Investigation the Effect of Low, Medium and High Dose of X-Radiation on the Expression of E-cadherin in Colorectal Cancer Cell Line

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ABSTRACT

Background: Radiotherapy has become a part of therapeutic process of more than 50 percent of patients suffering from cancer. However, recent studies have shown that radiation therapy might affect the expression of adhesive molecule related genes such as E-cadherin and cause cancer cells to move and migrate. Besides, various studies have reported that the expression of E-cadherin changes differently after radiation treatment. There are several studies which showed the loss of E-cadherin function after radiation; however, this reduction has not been observed in others.

Objective: This study aims to investigate the effect of different radiation doses of X-ray on changes that might occur in the expression of E-cadherin gene in colorectal cancer cell line HT-29.

Material and Methods: In this experimental study, the cells cultured in flasks were irradiated with X- rays in different doses, including 0.1, 2.5, 5, and 10 Gy; then, the expression of E-cadherin gene was measured using real-time PCR.

Results: The expression of E-cadherin did not change significantly in post-irradiated HT-29 cell line after different radiation doses of X-ray.

Conclusion: The results showed that low, medium and high doses of X- radiation did not change the expression of E-cadherin gene in HT-29 cancer cells. However, it has been reported that radiation mostly downregulated the expression of E-cadherin and mediated metastasis formation and invasiveness in different cancer cell lines. Therefore, further studies need to be conducted to investigate the effects of radiation dose on the molecular pathways contributing to regulation of E-cadherin in HT-29 cell line.

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Keywords

Radiation; Colorectal Cancer; E-cadherin; Real-Time PCR; X-Rays; Radiation Dosage

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Introduction

Radiation therapy has become a part of therapeutic process of more than 50 percent of patients suffering from cancer [1, 2]. However, according to recent clinical and experimental studies, ionizing radiation might induce invasion and enhance metastasis potential in cancer cells [3-8]. One possible mechanism for cancer cells to acquire invasive feature and metastasis potential is loss of cell-cell

adhesion and cell-extracellular matrix interaction [9-11]. Adhesion molecules, which mediates the interaction between cell-cell and cell-extracellular matrix, have a central role in different physiological and also pathological mechanisms [9]. A calcium-dependent adhesion molecule, E-cadherin, mediates Ca^{2+}-dependent hemophilic interaction which is essential for establishment and maintenance of different junctional structures, including tight junctions, adherent-type junctions and desmosomes. These junctions are crucial for epithelial cells to interconnect [12, 13]. According to different studies, E-cadherin's adhesion function is lost during the most epithelial cancers, including colon, prostate, esophagus, kidney, breast, skin, liver and lung [10, 13]. Decreased E-cadherin function in cancer cells correlates with de-differentiation, tumor development, invasion and metastasis [13]. Besides, loss of epithelial markers, including E-cadherin, might induce epithelial-mesenchymal transition (EMT) which causes a pro-metastatic properties and promotes stem-like phenotype in cancer cells [14, 15]. The possible mechanisms explaining the reduction of E-cadherin expression in cancer cells are: mutation of E-cadherin gene, chromatin rearrangements, hypermethylation and loss of trans-factor binding [10, 13, 16]. Moreover, recent studies have shown that radiation therapy might reduce E-cadherin gene and cause cancer cells to move and migrate. However, as summarized in Table 1, various studies have reported that the expression of E-cadherin changes differently after radiation treatment. There are several studies which claimed the loss of E-cadherin function after radiation therapy was

Table 1: Expression of E-cadherin gene in different Post-irradiated cells with various doses of X-ray

Cell line / Tissue	Origin	Radiation	Dose Rate	Assay	E-cadherin expression	Ref.
A549	Human Lung Cancer	X-ray (2-15 Gy) 0.98 Gy/min	0.98 Gy/min	Immunoblot	↑	[17]
T-Scc	Human squamous cell Carcinoma	X-ray (2-10 Gy)	0.98 Gy/min	Immunoblot	↑	[18]
A549	Human Lung Cancer	X-ray (10, 20 Gy) 5 FR &10 FR	200 cGy/min	Real time PCR	---	[19]
HT-29	Colorectal Cancer	X-ray (10 Gy) 5 FR	200 cGy/min	Real time PCR	↓	[19]
CaR1 **DLD1**	Colorectal Cancer	X-ray 5 Gy		Real time PCR	↓	[3]
Siha **C33A**	Human Cervical	X-ray (7 5Gy; 2 Gy, 5 times/weeks)	0.36 Gy/min	Real time PCR	↓	[20]
***Eca109R**	Human ESC	X-ray (4-8 Gy)	200 cGy/min	Real time PCR	↓	[21]
MCF7	Breast Cancer	X-ray (20 Gy) 20 FR & 10 FR	0.4 Gy/min	Real time PCR	↓	[22]
NIH3T3	Mouse Fibroblast					

ESC: Esophageal Squamous Carcinoma

FR: Fraction

PCR: Polymerase chain reaction

*Radio resistant Eca109 cell line was established by exposing Eca109 cells to 25 doses of 2 Gy

followed by EMT transition, metastasis and cancer stem cell induction in residual cancer cells; however, this reduction has not been observed in others [3, 17-22]. Various radiation process, accumulative dose, dose rate might lead to this discrepancy. Owing to this difference, the present study aims to investigate the effect of different radiation doses on changes occurring in the expression of E-cadherin gene in colorectal cancer cell line HT-29.

Material and Methods

Cell line and cell culture

In this experimental study, the colorectal cell line, HT-29 provided from Pasteur Institute (Tehran, Iran), were grown in Roswell Park Memorial Institute 1460 (Bioidea) supplemented with 10% fetal bovine serum (Gibco), 100 u/ml penicillin and 100 µg/ml streptomycin. The cells were incubated at a humidified 5% Co_2 atmosphere at 37 °C. The medium was renewed every 2 days and the cell was subcultured using 0.25% trypsin-0.5 mM EDTA solution, whenever required.

Irradiation

HT-29 cells were plated in the 12.5 cm^2 tissue culture flask. 70% confluent cells were irradiated with various single doses of x-ray, including 0.1, 2.5, 5 and 10 Gy, emitted from an X-ray unit (Philips, serial number 2.625, Netherland, dose rate: 1.365 Gy/min with 100 kVp and 8 mA) at room temperature. The cells, which received no radiation, were used as a control group.

RNA Extraction

Total cellular RNA was extracted from irradiated cells and their relevant group, 20 h after radiation according to manufacturer's instruction (Yekta Tajhiz Azma Kit, Tehran, Iran). The extracted RNA was then checked for concentration, purity and integrity using nanodrop® spectrophotometer (Thermo Scientific) and agarose gel electrophoresis. To avoid DNA contamination, extracted RNAs were treated with RNAs-free DNase I (Thermo Scientific kit, Massachusetts, USA) and inactivated by EDTA.

cDNA Synthesis

To synthesis cDNA, Suprime Script RTase, Oligo-dT and dNTPs (GeNet Bio, Korea) were reversely transcribed according to the manufacturer's instructions. The cDNA samples then were stored at -20 °C until further processes. The fidelity of synthesized cDNA was then confirmed by polymerase chain reaction (Ampliqon Taq DNA polymerase Master Mix RED kit, Denmark) using GAPDH primers. The final products were loaded on 2% agarose gel for electrophoresis (Figure 1). Cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s and final extension for 5 min at 72 °C.

Quantitative real-time PCR

Finally, the Ampliqon SYBER Green PCR kit (Denmark) was used to perform real-time polymerase chain reaction (real-time PCR). Light Cycler 96 System (Roche, Basal, Switzerland) was used to perform real-time PCR. To study the expression of E-cadherin, the following specific primers were used;

GAPDH: forward, 5′- GACCACTTTGTCAAGCTCATTTCC -3′;

Reverse, 5′- GTGAGGGTCTCTCTCTTCCTCTTGT-3′;

E-cadherin: forward, 5′- ACATATCGGATTTGGAGAGACACT -3′;

Reverse, 5′- CAACTGGAGAACCATTGTCTGTAG -3′;

The CT number of E-cadherin was normalized to GAPDH in each sample. PCR efficiency was measured for the gene of interest using Linreg PCR software, and relative changes for mRNA level were calculated based on the ΔΔCt method.

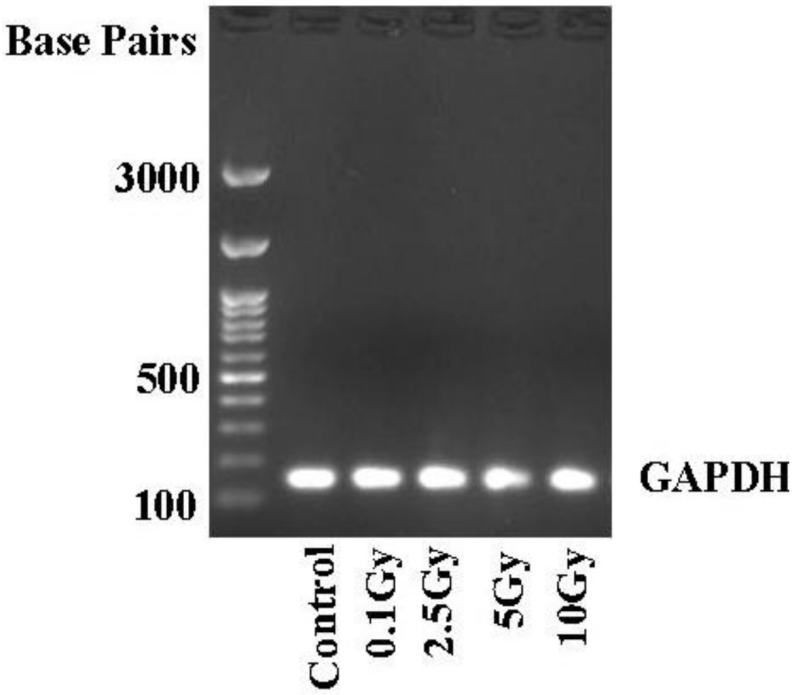

Figure 1: Gene expression pattern of GAPDH primers in post-irradiated HT-29 cell line and their relevant control group.

Statistical analysis

The data were statistically analyzed by one-way ANOVA using Graph Pad Prism version 8.0. Results were reported as mean ± SD and $P<0.05$ was considered to be statistically significant.

Results

Expression of EMT gene, E-cadherin, after exposing to different doses of X-ray

According to the data shown in Figure 2, different doses of X-ray (0.1, 2.5, 5 and 10 Gy) did not change the expression of E-cadherin gene in HT-29 cells significantly ($p>0.05$).

Discussion

Radiotherapy is one of the most important modalities for cancer treatment. However, according to recent clinical and experimental studies, ionizing radiation might induce invasion and enhance metastasis potential in cancer cells [3, 8, 19, 20, 23, 24]. Moreover, recent studies have demonstrated that changes undergoing in cell-cell adhesion and cell-extracellular matrix interaction in post-irradiated tumor cells might cause them to loss their epithelial characters and acquire mesenchymal morphology which lead to enhanced potential for migration, invasion and metastasis [9, 18, 22]. Therefore, overcoming the enhancement of metastatic potential through adhesion molecules after radiation treatment is an important issue. Among different adhesion molecules, dysfunction of E-cadherin, with a key role for establishment and maintenance of different junctional structures, has been recognized in various physiological and pathological diseases including cancer [10, 11, 25, 26]. Several studies demonstrated that the expression of E-cadherin decreases after radiation and causes cancer cells to acquire metastasis potential; On the other hand, there are a few research-

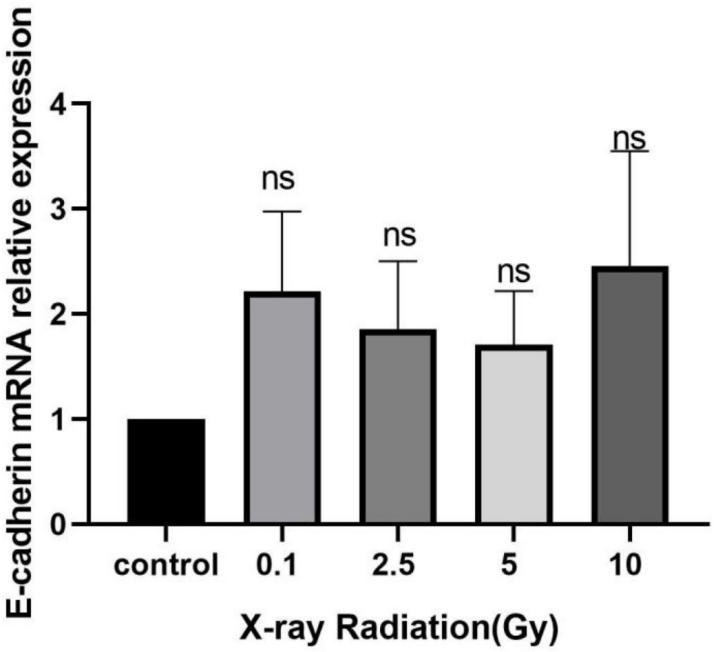

Figure 2: Expression of E-cadherin gene after irradiation of HT-29 cells with different single doses (0.1, 2.5, 5 and 10 Gy) of X-ray. Total RNA was extracted after 20 h and analyzed for mRNA level of E-cadherin gene. Gene expression values obtained from irradiated cells were compared with control group which was standardized to a value of 1. The experiments were performed at least three times in duplicate and results were presented as mean foldchange±SD.

ers who have observed increased expression of E-cadherin in post-irradiated cancer cells. Owing to this difference, the current study was planned to investigate the effect of different radiation doses on changes occurring in the expression of E-cadherin gene in colorectal cancer cell line HT-29.

Our results indicated that the expression of E-cadherin, due to various radiation doses of X-ray (including 0.1, 2.5, 5 and 10 Gy), enhanced, although compared to control group, it was not statistically significant. In consistent with this result, Akimoto et al., reported that the expression of E-cadherin did not change significantly at earlier times of 1 and 3 h after exposing lung cancer cells (A549 cell line) into 10 Gy of X-ray [17]; however, they found that the expression of E-cadherin increased 24 h after various doses of X-ray (2-15 Gy) in the same cell line (A549) [17]. The same result was reported by Ebara et al., in post-irradiated T-SCC cells (human squamous cell carcinoma) after different doses of 2-10 Gy of X-ray [18].

Tahmasebi et al., demonstrated that the expression of E-cadherin gene did not change significantly after irradiating A549 cells. However, in contrast to our result, they observed that the expression of E-cadherin gene decreased in HT-29 cell line. Regarding the two different irradiation regimens used in the two studies, different changes in E-cadherin gene expression in HT-29 cell line, may be explained. We irradiated the cells with single doses of 0.1, 2.5, 5, and 10 Gy, while, they applied fractionated irradiation regimen (cumulative doses of 10 and 20 Gy with 2 Gy/day fractions) [19]. There are more evidence that

fractionated radiation has rendered reduced expression of E-cadherin gene. In a study carried out by Zhang et al., doses of 20 Gy (1 Gy/day and 2 Gy/day) and greater induced epithelial-mesenchymal transition and reduced the expression of E-cadherin gene in breast cancer cells [22]. Downregulation of E-cadherin expression after fractional radiation with a total dose of 75 Gy has also been reported in cervical cancer cell lines [20].

Although our data show no significant changes in the expression of E-cadherin, it does not necessarily mean that radiation does not promote invasiveness and metastatic potential in cancer cells, due to the fact that the change of E-cadherin gene is not the just activator of cell migration and invasion. Moreover, according to Ikeguch et al., and Akimoto et al., there is a complex process behind the expression of E-cadherin in metastatic foci of colorectal cancer cells, which might lead to E-cadherin overexpression with negative function [17-27]. In addition, according to molecular studies, Wnt/βcatenin, STAT3 and ZEB1 are reported as the most important signaling pathways associated with E-cadherin expression in colorectal cancer cells [12, 28]. Therefore, it is necessary to carry out more studies to investigate the effect of radiation dose on these signaling pathways.

Conclusion

Current study demonstrated that different doses of X-ray did not change the expression of E-cadherin in HT-29 colorectal cancer cells. However, to investigate whether radiation can promote tumor progression, invasion and metastasis in cancer cells through dysregulation of adhesion molecules, examining the effect of dose radiation on the expression of cadherins (P and N-cadherins) and catenins (α, β and γ catenins), as the major components for E-cadherin function, needs to be conducted in future work.

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Conflict of Interest

None

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