. Mutation of the p53 gene is the most frequently reported genetic defect in human cancers. The best characterized and most important function of wild-type p53 is the sequence specific transactivation of target genes. In this experiment, the steady state level of p53 protein is very low before irradiation. However, DNA damage caused by irradiation induces a prominent increase in p53. The increased wild-type p53 protein transactivates expression of p21, bax and other important genes, which are involved in G_2/M arrest of cell cycle and cellular apoptosis increase[9-11, 14, 15]. Loss of wild-type p53 not only disrupts cell cycle checkpoints and apoptosis, but also results in cell radiosensitivity changes. Recent reports documented that the combination of ionizing radiation and adenoviral p53 gene therapy changed radiosensitivity of both p53-mutant and wild-type cancer cells[16-18].

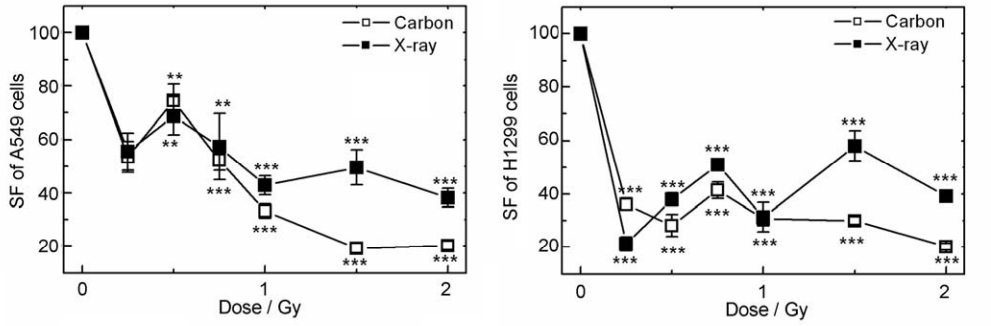

Fig. 1 Survival curves for lung carcinoma cell line irradiated by X-rays or 100 MeV/u $^{12}C^{6+}$ ions. (*p<0.05, **p<0.01 and ***p<0.001, compared with the controls).

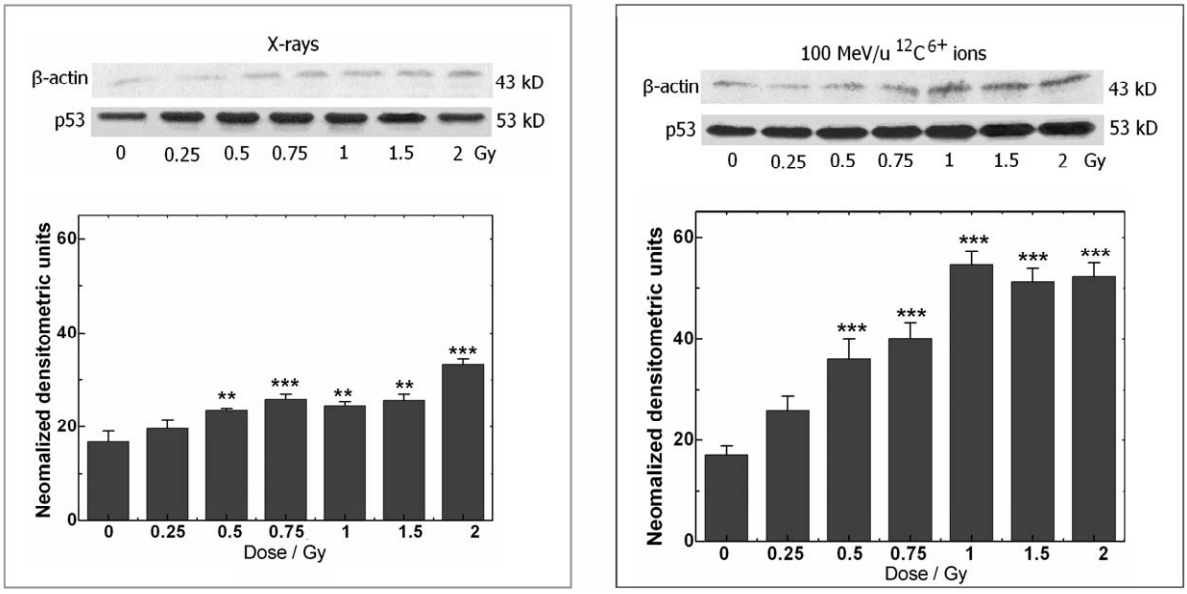

Fig. 2 Western blot analysis of A549 cells differentiation p53 protein expression after the ^{12}C ion and X-ray irradiation. (**p<0.01 and ***p<0.001, compared with the controls).

Table 1 Changes (in %) in cycle and apoptosis of A549 cells after $^{12}C^{6+}$ ion or X-ray irradiation. ($\overline{x}$ ±SD)

Groups			G_0/G_1	S	G_2/M	Apoptosis
Control			62.97±0.87	5.67±0.34	31.35±0.66	0.97±0.26
$^{12}C^{6+}$ ions	0.5 h	0.25 Gy	63.66±0.88	7.67±0.88*	28.67±0.33	2.17±0.44
		0.5 Gy	65.15±0.41	2.56±0.37**	32.29±0.12	1.68±0.52
		0.75 Gy	60.36±0.24	7.14±0.23	32.51±0.25	2.66±0.18
		1 Gy	64.33±1.20	4.17±0.44	31.50±0.87	3.67±0.88**
		1.5 Gy	85.33±0.47	5.95±0.45	8.72±0.14	3.69±0.18**
		2 Gy	60.61±1.02	5.45±0.45	33.94±1.13	2.92±0.46*
	4 h	0.25 Gy	64.89±2.25	13.36±1.92***	21.74±4.13*	6.24±0.80***
		0.5 Gy	56.6±8.66	3.86±0.18*	39.54±0.68*	2.11±0.35
		0.75 Gy	62.17±0.16	2.84±0.13**	34.99±0.24	2.78±0.19
		1 Gy	58.37±0.74	9.13±0.18**	32.49±0.61	7.09±0.49***
		1.5 Gy	62.58±0.30	1.84±0.92***	35.58±0.71	2.71±0.51
		2 Gy	54.64±0.35*	8.19±0.67*	37.17±0.70	8.5±0.44***
	12 h	0.25 Gy	82.15±8.10***	0.83±0.17***	17.02±8.01**	1.63±0.36
		0.5 Gy	57.05±0.74	2.44±0.98**	40.51±7.68*	2.29±0.17
		0.75 Gy	54.57±0.64*	1.09±0.50***	44.34±1.07**	5.53±0.65***
		1 Gy	70.32±0.70	1.19±0.47***	28.49±0.33	9.85±0.57***
		1.5 Gy	56.37±1.16	29.75±0.37***	13.89±0.85***	5.56±0.18***
		2 Gy	46.28±1.10***	2.69±0.28**	51.03±0.82***	19.35±1.9***
X-rays	0.5 h	0.25 Gy	58.93±1.64*	17.52±8.57***	23.55±10.49	1.46±0.16
		0.5 Gy	53.39±2.19***	6.35±1.84	40.25±3.97	1.13±0.25
		0.75 Gy	62.42±0.30	2.04±0.03	35.53±0.33	2.81±0.43
		1 Gy	60.8±1.59	7.17±0.44	32.03±1.82	1.3±0.35
		1.5 Gy	60.35±0.71	6.72±0.28	32.93±0.93	1.84±0.26
		2 Gy	59.81±1.57	6.83±0.17	33.36±1.44	2.49±0.53
	4 h	0.25 Gy	64.2±2.23	19.87±1.04***	15.93±3.27**	2.37±0.19
		0.5 Gy	58.2±0.61**	3.33±0.88	38.47±0.29	2.83±0.44
		0.75 Gy	59.32±0.94	7.42±0.59	33.25±1.53	2.24±0.39
		1 Gy	62.76±1.49	7.13±0.47	30.11±1.16	2.18±0.43
		1.5 Gy	60.47±0.29	6.33±0.33	33.2±0.61	2.37±0.32
		2 Gy	56.32±0.88	6.67±0.73	77.01±0.88	6.31±1.51**
	12 h	0.25 Gy	60.23±1.05	7.08±0.33	32.69±0.98	1.31±0.19
		0.5 Gy	53.93±0.68***	7.6±0.54	38.46±0.93*	1.88±0.60
		0.75 Gy	77.52±0.78***	2.24±0.91	20.24±0.40*	4.27±4.02*
		1 Gy	58.83±0.18	6.43±0.29	32.74±0.38	2.05±1.49
		1.5 Gy	54.32±0.63**	6.67±0.67	39.01±0.22**	6.09±1.89**
		2 Gy	59.58±0.87***	1.69±0.31	38.73±3.66	14.09±2.02***

Note: *p<0.05, **p<0.01, ***p<0.001

Table 2 Changes (in %) in cycle and apoptosis of H1299 cells after $^{12}C^{6+}$ ionand X-ray irradiation. ($\overline{x}$ ±SD)

Groups			G_0/G_1	S	G_2/M	Apoptosis
Control			65.68±0.25	11.08±0.48	23.24±0.16	0.84±0.45
$^{12}C^{6+}$ ions	0.5 h	0.25 Gy	63.4±0.31	14.48±0.29	22.12±0.12	3.14±0.46**
		0.5 Gy	57.47±0.30**	7.79±0.14	34.74±0.18**	1.54±0.74
		0.75 Gy	59.61±0.24*	10.21±0.49	30.18±0.61*	3.28±0.37
		1 Gy	67.83±3.02	17.57±0.56**	14.6±2.55**	11.48±0.59***
		1.5 Gy	65.54±3.36	14.33±2.33	20.13±1.04	8.07±0.58***
		2 Gy	66.14±2.21	16.85±1.39**	17.01±3.51	3.68±0.88***
	4 h	0.25 Gy	54.82±0.82***	9.04±0.36	36.14±0.61***	2.26±0.39
		0.5 Gy	51.60±0.22***	10.21±0.11	38.19±0.20***	2.07±0.35
		0.75 Gy	68.33±0.88	10.17±0.61	21.5±0.29	5.63±0.32***
		1 Gy	56.91±1.98**	7.36±0.33*	35.72±2.01***	5.26±0.21***
		1.5 Gy	47.87±1.34***	7.19±0.65*	44.93±0.69***	3.86±0.34***
		2 Gy	52.19±1.53***	17.15±1.17**	30.67±2.60*	7.83±0.44***
	12 h	0.25 Gy	54.25±2.07***	14.43±1.45	31.32±2.68*	8.75±0.42***
		0.5 Gy	48.45±0.23***	13.38±0.78	38.17±0.47***	1.99±0.31
		0.75 Gy	52.5±0.60***	16.16±0.40*	31.34±0.99*	2.49±0.63***
		1 Gy	49.52±2.24***	19.96±4.07***	30.52±6.28*	3.67±0.61***
		1.5 Gy	45.03±2.46***	22.57±0.16***	32.40±2.56**	3.56±0.38**
		2 Gy	40.72±2.93***	20.17±0.28***	39.11±2.68***	13.17±0.72***
X-rays	0.5 h	0.25 Gy	63.32±1.07	13.08±8.25	23.6±12.25	2.81±0.91
		0.5 Gy	67.38±2.06	13.7±3.87	18.92±4.87	2.02±0.29
		0.75 Gy	58.85±3.64	20.36±1.38	20.78±2.26	3.34±0.23
		1 Gy	63.53±1.36	16.15±7.26	20.32±7.05	1.64±0.78
		1.5 Gy	54.61±5.89*	16.43±3.36	28.96±4.90	2.3±0.63
		2 Gy	52.42±5.87*	25.19±2.60*	22.39±8.31	5.23±2.97*
	4 h	0.25 Gy	70.06±3.61	10.72±2.85	19.23±0.76	2.02±0.09
		0.5 Gy	64.92±2.21	12.7±3.96	22.37±2.37	2.37±0.32
		0.75 Gy	62.12±2.38	24.36±1.59*	13.52±3.41	1.22±0.58
		1 Gy	53.99±10.97*	15.28±1.27	30.73±10.38	1.44±0.92
		1.5 Gy	45.44±2.37**	9.25±2.84	45.32±2.26**	3.67±0.67
		2 Gy	48.33±2.56**	24.43±7.9*	27.24±5.35	4.91±2.61
	12 h	0.25 Gy	55.94±2.92	17.38±0.60	26.68±2.92	0.51±0.25
		0.5 Gy	62.93±0.32	16.92±0.97	20.14±1.36	0.65±0.05
		0.75 Gy	63.56±5.40	20.1±3.63	16.34±3.19	2.22±1.05
		1 Gy	54.41±2.58	16.65±5.86	28.93±3.37	3.54±3.61
		1.5 Gy	37.87±1.93***	30.95±1.47	31.17±0.48	0.43±0.14
		2 Gy	42.46±1.50***	34.04±1.15	23.5±2.18	7.71±3.32**

Note: *p<0.05, **p<0.01, ***p<0.001

In the $^{12}C^{6+}$ ion irradiation group, the G_2/M of H1299 and A549 cell cycle distribution increase significantly with dose, but no significant changes of cell cycle and apoptosis in the X-ray irradiation groups (Table 1 and 2), and the expression of p53 induced by the carbon ion is different from that of X-ray (Fig. 2). As we know, heavy ion irradiation has higher RBE than that of low- LET irradiation[9]. Ions deposit energy along their tracks in high density, the local concentration of the irradiation could have different impacts by heavy ion irradiation and low-LET irradiation[19].

4 Conclusion

In this study, the effects of carbon ions or X rays irradiation on lung carcinoma cells were different, $^{12}C^{6+}$ ion irradiation could have more effect on upregulating the expression of p53 than X-ray, and the upregulated expression of p53 might produce the cellular cycle G_2/M arrested, apoptosis increasing, and p53 gene might affect the lung carcinoma cells radiosensitivity.

Acknowledgments

We thank the accelerator crew at the National Laboratory of Heavy Ion Accelerator in Lanzhou.

References

1 Dikomey E, Borgmann K, Brammer I *et al*. Toxicology, 2003, **193**(1-2): 125-135.

2 Distel LV, Neubauer S, Keller U *et al*. Radiother Oncol, 2006, **81**(3): 257-263.

3 Peacock J H, Edwards H A, Mcmillan T R *et al*. Int J Radiat Biol, 1989, **56**(5): 543-547.

4 Komarova E A, Chernov M V, Franks R, *et al*. EMBO J, 1997, **16**(6): 1391-1400.

5 Frenkel J, Sherman D, Fein A, *et al*. Oncogene, 1999, **18**(18): 2901-2907.

6 Komarova E A, Gudkov A V. Biochemistry (Mosc), 2000, **65**(1): 41-48.

7 Vanparys C, Maras M, Lenjou M, *et al*. Coen Toxicol in Vitro, 2006, **20**(7): 1238-1248.

8 Farmer G, Bargonetti J, Zhu H, *et al*. Nature, 1992, **358**(6381): 83-86.

9 El-Deiry W S, Tokino T, Velculescu V E, *et al*. Cell, 1993, **75**(4): 817-825.

10 Harper J W, Adami G R, Wei N, *et al*. Cell, 1993, **75**(4): 805-816.

11 Xiong Y, Hannon GJ, Zhang H, *et al*. Nature, 1993, **366**(6456): 701-704.

12 Vousden K H. Cell, 2000, **103**(5): 691-694.

13 Vogelstein B, Lane D, Levine A J. Nature, 2000, **408**(6810): 307-310.

14 Deng Y, Wu X. Proc Natl Acad Sci U. S. A, 2000, **97**(22): 12050-12055.

15 Brugarolas J, Chandrasekaran C, Gordon J I, *et al*. Nature, 1995, **377**(6549): 552-557.

16 Harris M P, Sutjipto S, Wills K N, *et al*. Cancer Gene Ther, 1996, **3**(2): 121-130.

17 Nielsen L L, Maneval D C. Cancer Gene Ther, 1998, **5**(1): 52-63.

18 Colletier P J, Ashoori F, Cowen D, *et al*. Int J Radiat Oncol Biol Phys, 2000, **48**(5): 1507-1512.

19 Koizumi H, Taguchi M, Kobayashi Y, *et al*. Instrum Method Phys Res, Sect B, 2003, **208**(43): 161-165.