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Original Article

The cellular radiosensitivity of different adenocarcinoma cells is related to their Egr-1 expression levels

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Abstract: Aims: To investigate the radiosensitivity of three adenocarcinoma cell lines with radioresistance ability and the role of Egr-1 in tumor radiosensitivity. Methods: Lung adenocarcinoma GLC-82 cell, colorectal cancer HT29 cell and cervical cancer Hela cell were used. Before and after radiation, colony formation ability was detected by colony formation assay, damages of DNA was determined by comet assay, and cell apoptosis induced by radiation and cell cycle distribution was detected by flow cytometry. Egr-1 expression level was analyzed by FQ-PCR and Western blot (WB). Results: Radiosensitivity of GLC-82 (SF_2 = 0.532±0.003), HT29 (SF_2 = 0.641±0.005) and Hela (SF_2 = 0.641±0.005) cells decreased in sequence after irradiation. DNA damages and cell apoptosis of these cells were also decreased in sequence after irradiation. Cell cycle analysis showed G2/M phase arrest in these cells after irradiation. FQ-PCR showed that Egr-1 basic expression levels in GLC-82, HT29 and Hela cells were significantly different (F = 43.143, P = 0.000), from high to low in GLC-82 (1.094-fold), HT29 (0.793-fold), and Hela (0.529-fold) cells. The results of WB test were in accordance with that of FQ-PCR (F = 153.948, P = 0.000). Egr-1 expression induced by radiation in the three cell lines all peaked within 1 h. Irradiation-induced DNA damage, cell apoptosis and Egr-1 basic expressions were related to radiosensitivity parameters D_0 and SF_2. Conclusion: Irradiation-induced DNA damage and cell apoptosis partly contributed to radiosensitivity of tumor, which may be positively related to basic expressions of Egr-1 gene in different cancer cells. Therefore, Egr-1 gene could hopefully be used for predicting radiosensitivity of tumor.

Keywords: Egr-1, adenocarcinoma, radiosensitivity, cell apoptosis

Introduction

Radiotherapy is one form of cancer treatment that utilizes ionizing radiation to induce cell apoptosis and mitotic catastrophe, generally via inflicting DNA double-strand breaks [1, 2]. The ability of cells to detect and repair DNA damages will affect the intrinsic radiosensitivity [3], which is genetically determined by tumor histological types and genotypes. Indeed, accumulating preclinical and clinical data suggest that squamous cancer is sensitive to radiation whereas adenocarcinoma is resistant to it. Some studies have segregated intrinsic radiosensitivity of human tumor cell lines into distinct genotype-dependent radiosensitivity groups that associate with some genes such as mutATM, wtTP53, mutTP53, and an unidentified factor [3-7]. Most predictive factors of radiosensitivity are related to gene expression profiles [8]. Early growth respond-1 gene (EGR-1), mapped to chromosome5, is activated by a broad range of extracellular stimuli, and codes for an 80-82 kDa Cys_2-His_2-type zinc-finger transcription factor that mediates growth, proliferation, differentiation or apoptosis [9, 10]. In

particular, the functional role of EGR-1 in radiation-induced apoptosis is pivotal since the promoter of EGR-1 contains radiation-inducible CC $(A/T)_6$GG/CArG DNA sequences [11].

Early in 1991, Hallahan et al. [12] found that EGR-1 may participate in signal transduction of the cellular pathway responding to ionizing radiation as immediate early response gene, and the increased expression of EGR1 was observed within 0.5-3 h following an x-ray exposure. Radiation inducibility of EGR-1 is conferred by a region containing several CArG motifs that are targeted by reactive oxygen intermediates (ROIs) [13, 14]. Numerous reports further showed that EGR-1 protein can eliminate "induced-radiation resistance" by inhibiting the functions of radiation-induced pro-survival genes (NF-κB activity and bcl-2 expression) and activate proapoptotic genes (such as bax) to confer a significant radio-sensitizing effect [11]. Our previous studies showed the level of EGR-1 expression is related to the level of cancer apoptosis induced by radiation [15]. The expression of Egr-1 had a positive prognostic effect on survival of nasopharyngeal carcinoma patients who were treated by radiotherapy [16]. Therefore, association between Egr-1 and radiosensitivity of cancer may exist [11]. But recently, Pagel JI et al. reported that disease progression-mediated by Egr-1 were associated with signaling in response to oxidative stress [17]. Meirovitz A et al. reported that the role of heparanase in radiation-enhanced invasiveness of pancreatic carcinoma [18]. So the roles of Egr-1 in radiotherapy need to be further investigated [19].

Adenocarcinoma is a kind of cancer often found in epithelial tissues, which can happen in any tissues or organs having glandular epithelium cells. In radiotherapy, adenocarcinoma has relative resistance to radiation, and the radiosensitivity of adenocarcinoma from different positions is different. For example, after radiotherapy, the nidus of lung adenocarcinoma contracts obviously but that of cervical adenocarcinoma contracts undetectable. Though the radiosensitivity of cancer is affected by many factors, the inherent characteristics of cancer cells have critical effects. Although the mechanism is not yet elucidated completely [1, 20], Malaise and his colleagues have indicated that the radiosensitivity of cancer belonging to the same histopathological type is quite different [21]. In this study, we chose 3 cell lines of adenocarcinoma, including lung adenocarcinoma GLC-82 cells, colorectal cancer HT29 cells and cervical cancer HeLa cells. We preliminarily studied the relationship between the level of Egr-1 expression and the radiosensitivity of adenocarcinoma. A new method to improve the radiosensitivity of adenocarcinoma will provide more chances to achieve better prognosis of adenocarcinoma patients in the future.

Materials and methods

Cell culture

Three human adenocarcinoma cell lines were used. Lung cancer cell line GLC-82 was kindly supplied by department of genetics, Harbin Medical University, China. Colon cancer cell line HT29 was kindly supplied by Dr. R Huang, Department of general surgery, the Second Affiliated Hospital of Harbin Medical University, China, and cervical cancer cell line Hela was purchased from Scientific Research and Experimental Center, the Second Affiliated Hospital of Harbin Medical University, China. Cells were cultured in RPMI-1640 medium (Hyclone, Logan, Utah, USA) with 10% heat-inactivated fetal bovine serum, and supplemented with 1% penicillin and streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

Cell irradiation

All monolayers of cells in a 6-well cell culture plate were irradiated with 6-MV X-ray beam from XHA600D linear accelerator (field = 20 cm x 20 cm, SSD = 100 cm, dose rate = 4 Gy min^{-1}).

Colony formation assay

Clonogenicity ability was examined by colony formation assay. To obtain the similar number of colonies with different radiation doses, an appropriate number of exponentially grown cells were seeded into 6-well plates and irradiated for 12 hours at the dose of 0, 1, 2, 4, 6 and 8 Gy respectively. After 2-3 weeks of incubation, the colonies were fixed by methanol and stained by Giemsa stain. Colonies containing more than 50% of the cells were scored as clonogenic survivors. Finally, the clone formation rate (PE) and cell survival fraction (SF) were cal-

Table 1. Primers and probes for FQ-PCR

Gene	Product length	Sequence (5'-3')
Egr-1	200 bp	Forward primer AAAGTTTGCCAGGAGCGAT
		Reverse primer CAGGGGATGGGTATGAGGTG
		Probe CCCTACGAGCACCTGACCGCAGA
β-actin	71 bp	Forward primer CCCTGGCACCCAGCAC
		Reverse primer GCCGATCCACACGGAGTAC
		Probe ATCAAGATCATTGCTCCTCCTGAGCGC

Table 2. Radiobiological parameters calculated by single-hit multi-target model and linear quadratic model

	GLC-82	HT29	Hela	*F*	*P*
D_0	2.227±0.004	2.675±0.008	3.363±0.004	34711.357	0.000
SF_2	0.532±0.003	0.641±0.005	0.764±0.003	3267.923	0.000
N	1.462±0.009	1.613±0.006	1.807±0.006	1825.966	0.000
Dq	0.845±0.012	1.267±0.014	1.989±0.012	6058.965	0.000
α/β	10.307±0.4000	7.743±0.204	5.408±0.158	238.486	0.000

D_0, mean lethal dose; SF_2, surviving fraction at 2 Gy; N, extrapolation number; Dq, quasi-threshold dose.

culated. PE = clone formation/inoculated cell number × 100%, SF = radiated cell PE/control cell PE × 100%.

Alkaline comet assay

Alkaline comet assay was performed as described [22] with a little modification. Cells were inoculated in 6-well plates after completely adherent when entered exponential phase, cells were irradiated with 5 Gy X-ray for 0.5 hr or 16 hrs. Single cell suspension after trypsin digestion were mixed with 85 μl of 0.5% low-melting agarose (LMA) at 37°C and pipetted onto an agarose layer on the frosted glass slides. After cells lysis by freshly prepared cold lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10, with 1% Triton X-100) at 4°C for 1 hr, slides were placed in horizontal gel electrophoresis units with alkaline electrophoresis buffer (1 mM Na_2EDTA, 300 mM NaOH, pH 13) for 30 min to allow the DNA to unwind. Then electrophoresis was carried out at 25 V (0.83 V/cm^{-1}) for 20 min. The slides were washed with neutralization buffer (0.4 Tris pH 7.5) for three times (5 mins each) to remove alkali and detergents. Each slide was stained with 2 μg/mL PI staining and covered with a coverslip for immediate analysis. Observations were made at a magnification of 400 times using an epi-fluorescence microscope (Leica BSF-30V, Leica, Germany).

Fluorescence quantitative analysis of cell cycle and apoptosis

Cells were inoculated in 6-well plates, after completely adherent when entered exponential phase, cells were irradiated with 5 Gy X-ray. Before (0 hr) and at 6 hr, 12 hr, 24 hr and 48 hr after irradiation, cell cycle and apoptosis were analyzed according to the manufacture's instruction by NucleoView NC-3000 (Chemometec, Denmark).

Fluorescence real-time quantitative RT-PCR (FQ-PCR)

Cells were inoculated in 6-well plates, and 24 hrs later the cells were irradiated by 5 Gy X-ray. Before (0 hr), 0.5 hr, 1 hr, 1.5 hr, 2 hr and 3 hr after radiation, cDNA was reverse transcriptased by Supermo III RT Kits (BioTeke Corporation, China) and FQ-PCR was performed using AccuPower® GreenStar™ qPCR PreMix (Bioneer, Alameda, CA, USA) on a Mx3000P qPCR System (Agilent Technologies, Santa Clara, CA, USA). Results were calculated as previously described [15]. All FQ-PCR analyses were performed in triplicates with the primers listed in **Table 1**. Results were shown as the mean of target gene mRNA levels relative to β-actin mRNA levels. Gene expression at each time point was normalized to that of 0 hr in GLC-82 to generate the relative fold induction ± SD.

Western blot

Cells were cultured and irradiated as in FQ-PCR, and the cells were lysed in RIPA Lysis Buffer (Beyotime Institute of Biotechology, China). The protein samples (20 μg each) were then separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. The membrane was incubated with mouse anti-EGR-1 antibody (Abcam, Cambridge, MA, USA) and mouse anti-β-actin antibody (Beijing Zhong Shan-Golden Bridge Biological Technology CO., Ltd., China) followed by HRP-conjugated goat anti-IgG (Beijing Zhong Shan-Golden Bridge Biological Technology CO., Ltd., China) and developed

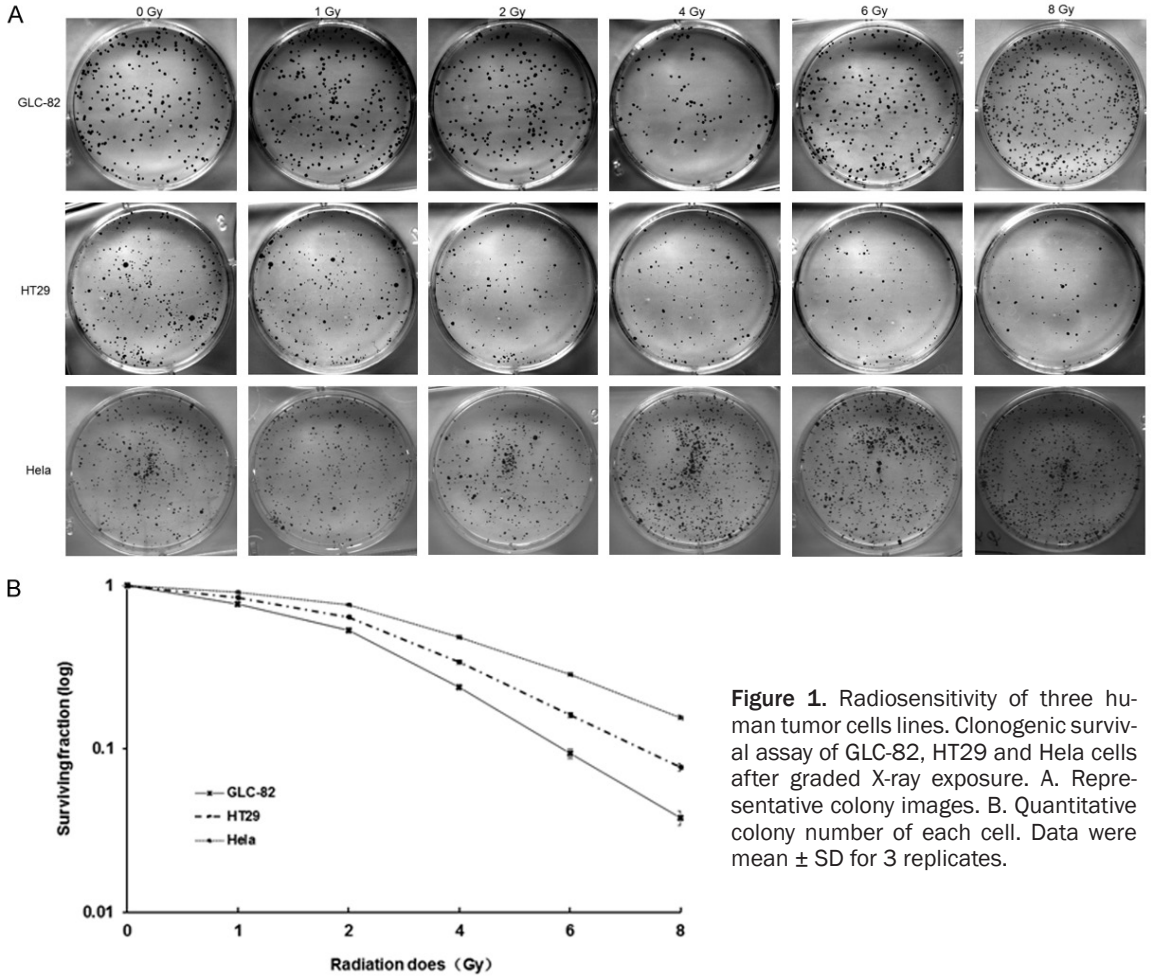

Figure 1. Radiosensitivity of three human tumor cells lines. Clonogenic survival assay of GLC-82, HT29 and Hela cells after graded X-ray exposure. A. Representative colony images. B. Quantitative colony number of each cell. Data were mean ± SD for 3 replicates.

Table 3. Tail factor calculation

F (I)-F (V)	Stages	GLC-82			HT29			Hela		
		0 h	0.5 h	16 h	0 h	0.5 h	16 h	0 h	0.5 h	16 h
2.5%	I	183±3	161±2	169±1	179±2	158±1	169±2	185±2	168±1	174±3
12.5%	II	8±0	24±1	15±1	10±1	26±2	16±1	9±1	25±2	18±1
30%	III	4±0	6±1	8±0	5±0	8±1	6±0	3±0	3±	3±1
67.5%	IV	2±0	4±0	3±0	1±0	3±1	3±0	1±0	1±0	2±0
97.5%	V	3±0	5±1	5±0	4±0	5±1	6±2	2±0	3±0	3±0
Tail factor		5.53±0.22	8.20±0.11	7.70±0.09	5.90±0.05	8.25±0.31	7.95±0.53	4.64±0.05	5.91±0.15	5.88±0.19

F (I)-F (V), amount of DNA in the tail at stages I-V.

using an enhanced chemiluminescence detection system (Beyotime Institute of Biotechology, China).

Statistical analysis

Each experiment was repeated at least three times, all data was analyzed using SPSS17.0 software (SPSS Inc., Chicago, IL, USA) and expressed as mean ± S.D. Data was analyzed by analysis of the variance (ANOVA) for difference, $P < 0.05$ was considered to be statistically significant. Correlation is significant at the 0.01 level by Pearson Correlation (2-tailed).

Results

Radiobiological parameters of GLC-82, HT29, Hela cells

The three cell lines were irradiated with graded doses of X-ray and the clonogenic survival was

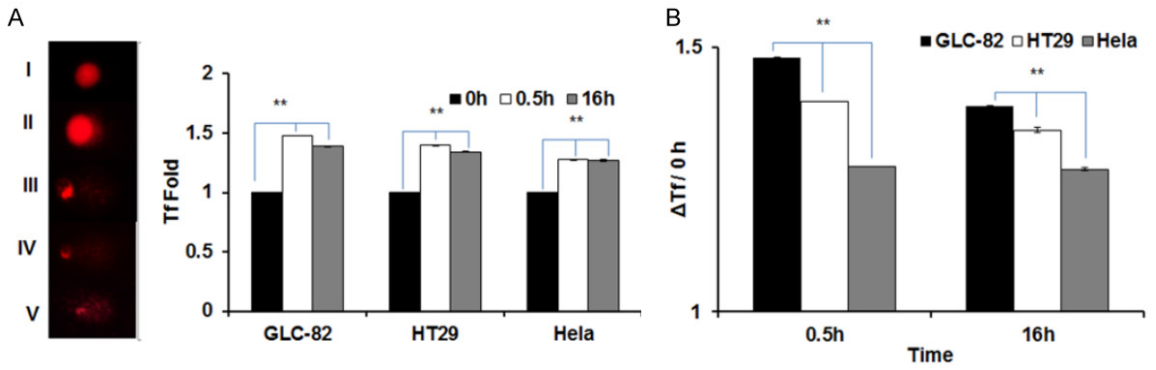

Figure 2. The visual scoring of alkaline Comet assays. A. Visual and automated data analysis of alkaline Comet assays were directly compared in the three cell lines after 5 Gy X-ray (0 hr, 0.5 hr and 16 hr) exposure. Cells of three independent experiments were scored in parallel with a fully automated Comet analysis system to obtain tail moments (right panels) and by the visual method assigning nuclei into the five different Comet stages I-V and calculating tail factors (TF, left panels). B. Relative Tail factor at 0.5 hr and 16 hr was calculated. The data were analyzed using analysis of the variance (ANOVA) for difference, and considered to be statistically significant among three cell lines and at each time point (*P < 0.05, **P < 0.005).

measured by colony formation assay. The radiobiological parameters of the survival curves were calculated by fitted single-hit multi-target model (SHMT, $S = 1-(1-\exp(-D/Do))^N$) and linear quadratic model (LQ, $S = \exp(-\alpha*D-\beta*D^2)$) in Spss17.0. Five radiobiological parameters were obtained (**Table 2**): D_0, mean lethal dose; SF_2, surviving fraction at 2 Gy; N, extrapolation number; Dq, quasi-threshold dose; and α/β value. D_0 and SF_2 were most significant to represent the radiosensitivity of cells, and they are "gold standardization" of delineating cellular radiosensitivity. N and α/β value represented the capacity of reparation to sublethal damage. The survival rates of GLC-82 dropped most quickly upon elevation of X-ray doses while the survival rates of Hela changed most slowly (**Figure 1A** and **1B**), and there was significant difference in all of the radiobiological parameters among the three cell lines (P < 0.001) (**Table 2**). These results indicated that the radiosensitivity of the three cell lines was different: GLC-82 had the poorest capacity of clonogenic survival and reparation to sublethal damage which was followed by HT29, and Hela had the strongest capacity.

DNA damage analysis and its correlation with D_0 and SF_2

DNA damage after radiation was analyzed by alkaline comet assay. Nuclei of 200 cells per group were analyzed by CAPS Software and classified into five stages as I-V according to their amount of DNA in the tail, that is cells showing <5% of DNA in the tail as stage I, cells with 5-20% DNA in tail as stage II, cells with 20-40% DNA in tail as stage III, cells with 40-95% DNA in tail as stage IV and cells with >95% DNA in tail as stage V. DNA damage was evaluated with Tail factor (Tf) [23, 24], and Tf was calculated with the following formula: Tf (%) = (I × F(I)+II × F(II)+III × F(III)+IV × F(IV)+V × F(V))/(I+II+III+IV+V) (**Table 3**). With increased time of radiation, Tf of every cell line was significantly different (F = 43326.241, P = 0.000 in GLC-82; F = 23276.829, P = 0.000 in HT29, and F = 7222.896, P = 0.000 in Hela, respectively). Tf obviously increased at 30 min and decreased at 16 hrs after radiation in GLC-82 and HT29 (**Figure 2A**). As shown in **Figure 2B**, the relative Tf at 30 min and 16 hrs. after radiation to that of 0 hr ($\Delta Tf_{0.5}$ and ΔTf_{16}, respectively) were significantly different in three cells (F = 2746.806, P = 0.000 and F = 777.553, P = 0.000, respectively). These results indicated that the degree of DNA damage was different among the three cell lines and the degree of DNA damage was also different in a same cell at different time of radiation. We further analyzed the correlation between $\Delta Tf_{0.5}$ and radiobiological parameters in these cells. There was negative correlation between $\Delta Tf_{0.5}$ and D_0 or SF_2 (r = -0.704, P = 0.009 and r = -0.873, P = 0.001, respectively), suggesting that DNA damage of cells was related to radiosensitivity.

Changes of cell cycle distribution

To investigate whether radiation affects cell cycle progression, cell cycle distribution was analyzed by flow cytometry. After 5 Gy of X-ray

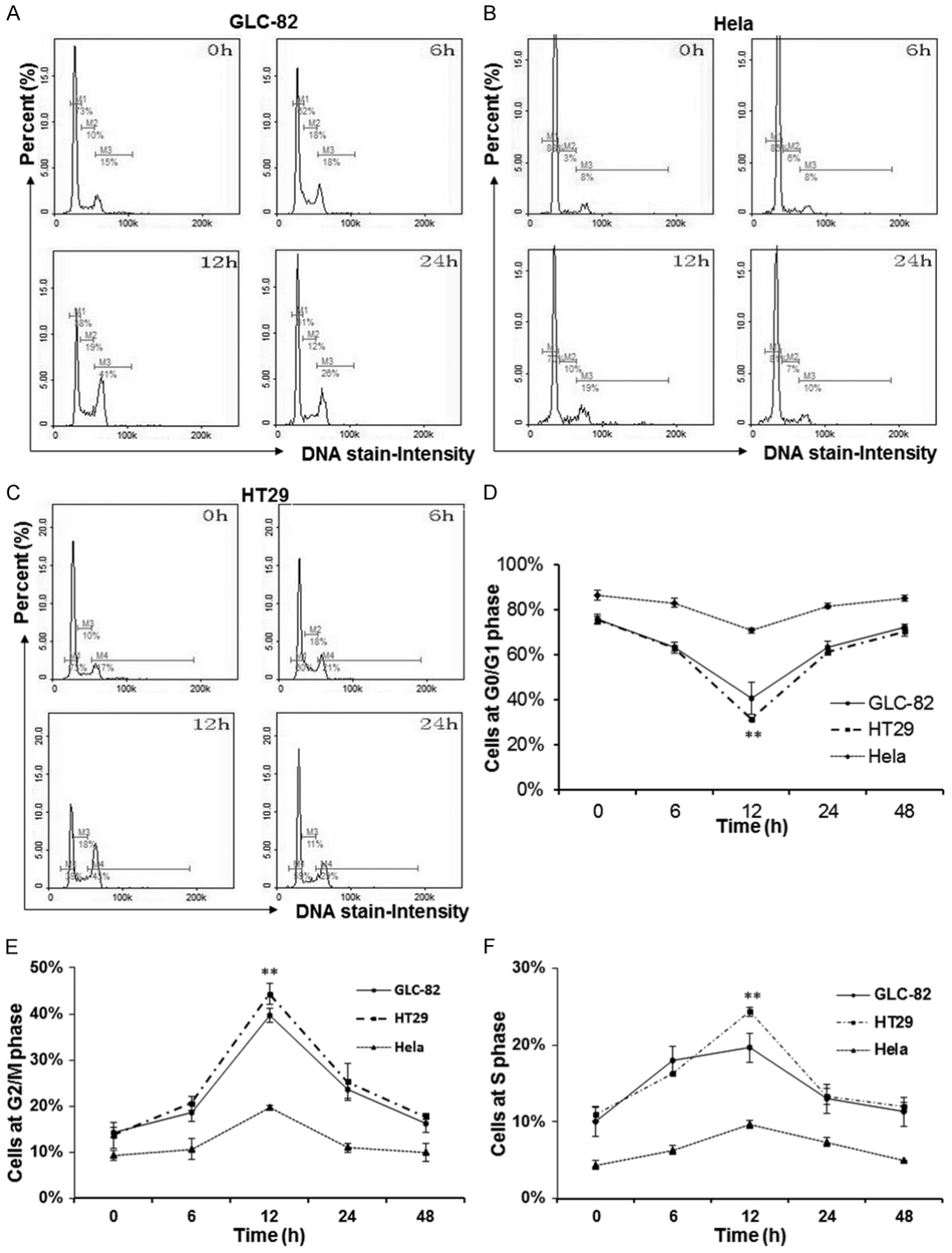

Figure 3. Cell cycle analysis in the three lines after radiation. GLC-82, HT29 and Hela cells were exposed to 5 Gy X-ray radiation for 0 hr, 6 hr, 12 hr, 24 hr and 48 hr, and cell cycle was analyzed by flow cytometry. A-C. Representative changes of cell cycle were shown. D-F. Percentages of cells at G0/G1, G2/M and S phase was shown. Data were analyzed using ANOVA, and difference in G2/M phase arrest was observed at 12 h after radiation in three cell lines.

radiation, the proportion of each phase in cell cycle was obviously changed in the three cell lines, with the most obvious change at 12 hr post treatment (**Figure 3A-C**). The percentage

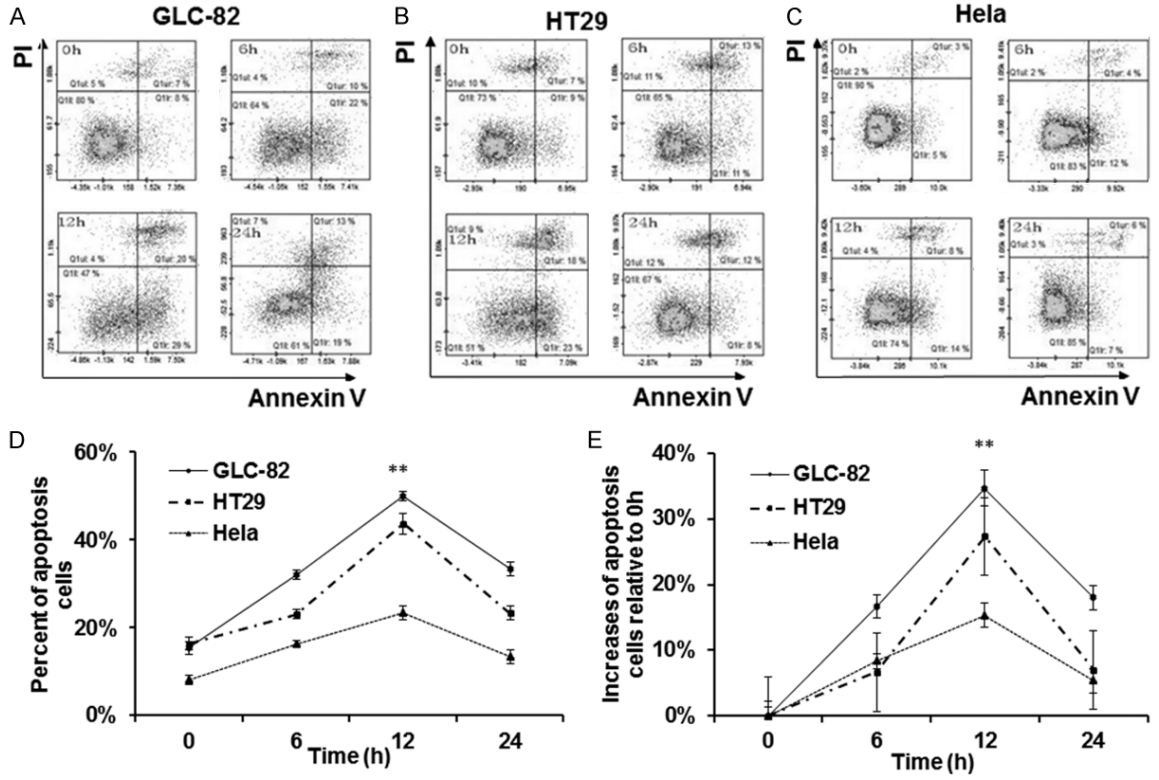

Figure 4. Changes of cell apoptosis in the three lines after radiation. GLC-82, HT29 and Hela cells were exposed to 5 Gy X-ray (0 hr, 6 hr, 12 hr and 24 hr), cell apoptosis was analyzed by Annexin V-PI staining and flow cytometry. A-C. Representative results of flow cytometry showing the changes of apoptosis in the three cell lines. D. Percentages of apoptotic cells after radiation. E. Changes on the percentages of apoptotic cells after radiation. Data were analyzed using ANOVA. And, **P < 0.005 among the three cell lines at 12 hr.

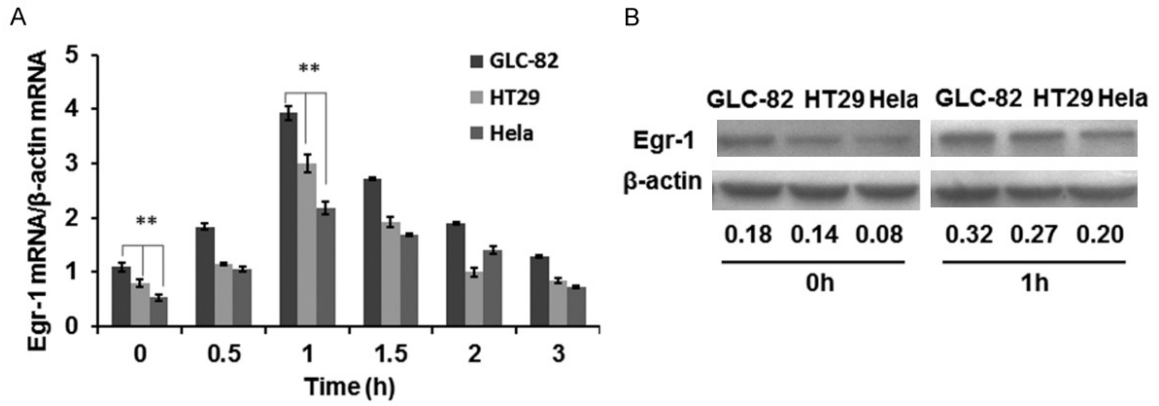

Figure 5. Changes of Egr-1 expression levels in the three lines. GLC-82, HT29 and Hela cells were exposed to 5 Gy X-ray (0 hr, 0.5 hr, 1 hr, 1.5 hr, 2 hr and 3 hr). A. Egr-1 mRNA expression levels in the three cell lines were analyzed by FQ-PCR. B. EGR-1 Protein expression levels in the three cell lines were analyzed by Western Blot, β-actin was used as internal control. Data were analyzed using ANOVA. And, **P < 0.005 among the three cell lines at 0 hr and 1 hr.

of cells in G2/M phase reached the highest at 12 h and decreased at 24 h after radiation, while the percentage of cells in G0/G1 and S phase also changed (**Figure 3D**, **3E**). Therefore, G2/M phase arrest was observed after radiation in these cells.

Changes of cell apoptosis and its correlation with D_0 and SF_2

Next the influence of radiation on cell apoptosis was analyzed by flow cytometry in the three cell lines. As is shown in **Figure 4**, the percentage of

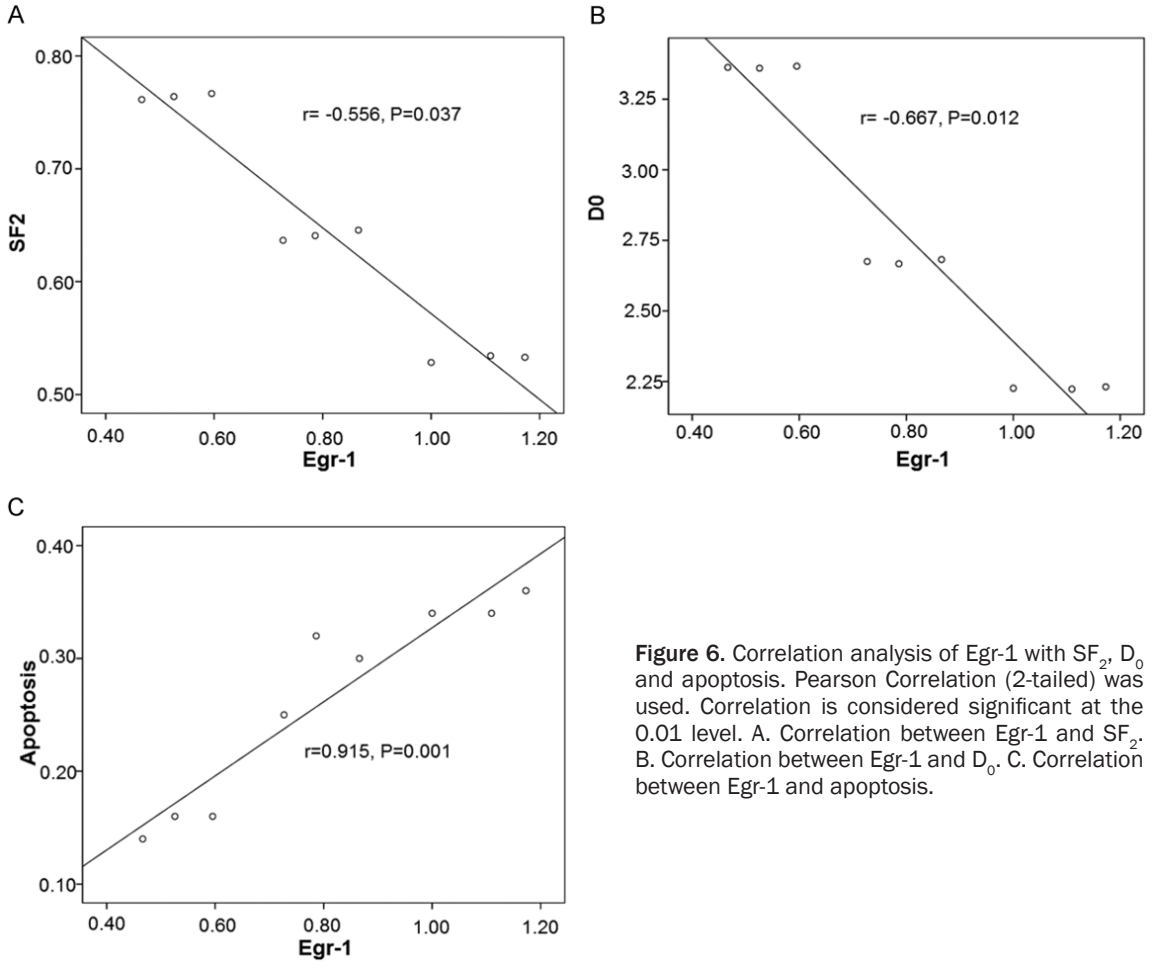

Figure 6. Correlation analysis of Egr-1 with SF_2, D_0 and apoptosis. Pearson Correlation (2-tailed) was used. Correlation is considered significant at the 0.01 level. A. Correlation between Egr-1 and SF_2. B. Correlation between Egr-1 and D_0. C. Correlation between Egr-1 and apoptosis.

apoptotic cells increased from 6 hr after radiation, peaked at 12 hr, and decreased at 24 hr. At 12 hr, the level of radiation-induced apoptosis is highest in GLC-82 cells (0.347±0.012), the lowest in Hela cells (0.153±0.012) with that of HT29 cells (0.273±0.025) in the middle, which was significantly different among them (F = 234.333, P = 0.000). We further analyzed the correlation between the level of radiation-induced apoptosis and radiosensitivity parameters in these cells. We found that there was negative correlation between apoptosis and D_0 (r = -0.667, P = 0.012) and between apoptosis and SF_2 (r = -0.556, P = 0.037). These results indicated that the radiation-induced apoptosis level of the three cell lines was correlated with radiosensitivity.

Egr-1 expression levels and its correlation with D_0 and SF_2

To further investigate the role of EGR-1 in cancer apoptosis induced by radiation, expression of Egr-1 mRNA and EGR-1 protein was analyzed. FQ-PCR showed that Egr-1 basic expression levels in GLC-82, HT29 and Hela cells were significantly different (F = 43.143, P = 0.000, **Figure 5A**), which was highest in GLC-82 cells and the lowest in Hela cells. There was also significant difference in Egr-1 expression between GLC-82 and HT29 (P = 0.003), and between HT29 and Hela (P = 0.005). Western blot showed similar results in the protein level before radiation (F = 153.948, P = 0.000) (**Figure 5B**). Expression of Egr-1 increased quickly after 5 Gy X-ray radiation in all the three cell lines, which peaked at 1 hr and declined gradually to the basic level at 3 hr. However, Egr-1 expression had significant difference at peak as determined by FQ-PCR and WB test in the three cell lines (F = 119.79, P = 0.000 and F = 153.95, P = 0.000, respectively). These results suggested that Egr-1 basic expression levels were significantly different before radiotherapy.

To investigatethe relationship between Egr-1 basic expression levels and radiosensitivity, we analyzed the correlation between Egr-1 basic

expression and radiosensitivity parameters. There was negative correlation between SF_2 and Egr-1 (**Figure 6A**) or between D_0 and Egr-1 (**Figure 6B**) ($r = -0.556$, $P = 0.037$ and $r = -0.667$, $P = 0.012$, respectively). We also analyzed the correlation between the levels of Egr-1 basic expression and radiation-induced apoptosis, and the result showed that there was a significantly positive correlation ($r = 0.915$, $P = 0.001$) (**Figure 6C**). These results suggested that Egr-1 basic expression level was associated with radiosensitivity.

Discussion

In the present study, we analyzed the radiosensitivity of three cell lines, GLC-82, HT29 and Hela, by colony formation assay, and found that radio sensitivity was relatively higher in GLC-82 cells ($SF_2 = 0.532\pm0.003$), relatively lower in Hela cells ($SF_2 = 0.764\pm0.003$) and moderate in HT29 cells ($SF_2 = 0.641\pm0.005$). To determine the underlying mechanisms for this difference in radiosensitivity, DNA damage, cell apoptosis and cell cycle distribution in the three cell lines was analyzed. Using comet assay, relatively higher level of DNA damage ($\Delta Tf_{0.5} = 1.480\pm0.002$), relatively lower level of DNA damage ($\Delta Tf_{0.5} = 1.274\pm0.003$) and moderate level of DNA damage ($\Delta Tf_{0.5} = 1.398\pm0.005$) was found in GLC-82 cells, Hela cells and HT29 cells respectively. Therefore, the irradiation-induced double-stranded DNA damage contributed to radiosensitivity of tumor cells to some extent.

Golden, Eriksson and Huerta et al. had reviewed current studies on the molecular signaling pathways, including p53-dependent and p53-independent pathways, in irradiation-induced DNA damage and the following irradiation-induced cell death. They considered that radiation-induced apoptosis is one of the main cell death mechanisms following exposure to irradiation [1, 2, 20]. Apoptosis firstly brought up by Professor Kerr at 1972 basing on morphological criteria is a distinctive morphological event which is essential in maintaining cellular homeostasis, This highly conserved biological process is initiated by different environmental stimuli and tightly regulated by multiple genes involving in different cell signaling pathways [25]. In fact, tumors that are susceptible to p53 dependent apoptosis are quite radiosensitive, whereas tumors that overexpress anti-apoptotic proteins (BCL2, Bcl-XL, and Survivin) or lose expression of proteins involved in the apoptotic machinery are radioresistant. In cancers with p53 mutations, unchecked cell proliferation occurs in spite of DNA damage by irradiation (IR) [26]. Moreover, the tumor cells accumulate DNA mutations, become aneuploid, and develop micronuclei, leading to mitotic catastrophe (MC) and subsequent cell death [27].

Here we used three cell lines in this study. GLC-82 was obtained from Tumor Institute of Yunnan Province, and it was established from the lung cancer tissue of a female retired worker in Yunnan Tin Company Group Ltd [28]. The karyotype of GLC-82 was stable after long-term *in vitro* culture [29], and this cell has wild type p53 and c-myc gene translocation [30-32]. HT-29 cells were derived from a 44-year-old Caucasian woman with colorectal adenocarcinoma, and have mutations of both alleles of the p53 gene (p53-null) [33], low levels of p21, and relatively higher levels of survivin and Bcl-2 (compared to p53-wild-type HCT-116 cells). This phenotype of HT-29 cells was associated with decreased apoptosis and increased survival, and the p53-CDKI-BAX/Bcl-2 axis might play an important role in radioresistance [34, 35]. Hela was established from an epidermoid carcinoma of the cervix in 1951 [36], which also has wild type p53. However, the viral oncoprotein E6 from this high-risk HPV type has the ability to promote p53 degradation and neutralize the function of p53 [37]. Hela cells have almost completely lack of endogenous connexins [38]. Here we found that G2/M phase arrest was obvious at 12 hr after radiation in the three cells lines. These results indicated that differentirradiation-induced apoptotic levels in the three cells may be resulted from the distinct genetic background of these cells.

Radiation inducibility of Egr-1 is pivotal in radiotherapy. In a variety of malignancies, the level of Egr-1 basal expression is different, and irradiation induced elevated expression levels of Egr-1. However, the mechanism is not fully understood [9-11, 39]. Levin et al. studied expression patterns of Egr-1 in samples of human lung adenocarcinomas, and found that the down-regulation of Egr-1 expression may be involved in the pathogenesis of lung cancer [40, 41]. Egr-1 overexpression may be related to the sen-

sitivity of uterine cervical squamous cancer to radiation [39]. Downregulated Egr-1 expression in A549 and Hela cells compared with that in peripheral blood mononuclear cells (PBMCs) from healthy donors may be related to radiation-resistance of these two cells [42, 43]. In our research, we found the basal expression level of *Egr-1* gene was relatively higher in GLC-82 cells, relatively lower in Hela cells and moderate in HT29 cells. After irradiation of the three cell lines, Egr-1 expression was obviously elevated, but no difference in Egr-1 expression after irradiation between the three cells was observed. Further analysis showed that radiosensitivity was positive correlation with the basal expression level of *Egr-1* gene.

In conclusion, we found that the radiosensitivity of three cell lines, GLC-82, HT29 and Hela, was decreased in sequence as determined by clone formation assay. This difference in radiosensitivity may be resulted from DNA damage and irradiation-induced apoptosis, and positively related to the basal expression level of *Egr-1* gene. Therefore, *Egr-1* gene may become a candidate for the prediction of tumor radiosensitivity.

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Disclosure of conflict of interest

None.

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