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Effect of ionizing radiation on transcription of colorectal cancer MDR1 gene of HCT-8 cells

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ABSTRACT

Objective: To discuss effect of ionizing radiation on transcription of colorectal cancer multidrug resistance (MDR) 1 gene of HCT-8 cells. **Methods:** Total RNA was extracted by guanidine thiocyanate one-step method. Northern blot was applied to detect transcription level of MDR1 gene. The expression of P-gp protein was detected by flow cytometry. **Results:** The expression of MDR1 of normal colorectal cancer HCT-8 cells was low. It was increased by 8.35 times under stimulus with 2 Gy. When treated with low doses in advance, high expressed MDR was decreased significantly under 0.05, 0.1 Gy, which was 69.00%, 62.89% in 2 Gy group and 5.77 times, 5.25 times in sham irradiation group. No obvious difference was detected between (0.2+2) Gy group and 2 Gy group. Compared with sham irradiation group, the percentage of P-gp positive cells after radiation of a high 2 Gy dose was increased significantly ($P<0.01$). When treated with high radiation dose following low radiation dose (0.05 Gy, 0.1 Gy) in advance, the percentage of P-gp positive cells were also increased significantly. The percentage of P-gp positive cells were increased obviously in 0.2 Gy and 2 Gy groups. Compared with simple high radiation 2 Gy group, the percentage of P-gp positive cells was decreased significantly ($P<0.05$). **Conclusions:** Low radiation dose can reverse multidrug resistance of colorectal cancer cells caused by high radiation dose.

1 Introduction

Colorectal cancer is a common gastrointestinal malignant cancer and is mainly treated with surgery. It is also a systemic disease, and comprehensive treatment such as chemotherapy and radiotherapy should be applied to patients after surgery, patients who lost their chances for surgery or patients suffered from metastasis colorectal cancer, in order to improve their quality of life, decrease recurrence rate of cancer, prolong their survival time, and increase recovery rate[1]. However, the biggest obstacle for chemotherapy is multidrug resistance of tumor cells, especially the primary and secondary high expression of multidrug resistance (MDR) 1 gene of colorectal cancer cells, rendering much more severe multidrug resistance and lower effectiveness of chemotherapy than other tumor. By detecting the effect of different radiation doses on multidrug resistant gene MDR1 and its protein P-gp of colorectal cancer cells, our research was to discuss the approaches to the reversion of tumor multidrug resistance.

2. Materials and methods

2.1. Main reagents

Human colorectal cancer HCT-8 cells were provided by MH Radiobiology Research Unit. Cells were cultured in RMPI1640 containing 1/100 FBS and placed in an incubator with saturated humidity at 37 ℃, 50 mL/L CO_2.

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Trypsin at 2.5 g/L was used for digestion and passage. Primers for mGAP were: 5′TATTGGGCGCCGGGTCACCA; 3′CCACCTTCTTGAT GTCATCA, product length 746 bp. Primers for mMDR1 were: 5′CCCATCATTGCAATAGCAGG; 3′GTTCAAACTTCTGCTCCTGA, product length 417 bp. Primers were produced by Takara Biotechnology (Dalian) CO., LTD.

2.2. Methods

2.2.1. Irradiation condition

Irradiation with domestic fixed X-ray deep therapy apparatus type X.S.S.250 (FZ),with voltage at 200 kV, current at 10 mA and filter plate at 0.5 mm Cu+1.0 mm Al. Systemic irradiation at low radiation dose (0.05-0.2 Gy), with target skin distance 247.3 cm, dose rate 0.012 5 Gy/min. Systemic irradiation at higher dose (2 Gy), with target skin distance 56 cm, dose rate 0.287 Gy/min.

2.2.2. Preparation for the model of drug resistant cells

Under sterilized conditions, cells in logarithmic growth phase were incubated with adriamycin (ADM) at concentration 35, 350, 3 500 μg/L, respectively for 3 d, then recovered for 3 d. We observed shape as well as number of cells and discovered that cells in 350 μg/L group grew with moderate amount and intact shape, hence 400 μg/L ADM was applied as stimulus for the model of drug resistant cells. Under sterilized conditions, cells in logarithmic growth phase were incubated with 50 μg/L ADM for 3 d. After recovering for 3 d, cells were incubated with doubled concentration (100 μg/L) for 3 d, then recovered again for 3 d. The above procedure was repeated until concentration reached 400 μg/L, cells were collected for later work. Meanwhile a blank control (without drug stimulus) and a parallel control which was stimulated with fixed concentration were set.

2.2.3. Preparation for the model of irradiation

Drug resistant cells were divided into 5 groups: A: sham control group; B: high dose group; C: 0.05 Gy+2 Gy group (a low dose of 0.05 Gy was offered, followed by a high dose of 2 Gy. The same for the follow); D: 0.1 Gy+2 Gy group; E: 0.2 Gy+2 Gy group. After low dose irradiation, there was an interval for 4 h before high dose irradiation. Cells were collected for later detection 24 hours after high dose irradiation.

2.2.4. Northern blot

2×10^7 cell and 1 mL guanidine isothiocyanate were mixed speedily. 100 μL sodium acetate (pH 4.0), phenol water with the same volume and 200 μL CH_4Cl_3/ISA (49:1) were added afterward and fully mixed. The mixture was posited for 15 min at 4 ℃ and centrifuged at 12 000 r/min for 10 min. Upper water was pipetted and isopropanol with the same volume was added. The mixture was then store at -20 ℃ for at least 30 min for depositing RNA and centrifuged for 15 min at 4 ℃ at 12 000 r/min. Upper water was pipette. After deposition dried and dissolved in RNase free water, ultraviolet spectrophotometer was applied for purity and content of RNA, thereafter it was stored at -70 ℃. See formaldehyde degradation gel electrophoresis, transfer, hybridization and washing membrane in references[2]. Probes were marked by random priming, American Phosphoimager laser scanner was used for average grey value of hybridized bands, the result was presented as the ratio of average grey value between specific hybridized band and GADPH probe hybridized band.

2.2.5. Expression of P-gp protein detected by flow cytometry (FCM)

FAC Scan flow cytometry manufactured by American B-D company was applied in our experiment. Its excitation light source was 15 mW argon laser, wavelength was 488 nm, indirect immunofluorescence was used in FCM. Suspension of HCT-8 single cell was washed by PBS twice. Upper water was pipetted. Cells were sucked up and infused into 75% cold ethanol for fixation, then posited at 4 ℃ overnight. HCT-8 cells were washed by PBS to remove ethanol and centrifuged at 1 200 rpm for 5 min twice. 50 μL (1:100) P-gp monoclonal antibody as first antibody was added to each sample. Samples were posited at 4 ℃ for 45 min for reaction, washed by PBS twice and centrifuged at 1 200 rpm for 5 min. Goat anti-mouse IgG-FITC(diluted at 1:100) as second antibody was added. Samples were posited at 4 ℃ for 45 min for reaction and washed by PBS twice. After adding 500 μL PBS, samples were detected by FCM.

2.3. Statistical analysis

Results in this essay are expressed by mean±standard deviation. *t*-test was applied in pairwise comparison.

3. Results

3.1. Changes of MDR1 gene

As Table 1 showed, expression of MDR1 was low in normal HCT-8 cells, but it was increased by 8.25 times after stimulation with 2 Gy. When treated with low doses in advance, high expressed MDR was decreased markedly in 0.05 Gy group and 0.1 Gy group, which was 69.00%, 62.89% of 2 Gy group and 5.77 times, 5.25 times of sham irradiation group. No significant difference was detected between (0.2+2) Gy group and 2 Gy group.

3.2. Effect of X-ray on expression of P-gp of HCT-8

Compared with sham irradiation group [(45.67±5.30)%], the percentage of P-gp positive cells after radiation of a high 2 Gy dose increased significantly [(81.74±3.47)%] ($P<0.01$). When treated with high radiation dose following low radiation dose (0.05 Gy, 0.1 Gy) in advance, the percentage of P-gp positive cells also increased significantly [(70.51±6.40)%

in (0.05+2.0) Gy, group, (67.37±4.20)% in (0.1+2.0 Gy) group]. The percentage of P-gp positive cells increased significantly in 0.2 Gy and 2 Gy groups [(83.04±7.07)%]. Compared with single high radiation 2 Gy group, the percentage of P-gp positive cells decreased significantly (P<0.05).

Table 1

Effect of X-ray on MDR1 mRNA of HCT-8 cells.

Dose (Gy)	GAPDH mRNA	MDR1 mRNA	Ratio
Sham irradiation	107.38	17.42	0.162
2.0	89.91	121.67	1.353
0.05+2.0	96.57	90.25	0.935
0.1+2.0	128.36	109.25	0.851
0.2+2.0	93.42	123.49	1.322

4. Discussion

Comprehensive treatment such as chemotherapy and radiotherapy should be applied to patients after surgery, patients who lost their chances for surgery or patients suffered from metastasis colorectal cancer, in order to improve their quality of life, decrease recurrence rate of cancer, prolong their survival time, and increase recovery rate[3-5]. However, the biggest obstacle for chemotherapy is multidrug resistance of tumor cells. MDR exists commonly in the process of chemotherapy for human tumors. One significant mechanism for MDR is overexpression of MDR1 gene. One currently conceived reason for multidrug resistance is that it is congenital[6]. During the mutation of cancer cells, MDR-1 gene was activated, transcribed and interpreted, thus P-glycoprotein was produced, rendering cancer cells self-protective, also known as drug resistance. The other believed reason is that during chemotherapy, drugs can not kill cancer cells directly because of inadequate dose or low sensitivity to drugs, incurring activation of MDR-1 gene in cancer cells, which leads to drug resistance of cancer gene, also known as acquired drug resistance. Both congenital and acquired drug resistance will severely affect chemotherapy and recovery of patients. Many research show that MDR1 is closely related to efficiency of treatment. Patients who have positive MDR1 present had shorter survival time, lower recovery rate and higher recurrence rate. Therefore, analysis of MDR1 can be used as an indication for prediction of therapeutic outcome of cancer and realize individualized chemotherapy. Clinicians may judge from the expression of MDR1 of patients, evaluate the extent of drug resistance and make proper therapeutic schedule. MDR1 can be used as indicative reference for treatment[7,8], hence, it is important to detect MDR1.

In this research, we extracted total RNA by guanidine thiocyanate one-step method, and applied Northern blot to detect changes in MDR1 gene on gene transcription level. Research showed that the expression of MDR was low in HCT-8 cells of normal colorectal cancer, it was increased markedly after being stimulated by 2 Gy. This result indicates that high radiation dose can significantly intensify multidrug resistance of cancer cells, which is in accordance with relevant reports[9-11]. One potential mechanism is the expression of protein kinase C increased prominently by high radiation dose further induces overexpression of MDR1 gene and accelerates phosphorylation of P-gp, finally generates MDR[12]. However, by treating with low radiation dose 0.05, 0.1 Gy, high expressed MDR decreased markedly, manifesting that low radiation dose can reverse multidrug resistance caused by high radiation dose. Low radiation dose following with high radiation dose reduce high expressed PKC protein might be the reason for this phenomenon.

Conflict of interest statement

We declare that we have no conflict of interest.

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