Nuclear Science and Techniques **21** (2010) 294–301

# Regulation of the radiosensitivity of tumor cells through HIF-1α dependent intracellular redox status after fractionated radiation

JIN Wensen [1,*] KONG Zhaolu [2] SHEN Zhifen [2] JIN Yizun [2,*] ZHANG Wukui [1]

[1] *Department of Nuclear Medicine, An-hui Medical University, Hefei, China*

[2] *Eighth Laboratory, Institute of Radiation Medicine, Fudan University, Shanghai, China*

**Abstract** Tumor cells often develop resistance to radiotherapy by fractionated radiation possibly due to the heterogeneity and hypoxia in tumor tissue. However, the mechanism of refractory effect remains unclear. In the present study, a radioresistant variant HepG2/R60 cell line was isolated from human HepG2 cells by repeated exposure to radiation. The results showed that, after irradiation, the higher survival rate was in HepG2/R60 cells compared to parental cells. Hypoxia treatment could further increase the radioresistance of HepG2/R60 cells concomitant with high level of intracellular GSH and overexpression of HIF-1α. When hypoxic HepG2/R60 cells were pretreated with BSO, a GSH depleter, the refractory response was significantly reduced showing a decrease in intracellular GSH level, followed by the suppression of HIF-1α in hypoxic cells. Subsequent study found that the level of BCL-2 was down-regulated, targeted by HIF-1 prompting transcription in hypoxic cells. The effect of HIF-1α on the radiosensitivity of hypoxic cells was confirmed using YC-1, a specific inhibitor of HIF-1α. Consequently, our results suggest that the radiosensitivity of tumor cells might be regulated by fractionated radiation, and the radioresistance of cells induced by repeated exposure, under hypoxic condition, could be correlated with overexpression of HIF-1α dependent on the alteration of intracellular GSH contents.

**Key words** Radiation, Hypoxia inducible factor-1α, Radiosensitivity, Reduced glutathione, Redox status.

## 1 Introduction

Radiotherapy is an effective tool for malignant tumor treatments. It is particularly beneficial for rapid growth tumor because of their high susceptibility to radiation[1, 2]. However, the same type of carcinoma may differ from patient to patient in the sensitivity to irradiation, and recurrent or metastatic tumors are very refractory to secondary radiation therapy[3,4]. This indicates the presence of radioresistant tumor cells. Studies on mechanisms of radiosensitiviy and resistance have found that the factors contributing to development of the radioresistance may include cellular environment and biological features of cancer cells, such as the heterogeneity and hypoxia in tumor tissue, intracellular redox status and the alteration of refractory gene expression[5–8].

Tumor radiation biology have demonstrated that hypoxia can induce resistance to radiation in solid tumors[9–11]. At molecular level, hypoxia inducible factor-1 (HIF-1) plays an important role in tumor invasion, angiogenesis, metastasis, and failure of radiotherapy[12, 13]. As a heterodimeric protein, HIF-1 consists of two subunits, i.e. the constitutively expressed HIF-1β/ARNT, and the highly regulated HIF-1α. Under aerobic condition, the HIF-1α subunit is hydroxylated by prolyl hydroxylases (PHDs), and targeted rapidly for proteasome-mediated degradation through a protein ubiquity ligase complex[14, 15]. When cells are exposed to hypoxic environment, this degradation pathway is blocked. Therefore, HIF-1α is accumulated and migrated to nucleus where more than 60 genes have been identified as the direct targets of

Supported by National Natural Science Foundation of China (Grant No. 30500143), Science Research Foundation of Anhui Medical University (Grant No. 010503101)and College' Science Research Program of Anhui Province (Grant No.KJ2010A189).

*Corresponding author. *E-mail address*: wensenjn@126.com; yzjin@shmu.edu.cn

Received date: 2010-06-27

HIF-1α[16], of which many are responsible for poor prognosis, metastasis and resistance to tumor therapy.

Studies on tumor radiobiology have revealed that high level of HIF-1α in tumor tissue could result in tolerance of cells to irradiation[16,17]. It has been shown that the up-regulation of HIF-1α in tumor cells, under hypoxic condition, targets many genes involving DNA repair and cellular resistance to various physical and chemical damages[18,19]. Clinical data showed that, with high level HIF-1α, some tumor tissues had poor response to radiotherapy[20–24]. Although fractionated radiation can kill the most cells in the tumor tissue, due to the heterogeneity and hypoxia in tumor tissue, a small amount of cells under repeated exposure may form escape response to radiation, hence neoplasm recurrence by modified expressions of refractory genes and intracellular redox status[8,25]. It is unclear, however, whether this is correlated with the alteration of HIF-1α in those cells or not.

The expression level of HIF-1α can be regulated by different factors, such as various cytokines, heat-shock protein 90, reactive oxygen species (ROS) and nitric oxide (NO)[9, 10]. As a primary consequence of equilibrium between the levels of oxidants and endogenous reducing reagents, intracellular redox status directly influences the survival of irradiated cells in terms of the ability of scavenging oxidants produced from ionizing radiation. Among these reducing reagents, intracellular antioxidant systems, such as glutathione (GSH), superoxide dismutase, glutathione peroxide, etc, are mainly responsible for the change of redox status to eliminate the excessive oxidants generated by irradiation and keep oxidants at a low level[26]. GSH by oxidizing action is converted into glutathione oxidized disulfide (GSSG), and then maintains intracellular redox equilibrium[27]. Studies have shown that GSH could protect cells from various oxidants, including oxide free radicals, reactive oxygen species (ROS) and nitrogen radicals derived from radiation exposure, and impact on HIF-1α expression in hypoxic cells[28–30]. However, so far the relationship remains unclear among the expression of HIF-1α, intracellular redox status and survival of hypoxic cells after fractionated radiation.

The purpose of the present study was to investigate whether the mode of fractionated irradiations influence HIF-1α expression in hypoxic cells, whether changes in intracellular redox status involve with the HIF-1α level in cells irradiated repeatedly, and for hypoxic radioresistant cells whether the refractory response to irradiation can be regulated by level alteration of HIF-1α.

## 2 Methods

### 2.1 Cells culture and treatment

HepG2 cells (Cell Bank, Chinese Academy of Sciences) were cultured in RPMI-1640 medium (GIBCO BAL, USA) supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL) at 37°C in an incubator in 95% humidity containing 5%$CO_2$. Cells were propagated according to protocol given by the American Type Culture Collection. Hypoxia treatment was in a controlled chamber maintained with 1% $O_2$ and 99%$N_2$ for 4 h. The medium was changed prior to experiments. To investigate the effect of redox state on the HIF-1α expression, the cells were cultivated for 12 h in the absence or presence of 100 μM DL-Buthionine sulphoximine (BSO, Sigma, USA) before the 4-h hypoxia treatment. Also, 10 μM 3-(5'-hydroxymethyl -2'-furyl)-1-benzylindazole (YC-1), an inhibitor of intracellular HIF-1α, were pretreated to hypoxic cells.

### 2.2 Irradiation

A $^{137}Cs$ source (Gammacell-40 Exactor) was used for γ-ray irradiation at a dose rate of 0.8 Gy/min. Logarithmic growing HepG2 cells were exposed twice a week to 30 fractions of 2-Gy irradiation to a total absorbed dose of 60 Gy. The medium was changed after each exposure. Monoclonal cell was isolated by Difco soft-agar gel method, and was extensively cultured to over 50 generations. The irradiated cell subline HepG2/R60 was derived from parental cell line HepG2 *in vitro*.

### 2.3 Clonogenic assays

HepG2 cells were seeded and cultured overnight at an appropriate density in T25 flasks. They were moved into a special chamber with a gas mixture of 1% $O_2$ and 99% $N_2$ for 4 h before irradiation. In some

experimental groups, cells were pretreated with 100 μM BSO or 10 μM YC-1 for 12 h before hypoxia treatment. After irradiated to different doses (0, 1, 2, 3, 5 and 7 Gy), the cells were incubated for 2 h under hypoxia. With fresh medium, the cells were allowed to grow colony formation for 9–12 days. The cell colonies were fixed by absolute methanol and stained with Giemsa for counting. Clonogenic survival ($S_F$) was calculated as colony number/(number of seeded cells × plating efficiency). The plating efficiency was defined as colony number/number of seeded cells of the control. Survival curve was fitted with the single target multi-model of $S = 1-(1-e^{-D/D_0})^N$.

### 2.4 Intracellular GSH assay

The GSH/GSSG ratios in cells treated under different conditions were measured with the glutathione reductase/5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) recycling assay kit (Beyotime, China). The protocol was carried out according to the manufacturer. The standard sample and checking sample cuvettes were placed into a dual-beam spectrophotometer, and the increases in absorbance at 412 nm were followed as a function of time. The standard curves of total glutathione and GSSG concentrations were fitted with the absorbance, followed by determining concentration of the checking samples. The concentrations were converted to nmol/mg protein, and the reduced GSH concentrations were obtained by subtracting two times GSSG from total glutathione. Finally, GSH/GSSG ratio, with different treatment, was calculated through cellular GSH concentration divided by GSSG concentration.

### 2.5 Western blot analysis of HIF-1α expression

Cells were scraped off from culture flasks and lysed in lysis buffer containing 10% glycerol, 10 mM Tris-HCL (PH 6.8), 1%SDS, 5 mM dithiothreitol (DTT) and 1×complete protease inhibitor cocktail (Sigma, USA). The method of Bradford was used to assay concentrations of protein in diverse samples. Protein concentration was measured using an auto multifunction microplate reader. Fifty micrograms of proteins were separated by 8% polyacrylamide-SDS inconsecutive gel electrophoresis. The separated proteins were electrophoretically transferred to polyvinylidene difluoride membrane. Membranes were blocked at room temperature for 1 h with a 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween 20, and then incubated with monoclonal mouse HIF-1α antibody (Abcam, USA) at a 1:500 dilusion overnight at 4°C, followed by goat anti-mouse IgG for 1 h at room temperature. Signals were detected with enhanced chemiluminescence (ECL plus, Amersham, USA). Microtubule protein (Tubulin, Abcam, USA) at a 1:1000 dilution was used as internal control to observe the changes of HIF-1α bands.

### 2.6 RT-PCR

Two micrograms RNA was used for cDNA synthesis using Olig-$(dt)_{18}$ as primer and AMV reverse transcriptase. The RT reaction was started with 10 min incubation at room temperature and 60 min at 42°C, followed by 10 min at 70°C to terminate the reaction. Subsequently, a 2 μL aliquot of cDNA was amplified by PCR in a total volume of 25 μL containing 2.5 μL 10×PCR buffer (0.2 M Tris–HCl, pH 8.4, 0.5 M KCl), 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 0.2 μM of each primer and 1.25 units of Platinum Taq DNA polymerase (Invitrogen, USA). The thermal cycler was set to run at 95°C for 5 min, 30 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 2 min, and 72°C for 10 min finally. The primers specific for B cell lymphoma/lewkmia-2(BCL-2) (upstream: 5'-GTTCA CTTGTGGCCCAGATAGG-3'; downstream: 5'-GG T GCCACCTGTGGTCCACCTG-3'; produced a 441-bp fragment) were used, and for β-actin (upstream: 5'-GTT GCG TTA CAC CCT TTC TTG-3'; downstream: 5'-GAC TGC TGT CAC CTT CAC CGT-3'; 157-bp fragment) were as the control. PCR products were analyzed by electrophoresis in 1.2% agarose gel. The specific bands were visualized with ethidium bromide and digitally photographed under ultraviolet light, furthermore scanned using Gel Documentation System 920 (Nucleo Tech, San Mateo, CA). Gene expression was calculated as the ratio of mean band density of analyzed specific products to that of the internal standard (β-actin).

### 2.7 Statistical analysis

Data were reported as the means ± s.e.m. of three separate experiments. Statistical significance was

measured by independent sample *t* test and analysis of variance. A value of $p<0.05$ was considered as statistically significant.

## 3 Results

### 3.1 Radiosensitivity of HepG2 cells after fractionated irradiation

Fig.1 shows that, under aerobic and hypoxic condition, the SFs of HepG2/R60 cells after acute exposure to irradiation were enhanced compared to those of HepG2 cells. Based on the single target multi-model, fitting curves of the irradiated HepG2 and HepG2/R60 cells have a gradually declined tendency with increasing doses. By calculating the $D_0$ values and oxygen enhance ratios (OERs) of HepG2/R60 cells and HepG2 cells, and the $D_0$ of HepG2/R60 cells, under aerobic and hypoxic condition, were significantly higher than those of HepG2 cells. Hypoxic treatment could effectively increase OERs of both HepG2/R60 and HepG2 cells, but a higher increase was found in HepG2/R60 cells. It is therefore clear that the fractionated radiation may induce the radioresistance of tumor cells, and hypoxic treatment further increases the refractory effect.

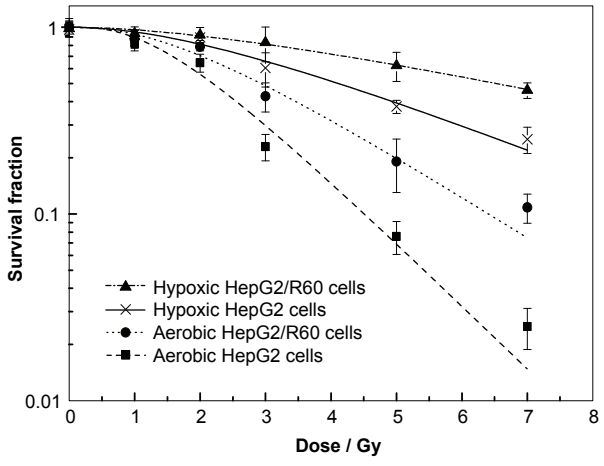


**Fig.1** Radiosensitivity of HepG2 cells by various pre-treatments. $D_0$ of HepG2/R60 and HepG2 cells under aerobic condition were 1.95 and 1.28, respectively. $D_0$ of hypoxic HepG2/R60 and HepG2 cells (irradiated) were 5.39 and 3.10, respectively. The oxygen enhancement ratios of HepG2/R60 and HepG2 cells were 2.76 and 2.42, respectively.

### 3.2 Changing of hypoxic HepG2/R60 cells radiosensitivity

To investigate the effects of redox status and HIF-1α on radiosensitivity of the cells after fractionated radiation, 100 μm BSO or 10 μm YC-1 was used to treat the hypoxic HepG2/R60 cells prior to irradiation. As shown in Fig.2, by both the BSO and YC-1 pretreatment the SFs of hypoxic HepG2/R60 cells exposed to γ-rays decreased significantly, hence the reduced $D_0$ values. Compared with YC-1, BSO showed more inhibitory effect on the radioresistance of hypoxic cells. The findings thus indicate that intracellular redox state and the level of HIF-1α expression can influence the vital activities of refractory cells by fractionated radiation inducting.

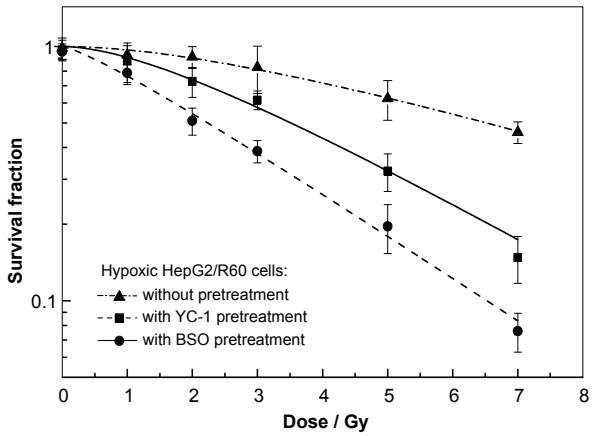


**Fig.2** The inhibitory effect of BSO and YC-1. $D_0$ values of HepG2/R60 cells under hypoxia condition by 10 μm YC-1 and 100 μm BSO pretreatment was 3.01 and 2.58, respectively.

### 3.3 Alteration of redox status in HepG2/R60 cells

After repeated exposure to radiation, the intracellular GSH level detected in hypoxic HepG2/R60 cells was higher than that of hypoxic HepG2 cells. The GSH/GSSG ratio revealed that redox status in hypoxic HepG2/R60 cells imposed intracellular reducing status. For the 100-μM BSO- pretreated HepG2/R60 cells, the intracellular GSH level decreased with the GSH/GSSG ratio (Fig.3). The GSH level in hypoxic HepG2/R60 cells pretreated by 10 μM YC-1, however, decreased insignificantly, compared to hypoxic HepG2/R60 cells in absence of YC-1.

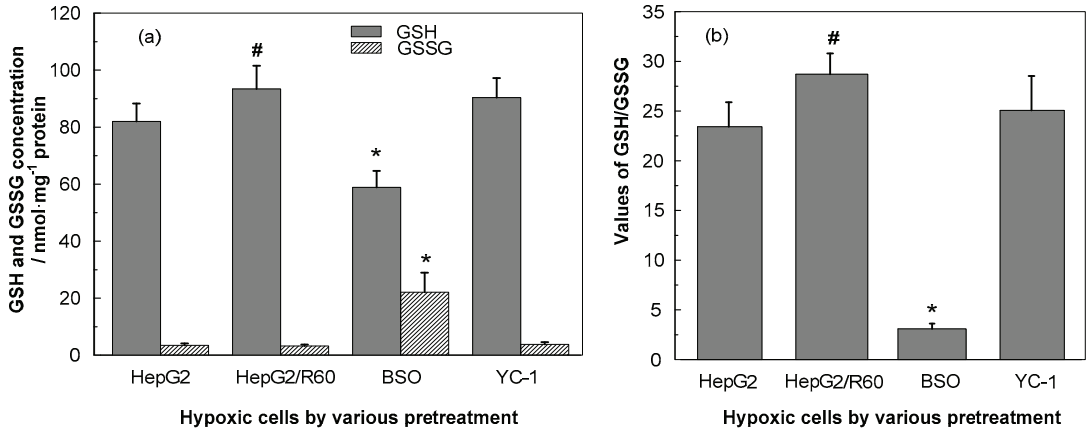


**Fig.3** Redox status of hypoxic HepG2 and hypoxic HepG2/R60 cells at a different pretreatment.

(a) the GSH and GSSG contents, (b) the GSH/GSSH rato.

#$P<0.05$, compared with hypoxic HepG2 cells; *$P<0.01$, compared with hypoxic HepG2/R60 cells without pretreatment.

### 3.4 Changes of HIF-1α expressions in hypoxic HepG2/R60 cells

The changes in HIF-1α protein level in HepG2/R60 cells by various pretreatment were examined using the Western blot assay. Fig.4 shows that the HIF-1α expression of HepG2/R60 cells are higher than that of HepG2 cells, and the increase in HIF-1α protein level was prevented in the irradiated cells in the 100 μM BSO or 10 μM YC-1 treatment. The YC-1 seems to have a slightly stronger inhibition on HIF-1α expression than BSO, but no significance was found by statistical analysis. Our results suggest that the fractionated radiation might induce the increase of HIF-1α in the hypoxic tumor cells, and alteration of HIF-1α might involve with intracellular GSH level.

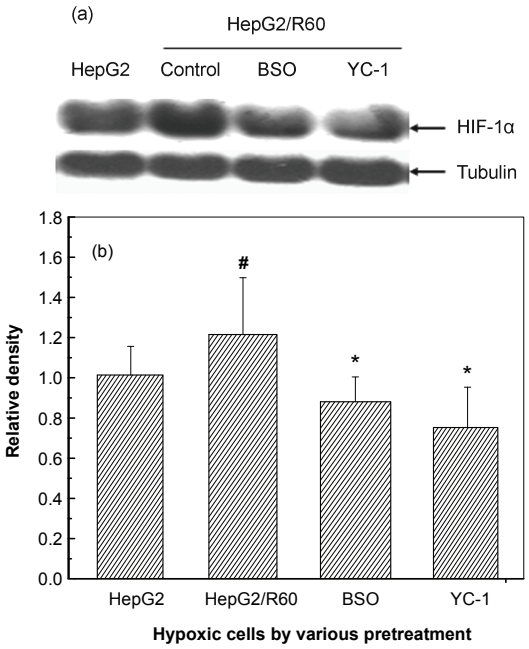


**Fig.4** Expression of HIF-1α protein in hypoxic HepG2 and hypoxic HepG2/R60 cells at a different pretreatment.

(a) typical gel picture taken from three separate experiments,

(b) the analysis of relative densities.

#$P<0.05$, compared with hypoxic HepG2 cells;

*$P<0.01$, compared with hypoxic HepG2/R60 cells without pretreatment.

### 3.5 Alteration of BCL-2 mRNA in hypoxic HepG2/R60 cells

The levels of BCL-2 mRNA were measured using semi-quantitative RT-PCR. Fig.5 shows the statistical analysis results. The levels of BCL-2 mRNA were up-regulated in hypoxic HepG2/R60 cells, followed by the overexpression of BCL-2 prevented by BSO or YC-1 pretreatment. It was found that the change tendency of BCL-2 was consistent with the alteration of HIF-1α. Therefore, it is possible that, in hypoxic resistant variant, the refractory effect of cell is up-regulated by BCL-2 expression dependent upon intracellular HIF-1α enhancement.

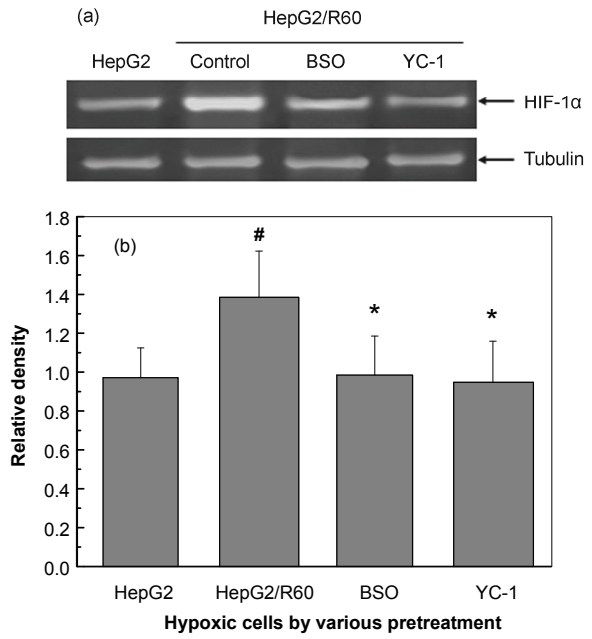


**Fig.5** Expression of BCL-2 mRNA in hypoxic HepG2 cells and hypoxic HepG2/R60 cells at a different pretreatment. (a) typical gel picture taken from three separate experiments, (b) the analysis of relative densities. $^{\#}P<0.01$, compared with hypoxic HepG2 cells; $*P<0.01$, compared with hypoxic HepG2/R60 cells without pretreatment.

## 4 Discussion

Growing evidences have revealed that high-level HIF-1α expression can result in the refractory effect of tumor cell to irradiation[24], while ionizing radiations up-regulate intracellular HIF-1α level during the radiotherapy[31]. The interaction HIF-1α with irradiation, therefore, is one of the main reasons of radioresistance in hypoxic cells. In the present study, we observed that, after fractionated radiation, the $D_0$ value of cells was enhanced from 1.28 to 1.95 (Fig.1), showing the production of radioresistant impact. Moreover, compared with parental cells, there was a higher refractory impact shown in the resistant cells by hypoxia treatment, concomitance with the high-level of HIF-1α. The subsequent experimental findings were observed that the inhibitory effect of HIF-1α expression in hypoxic cells by YC-1 pretreatment, a novel targeted inhibitor[32], resulted in the reduction in HepG2/R60 cells resistant response. Our results clearly indicate that the elevation of intracellular HIF-1α expression by repeated exposure to irradiation is responsible for the radioresistant response of hypoxic tumor cells.

High-level of intracellular GSH was found to be in favor of avoiding radiation injury and DNA-damage[33–35]. GSH, the main nonprotein cellular thiol, plays a prominent role in the defense against oxidative stress-induced cell injury. To fulfill such a role, GSH acts as substrate for the GSH-S transferase and GSH peroxidase, eliminating ROS generated by stimulators including ionizing radiations[36]. Furthermore, owing to its protective role in maintaining critical cellular functions, some tumor cells develop resistance against radiotherapeutic patterns by up-regulating the GSH contents[28, 37]. GSH is synthesized from its constituent amino acids in two sequential enzymatic reactions, catalyzed by γ-glutamylcysteine synthetase (γ-GCS) and GSH synthetase. γ-GCS catalyzes the rate-limiting step in de novo GSH synthesis, and is inhibited by GSH through a feedback mechanism[38]. However, there was rare data displaying whether the presence of higher level in hypoxic radioresistant cells compared with its parental cells under the same condition. The present results reveal that GSH levels in hypoxic cells by fractionated radiation are significantly increased, following the improved cells survival against acute exposure. According to Refs.[39, 40], the exposure of HepG2 cells to moderate doses of radiation increased the levels of γ-GCS heavy subunit (γ-GCS-HS) mRNA and activity leading to greater level of the radio-protective antioxidant, GSH. Conversely, the cloning viabilities of hypoxic HepG2/R60 cells were suppressed by BSO pretreatment, an irreversible inhibitor of γ-GCS and further blocking the formation of GSH[36], and the fitting curve showed the value of $D_0$ of HepG2/R60 cells was declined from 5.39 to 2.58. Therefore, the increased GSH level in hypoxic refractory cells may contribute to the modification of radiosensitzing.

Many reports showed that the expression of HIF-1α could be regulated by changing cytoplasmic redox state[41,42]. It can be thus conjectured that, due to the increase of intracellular GSH level, the overexpression of HIF-1α may be emerged from hypoxic HepG2/R60 cells. According to our results, the HIF-1α levels in HepG2/R60 cells being consistent with GSH overexpression are higher than those in HepG2 cells. Furthermore, the inhibitory effect on HIF-1α by BSO attenuating the high-level of GSH was

found to result in the decreased HepG2/R60 redioresistance. Mechanisms for the expression of HIF-1α regulated by redox status are yet to clarified, but possible reasons may include: (i) the biosynthesis of GSH impose a reducing micro-environment, subsequently prolonging the half-life of HIF-1α and protracting its stability in cytosol and favoring its translocation[43]; and (ii) GSH anti-oxidant system can effectively clear away free radicals and ROS that may suppress the expression of HIF-1α according to Refs.[44,45]. As a consequence, the refractory response of hypoxic cells induced by fractionated radiation may be augmented by the elevation of HIF-1α dependent upon intracellular GSH contents.

To further understand the mechanisms underlying the development of resistance to irradiation in hypoxic cells and verify the present results, we found that the changes in BCL-2 mRNA, the down-stream target gene by HIF-1α promote transcription in hypoxic cells. Coincident with the modification of HIF-1α, the high level of BCL-2 mRNA was detected in hypoxic HepG2/R60 cells and could be suppressed by both BSO and YC-1. As is well known, the BCL-2 gene is closely associated with the cells survival and its overexpression causes refractory action on the apoptosis induced by radiotherapy or and chemotherapy. Increased BCL-2 mRNA in hypoxic cells, was found in many experiments[46–48], where HIF-1 was combined with the hypoxia-responsive element (HER) of BCL-2 and induced its overexpression. In this work, we showed increased BCL-2 mRNA levels in the hypoxic HepG2/R60 cells and the overexpression of BCL-2 reversed by both BSO and YC-1 pretreatment. The overexpression of BCL-2 in tumor cells was associated with up-regulated GSH contents and with increased resistance to cells death after toxic agents[49]; otherwise, BSO treatment could completely abolished such BCL-2-mediated drug resistance[50]. Similarly, the inhibitory phenomenon was observed in the cells by YC-1 treatment[51]. Our redults, which show that BSO and YC-1 treatments are effective in inhibiting the up-regulation of BCL-2 expressions in the hypoxic refractory cells and diluting the radioresistance of cells, are in agreement with other reports [52, 53].

In conclusion, the results of our experiment indicate that the radiosensitivities of tumor cells are influenced by fractionated radiation, the radioresistant response in hypoxic cells is strongly correlated with the high-expression of HIF-1α, and this effect is dependent on the alteration of intracellular GSH level. The refractory effect from HIF-1α overexpression can involve with the up-regulated level of BCL-2. The development of resistant response to radiotherapy, arising from repeated irradiations, has always hampered clinical prognosis. It is therefore valuable that the design of new drugs is utilized to aim at the radiosensitivity of hypoxic refractory cells.

## Acknowledgements

We thank Mr. Shungao Tong and Mr. Huajun Ji with Institute of Radiation Medicine, Fudan University for constant supports, and Dr. Shengquan Zhang with College of Basic Medicine, An-hui Medical University for technical help.

## References

1 Rosen E M, Fan S, Rockwell S, *et al*. Cancer Invest, 1999, **17**: 56–72.

2 Withers H R. Acta Oncol, 2000, **39**: 569–577.

3 Erridge S C, Gaze M N, Price A, *et al*. Clin Oncol, 2005, **17**: 61–67.

4 Yau T K, Sze W M, Lee W M, *et al*. Head Neck, 2004, **26**: 1024–1030.

5 Yaromina A, Krause M, Thames H, *et al*. Radiother Oncol, 2007, **83**: 304–310.

6 Chan N, Koritzinsky M, Zhao H, *et al*. Cancer Res, 2008, **68**: 605–614.

7 Husbeck B, Peehl D M, Knox S J. Free Radic Biol Med, 2005, **38**: 50–57.

8 Xu Q Y, Gao Y, Liu Y, *et al*. Chin Med J. 2008, **121**: 1830–1837.

9 Ridder M D, Verellen D, Verovski V, *et al*. Nitric Oxide, 2008, **19**: 164–169.

10 He F, Li L, Kim D, *et al*. Cancer Res, 2007, **67**: 634–642.

11 Newcomb E W, Lymberis S C, Lukyanov Y, *et al*. Cell Cycle, 2006, **5**: 93–99.

12 Harada H, Itasaka S, Zhu Y, *et al*. Br J Cancer. 2009, **100**: 747–757.

13 Kizaka K S, Itasaka S, Zeng L, *et al*. Clin Cancer Res, 2009, **5**: 3433–3441.

14 Bruick R K, Mcknight S L. Science, 2001, **294**: 1337−1340.

15 Conaway R C, Brower C S, Conaway J W. Science, 2002, **296**: 1254−1258.

16 Semenza G L. Nat Rev Cancer, 2003, **3**: 721–732.

17 Pore N, Gupta A K, Cerniglia G J, *et al*. Cancer Res, 2006, **66**: 9252–9259.

18 Sullivan R, Graham C H. Mol Cancer Ther, 2009, **8**: 1702–1713.

19 Unruh A, Ressel A, Mohamed H G, *et al*. Oncogene, 2003, **22**: 3213–3220.

20 Aebersold D M, Burri P, Beer K T, *et al*. Cancer Res, 2001, **61**: 2911–2916.

21 Koukourakis M I, Giatromanolaki A, Sivridis E, *et al*. Int J Radiat Oncol Biol Phys, 2002, **53**: 1192–1202.

22 Koukourakis M I, Giatromanolaki A, Skarlatos J, *et al*. Cancer Res, 2001, **61**: 1830–1832.

23 Hui E P, Chan A T, Pezzella F, *et al*. Clin Cancer Res, 2002, **8**: 2595–2604.

24 Bachtiary B, Schindl M, Potter R, *et al*. Clin Cancer Res, 2003, **9**: 2234–2240.

25 Madhusoodhanan R, Natarajan M, Veeraraghavan J, *et al*. J Radiat Res, 2009, **50**: 311–324.

26 Chen H, Chow P H, Cheng S K, *et al*. J Androl, 2003, **24**: 704–711.

27 Griffith O W, Mulcahy R T. Adv Enzymol Relat Areas Mol Biol, 1999, **73**: 209–267.

28 Tiwari P, Kumar A, Balakrishnan S, *et al*. Mutat Res, 2009, **676**: 62–68.

29 Brouazin J V, Guitton N, LeguéF, *et al*. Anticancer Res, 2002, **22**: 257–262.

30 Geng C X, Zeng Z C, Wang J Y, *et al*. Docetaxel shows radiosensitization in human hepatocellular carcinoma cells. World J Gastroenterol, 2005, **11**: 2990–2993.

31 Moeller B J, Cao Y, Li CY, *et al*. Cancer Cell, 2004, **5**: 429–441.

32 Sun H L, Liu Y N, Huang Y T, *et al*. Oncogene, 2007, **26**: 3941–3951.

33 Kumar M, Samarth R, Kumar M, *et al*. Evid Based Complement Alternat Med, 2007, **4**: 343–350.

34 Di Toro C G., Di Toro P A, Zieher L M, *et al*. Neurotoxicology, 2007, **28**: 555–561.

35 Srinivasan M, Rajendra P N, Menon V P. Mutat Res, 2006, **611**: 96–103.

36 Haddad J J. Cell Signal. 2002, 14: 879–897.

37 Tani M, Goto S, Kamada K, *et al*. Jpn J Cancer Res, 2002, **93**: 716–722.

38 Haddad J J. Cell Signal, 2002, **14**: 799–810.

39 Teshima K, Yamamoto A, Yamaoka K, *et al*. Int J Radiat Biol, 2000, **76**: 1631–1639.

40 Morales A, Miranda M, Sanchez–Reyes A, *et al*. FEBS Lett, 1998, **427**: 15–20.

41 Sommani P, Yamashita K, Miyoshi T, Biol Pharm Bull. 2007, 30: 2181–2184.

42 Nikinmaa M, Pursiheimo S, Soitamo A J. J Cell Sci, 2004, **117**: 3201–3206.

43 Haddad J J, Olver R E, Land S C. J Biol Chem, 2000, **275**: 21130–21139.

44 Wellman T L, Jenkins J, Penar P L, *et al*. FASEB J, 2004, **18**: 379–381.

45 Liu Q, Berchner–Pfannschmidt U, Möller U, *et al*. Proc Natl Acad Sci USA, 2004, **101**: 4302–4307.

46 Diensthuber M, Potinius M, Rodt T, *et al*. J Neurooncol, 2008, **89**: 131–139.

47 Sasabe E, Tatemoto Y, Li D, *et al*. Cancer Sci, 2005, **96**: 394–402.

48 Zhou J, Callapina M, Goodall G J, *et al*. Cancer Res, 2004, **64**: 9041–9048.

49 Ortega A, Carretero J, Obrador E, *et al*. Nitric Oxide, 2008, **19**: 107–114.

50 Yoshida A, Takemura H, Inoue H, *et al*. Cancer Res, 2006, **66**: 5772–5780.

51 Pan S L, Guh J H, Chang Y L, *et al*. Cardiovasc Res, 2004, **61**: 152–158.

52 Hara T, Omura–Minamisawa M, Kang Y, *et al*. Int J Radiat Oncol Biol Phys, 2008, **71**, 1485–1495.

53 An J, Chervin A S, Nie A, *et al*. Oncogene, 2007, **26**: 652–661.