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Original Article

Gene alterations after ionizing radiation in human cervical carcinoma-associated endothelial cells

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Abstracts: More and more researchers pay attention to the response of endothelial cells during tumor radiotherapy, and most researches presented that endothelial cell apoptosis ratio influences the efficacy of radiotherapy. Nevertheless, radiation-induced gene conversions in human cervical endothelial cells (HCECs) remain rarely understood. This study would address the gene expression conversions after X-ray radiation in these cells and provide novel treatment strategies for human cervical cancer. We obtained endothelial cells from six human cervical cancer patients before and 4 h after 400 cGy X-ray irradiation. The significant genes were selected by a 22K Human Genome Oligonucleotide Microarray Kit and confirmed by real-time quantitative PCR and western blot. Our results showed that 46 genes consistently altered in all the microarray experiments. Compared with the control group, 26 genes were up-regulated and 20 genes were down-regulated. Most of the genes were identified as significant conversions (≥2 fold), including the regulation of the cell cycle, cell adhesion, the immune response, chemokines, the inflammatory response, growth factors, angiogenesis, DNA synthesis and repair, and protein synthesis. Four genes were randomly selected to confirm the microarray data. Radiation-induced gene alterations and gene-related signal pathways were related to vasculogenesis and radiosensitivity of human cervical cancer. This study elucidates that the identified genes and gene-related signaling pathways may provide meaningful biomarkers for radiation and anti-angiogenesis treatments against human cervical cancer.

Keywords: Cervical cancer, carcinoma-derived endothelial cells, radiosensitivity, gene microarray, anti-angiogenesis

Introduction

Radiotherapy remains the mainstay of the conventional treatment methods in cervical cancer, especially in advanced cervical cancer. Resistance to radiation detailed molecular mechanism is still not clear [1]. Therefore, understanding the mechanism may provide valuable information for malignant therapeutics. Patients with cervical cancer may have different responses to radiotherapy even if the pathological type and stage are similar. Someone heal, but others'disease may just progress. Based on cancer radiosensitivity, each patient can accept individualized treatment. This study would address the gene expression conversions after X-ray radiation in human cervical cancer vascular endothelial cells and provide novel treatment strategies.

Tumor vasculature can supply nutrient for growth and proliferation of human cervical cancer. In addition, cancer invasion, metastasis and radiosensitivity go hand in handwith angiogenesis, and the radiosensitivity determined the tumor response against radiotherapy. With the development of gene microarray, cancer radiosensitivity or resistance genes and their associated signal pathways can be further understood. Formerly, some researchers studied radiation-induced gene expression conversions in cervical cancer; nevertheless, it is rarely about tumor microenvironment. The endothelium plays a critical role in radiation-induced vascular injury. In the previous phase of work, we have studied ovarian endothelial cells [2] and endometrial endothelial cells [3] of gene expression changes after ionizing radiation and known that their radiation-induced

Table 1. Characteristics of patients with cervical epithelial cancer

Pathological pattern	Neoplasm staging	Age (years)	Gene microarray	qPCR
Cervical cancer	Stage IIIb	50	+	+
Cervical cancer	Stage IIIa	55	+	+
Cervical cancer	Stage Ib	37	+	+
Cervical cancer	Stage IIa	47	+	+
Cervical cancer	Stage IIb	51	+	+
Cervical cancer	Stage IIIa	43	+	+

qPCR, quantitative polymerase chain reaction. +, microarray/qPCR was performed.

Table 2. Primer pairs for qRT-PCR

Gene bank ID	Gene name	Primer sequence (5' to 3')
NM_199168.3	CXCL12	F-gattcttcgaaagccatgttg
		R-cactttagcttcgggtcaatg
NM_004994.2	MMP9	F-accaccacaacatcacctattg
		R-acaccaaactggatgacgatg
NM_004864	GDF15	F-gcggaaacgctacgagcac
		R-tgcaaggctgagctgacg
NM_006169.2	NNMT	F-agactccttcttcagccaacat
		R-accccaaagttcagagagacag
NM_003246.2	THBS1	F-gacatcccaaaatgaccctaac
		R-acttgcttccacatcacaacat
NM_053056	CCND1	F-gtgaagttcatttccaatccgc
		R-gggacatcaccctcacttac
NM_012329	MMD	F-acacacgcattcctcatt
		R-tcttttcccagcagtcatca
NM_004345.4	CAMP	F-cggtgtatggggacagtgac
		R-cagcagggcaaatctcttgt

gene alterations and gene-related pathways were associated with vasculogenesis and radiosensitivity, which may provide promising biomarkers for radiation and anti-angiogenesis treatments against ovarian carcinoma and endometrial carcinoma. Now, we extract and study human cervical endothelial cells by the same method. Due to the discovery of carcinoma-associated radiosensitivity genes, anti-angiogenesis therapies for cancer may be a promising treatment strategy.

Patients and methods

Patients

Six cases of cervical epithelial cancer were drawn from Shandong Cancer Hospital (Jinan, China). They had no history of treatment. Study was approved by the Medical Ethics Committee, Shandong Cancer Hospital on the basis of the consent. **Table 1** listed the circumstantial clinicopathologic characteristics of each patient.

Endothelial cell isolation, culture and irradiation

Fresh cervical endothelial cells were taken from six human cervical epithelial cancers before and 4 h after 400 cGy X-ray irradiation. The tissues were processed by dividing (0.2-mm^3 pieces), filtering (a 100 μm metal mesh), digesting (add 1 % collagenase I to digest under the condition of 37°C for 30 minutes), and filtering (a 200 μm metal mesh) to make a single cell suspension. Many negative selections were undergone, including erythrocyte hemolysis (NH4Cl) and eviction of monocytes, lymphocytes, and granulocytes using anti-CD14, anti-CD45, and anti-CD64 beads (DynaBeads, Dynal Biotech, Brown Deer, WI). Positive screening was done with anti-CD31 beads using a magnetic separator (Dynal Biotech). The purified cervical endothelial cells were cultivated with 20% fetal bovine serum, 100 U/ml streptomycin and penicillin, 40 μg/ml ECGS, 40 U/ml insulin and 20 U/ml heparin in a 37°C incubator with 5% CO_2.

Immunofluorescence staining

Cervical endothelial cells were processed by fixing (cold 4% paraformaldehyde solution for 20 minutes), perforating (0.1% Triton X-100 for 15 min in 37°C), blocking (1% bovine serum albumin for 30 min at 37°C) and cultivating with rabbit antihuman von Willebrand factor (vWF) antibody (Immuno Way, Newark, DE, USA) for 10 h at 4°C in order. After washing, fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G (1:100) was added for 1 h and 4', 6-diamidino-2-phenylindole (Sigma, St Louis, MO, USA) for 4 min.

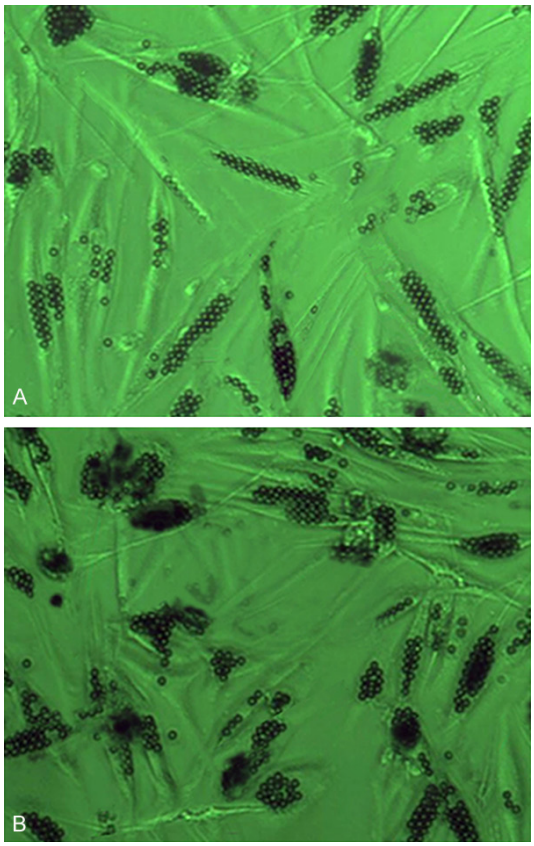

Figure 1. Morphology and characterization of purified endothelial cells. (A) Cervical cancer-derived and (B) cervical endothelial cells after X-ray irradiation contained anti-CD31 magnetic beads; all endothelial cells contained anti-cluster of differentiation 31 immunomagnetic beads, presented typical cobblestone morphology and contact-inhibition phenomenon.

RNA isolation and oligonucleotide array sequence analysis

The RNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract RNA, and the RNase-free DNase Set (Qiagen, Valencia, CA) was used to purify RNA. Gel electrophoresis was used to assess the quality and quantity of RNA, and the ratio of the optical density at 260 and 280/260 nm, respectively. Total RNA was obtained from unirradiated and irradiated cervical endothelial cells, synthesized by reverse transcription to cDNA, labeled with Cy3-and Cy5-dCTP, respectively, hybridized to the Human Genome U133 Plus 2.0 Affymetrix oligonucleotide microarray (Affymetrix, USA). Arrays were washed, stained and scanned by using a LuxScan™ scanner, and the images obtained were analyzed by LuxScan 3.0 software. Dye swap hybridizations were used to ensure accuracy, and the mean ratio of Cy5/Cy3 was calculated to evaluate the gene expression levels.

Quantitative real-time PCR

To confirm the reliability of the microarray assay, PCR was undertaken using a SYBR Green real-time PCR kit according to the manufacturer's instructions of ABI Prism 7000 Sequence Detection System (Applied Biosystems, USA). Four genes, which had different fold changes in expression, included CXCL12, MMP9, THBS1 and CAMP, were randomly selected to ensure the microarray data. Primer sequences can be found from **Table 2**. The total RNA extraction method was performed under the following conditions: started at 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 10 sec, annealed at 55°C for 10 sec and extended at 72°C for 15 sec. All samples of PCR reactions were undertaken in triplicate. By using melting curve analysis and agarose gel electrophoresis, the reaction data was evaluated. The 2-ΔΔCT method was used to calculate the average cycle threshold (Ct) values and the relative expression. These Ct values were standardized against for GAPDH for each gene [4].

Analysis of protein expression by Western blot

Seventy-five percent confluent cells were obtained and lysed with cold RIPA buffer. Total cell lysates were clarified by centrifugation (14,000 rpm) at 4°C for 20 minutes. Protein concentrations were measured with the MicroBCA kit (Pierce Inc. Rockford, IL). Then 25 μg of each soluble protein sample was separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to a nitrocellulose membrane. After blocking with 5%TBST-milk, membranes were incubated with mouse monoclonal anti-ADM (1:2 500), anti-LOX, anti-MMP10, rabbit polyclonal anti-IL23A (Santa Cruz Biotechnology, Santa Cruz, CA), anti-MYC and anti-HAS2 (Stressgen Biotechnologies) and anti-B-actin (Sigma) antibodies overnight at 4°C. Then the membranes were incubated with horseradish peroxidase (HP)-conjugated anti-rabbit or anti-mouse IgG antibody, respectively (Santa Cruz Biotech, Santa Cruz, CA). Membranes were incubated in SuperSignal RWest Pico Chemiluminescent Substrate (Pierce, Rockford, IL), exposed to CL-Xposure FilmTM (Pierce, Rockford, IL) and

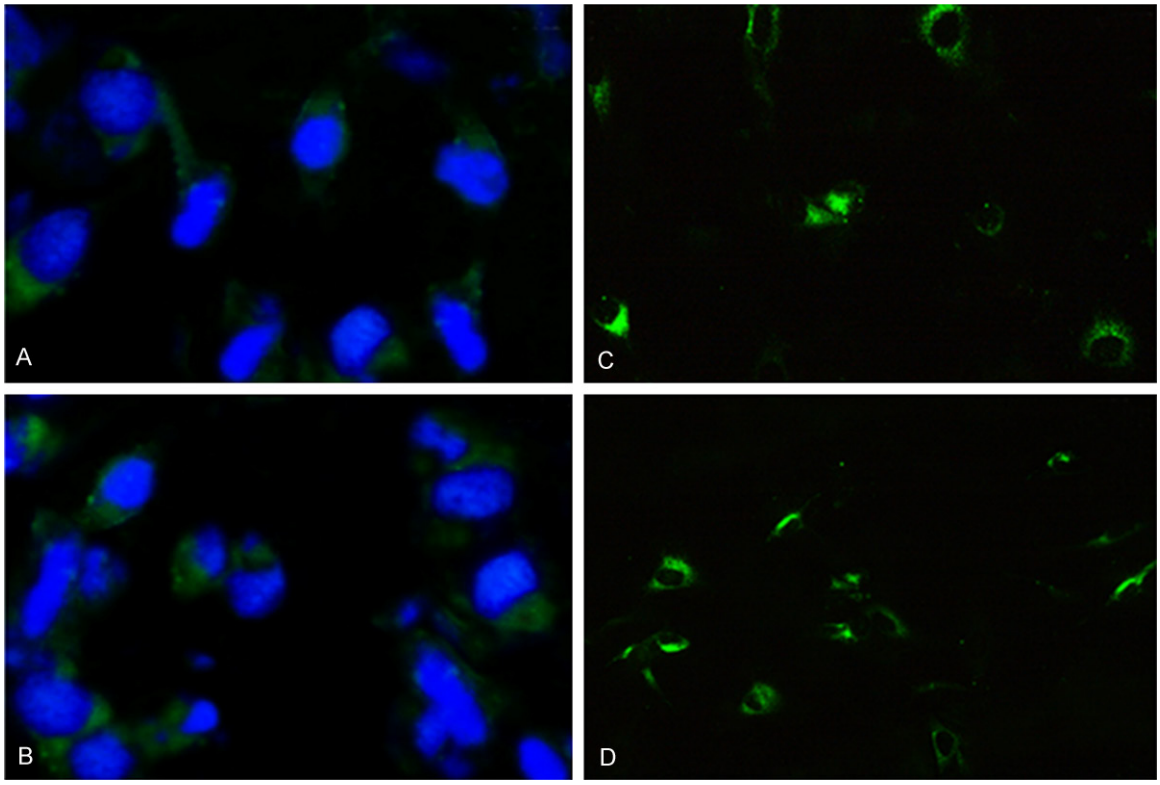

Figure 2. vWF immunofluorescent staining for cervical endothelial cells after X-ray irradiation (A, C). Cervical cancer-derived endothelial cells were used as the controlling group (B, D). The nuclei were stained with DAPI.

developed using an All-Pro 100 Plus automated X-ray film processor(All-Pro Imaging Corpo ration, Hicksville, NY). The resultant bands were quantified using AlphaEaseFC™ software (AlphaInnotech, San Leandro, CA).

Statistical analysis

The data distinctness between the two groups was analyzed using the Student's t test (SPSS19.0, $P < 0.05$ was considered to be statistically significant).

Results

Cervical endothelial cell characteristics

Primary cultured purified cervical endothelial cells, which were marked by anti-CD31 magnetic beads, showed characteristics including contact inhibition phenomenon, and typical cobblestone morphology (**Figure 1**). By using the immunofluorescence assay, carcinoma-associated (**Figure 2A**, **2B**) and radiation-derived (**Figure 2C**, **2D**) cervical endothelial cells showed the classical endothelial marker, vWF.

Gene alterations

Microarray assay was used to identify significant gene conversions (≥2 fold) in reaction to 400 cGy X-ray irradiation. A total of 63 genes were identified, and 46 genes were significantly and consistently changed. Of all the distinctly expressed genes, 26 genes were upregulated whilst 20 genes were down-regulated (**Tables 3** and **4**). Significant alterations (≥2 fold) included the regulation of cell cycle (CCND1), cell adhesion (SIGLEC1, MMP9, MMP1), the immune response (CAMP), chemokines (CCL2, CXCL1 and CXCL12), the inflammatory response (MMD, IL6 and IL18), growth factors (GDF15, NNMT), angiogenesis (THBS1), DNA synthesis and repair (GAPDH), protein synthesis (MRPS6).

Pathway analysis

MAS 3.0 was used to analyze this significant genetic data. **Table 5** displayed the interworking network of these gene-associated pathways. Chemokine signaling pathway, PI3K-Akt signaling pathway, and Cytokine-cytokine receptorwas the most important.

Table 3. Up-regulated genes in the carcinoma-associated endothelial cells after radiation

Gene	Accession number	Description	Function Average	Fold change
IL6	NM-000600	Human interleukin 6	Participation in a wide variety of inflammation associated disease states	6.9621
NNMT	NM-006169	Human nicotinamide N methyltransferase	Participating in nicotinamide metabolism	5.0247
GDF15	NM-004864	Growth differentiation factor 15	Growth differentiation factor	3.0671
MMP9	NM_004994	Matrix metallopeptidase 9 (gelatinase B,92 kDa gelatinase, 92 kDa type IV collagenase)	Breaking down extracellular matrix; leukocyte migration	7.3049
CXCL12	NM_199168	Chemokine (C-X-C motif)ligand 12, transcript variant 1	Regulation of hematopoietic cell trafficking and lymphoid-tissue architecture; associated with tumor metastasis	6.3402
GAPDH	NM_002046	Glyceraldehyde-3-phosphate dehydrogenase	DNA damage repair, histone transcription regulation,apoptosis, GAPDH phosphate kinase activity, catalytic functions	2.0173
PTEN	NM_031606	Phosphatase and tensin homolog deleted on chromosome 10	Cell growth, apoptosis and inhibition of proliferation and adhesion	6.0412
THBS1	NM_003246	Thrombospondin 1	Participation of platelet aggregation, angiogenesis, and tumorigenesis	3.0081
CCL2	NM_002982	Human chemokine (CC motif) ligand 2	Involved in immunoregulatory and inflammatory processes	2.0179
MAP17	NM_005764	A small 17 kDa non-glycosylated membrane protein	Increased oxidative stress, decrease in the tumorigenic capacity of carcinoma cells	8.1017

Table 4. Downregulated genes in the carcinomas-associated endothelial cells after radiation

Gene	Accession number	Description	Function	Fold change
CCND1	NM_053056	Human cyclin D1	Interacting with tumor suppressor protein Rb; altering cell cycle progression	0.1971
MMD	NM_012329	Monocyte to macrophage differentiation associated	Maturing macrophages	0.3741
CAMP	NM_004345	Cathelicidin anti-microbial peptide	Anti-microbial activity	0.2001
CXCL1	NM_001511	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	Chemokine	0.3621
MMP1	NM_002421	Matrix metallopeptidase 1 (interstitial collagenase)	Breakdown of extracellular matrix	0.3402
IL18	NM_001562	Homo sapiens interleukin 18 (interferongamma-inducing factor)	Pro-inflammatory cytokine	0.7621
MRPS6	NM_032476.2 NM_006933	Mitochondrial ribosomal protein S6	Participation of protein synthesis within the mitochondrion	0.3414
SIGLEC10	NM_033036.1	Sialic acid binding Ig-like lectin 10	Carbohydrate binding protein binding sialoadhesin	0.4176

Table 5. Significant gene-related pathways involved in radiosensitivity of endometrial cancer-associated endothelial cells

Pathway	Gene	*P*-value
Cytokine-cytokine receptor interaction	IL6, CCL2, CCL8, CXCL1, CXCL12, IL18, OSM	1.90×10^{-9}
Chemokine signaling pathway	CCL2, CXCL1, CXCL12	1.29×10^{-4}
NOD like receptor signaling pathway	CXCL1, CCL2	0.001931
Jak/STAT signaling pathway	CCND1	0.046731
Toll-like receptor signaling pathway	FOS	0.132941
ECM-receptor interaction	THBS1	0.024321
p53 signaling pathway	CCND1, IGF1, THBS1	0.047061
Focal adhesion	CCND1, IGF1, PDGFC, THBS1	0.074316
Toll-like receptor signaling pathway	FOS, IL6	0.100614
PI3K-Akt signaling Pathway	CCNE1; PDGFC; THBS1	0.047999

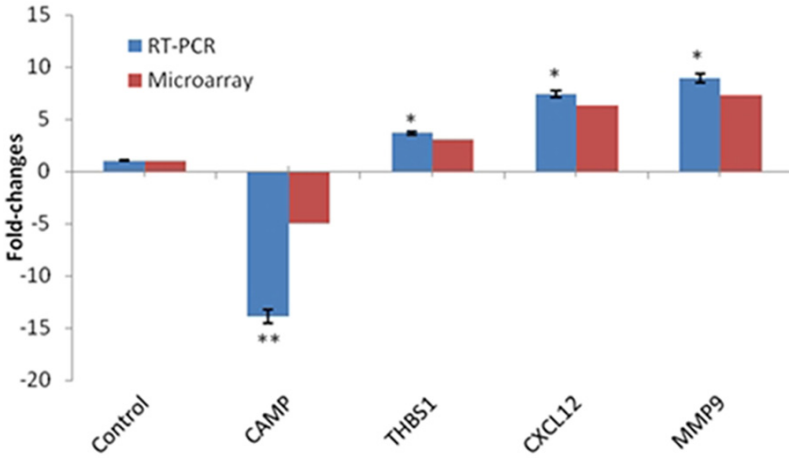

Figure 3. QRT-PCR confirmation of the microarray results. All qRT-PCR results were usually consistent with cDNA microarray result. QRT-PCR was done in triplicate and the ratio was calculated relative to the reference genes β-action. *P < 0.05 versus control; **P < 0.01 versus control.

Corroboration of microarray data using real-time PCR

Four significant changes of genes were randomly selected to confirm the reproducibility of the cDNA microarray analysis results. Up-regulated genes included CXCL12 (6.34 fold), MMP9 (7.30 fold) and THBS1 (3.00 fold), and down-regulated genes included CAMP (5.00 fold). For real-time PCR, each independent experiment was done at least three times; the end results are seen in **Figure 3.** The results of qPCR were observed to mutually agree with the microarray analysis and supported the reproducibility of the gene microarray data.

Corroboration of microarray data using Western blot

Western blot analysis showed higher CAMP, THBS1, CXCL12 and MMP9 protein in tumor-associated HEECs (**Figure 4**).

Discussion

Cervical cancer is the second most common cancer in women worldwide. Radiotherapy remains the mainstay of the treatment methods in cervical cancer, especially in advanced cervical cancer. Cervical tumors are often highly vascular and microvascular endothelial cell apoptosis ratio influences the efficacy of radiotherapy. Antiangiogenic activity can attenuate tumor progression. Hanahan et al demonstrated that the initial phase of tumor growth is characterized by avascular tumors that maintain a small diameter for a period of weeks until an angiogenic switch is activated and the tumors become vascular and begin to expand in size [5]. Treatment of such tumor with angiogenesis inhibitors blocked formation of these tumor colonies [6]. These results indicate that angiogenesis inhibition can lead to tumor regression and complete elimination of the tumor growth. Our team has researched on the gene expression conversions after X-ray radiation in human endometrial and ovarian cancer endothelial cells and obtained some findings for the combined treatment of antiangiogenesis and radiation therapy [2, 3]. Many researchers focused on the angiogenesis and tumor progression and the possible mechanisms in human cervical cancer [7, 8] but none in tumor microenvironment. This

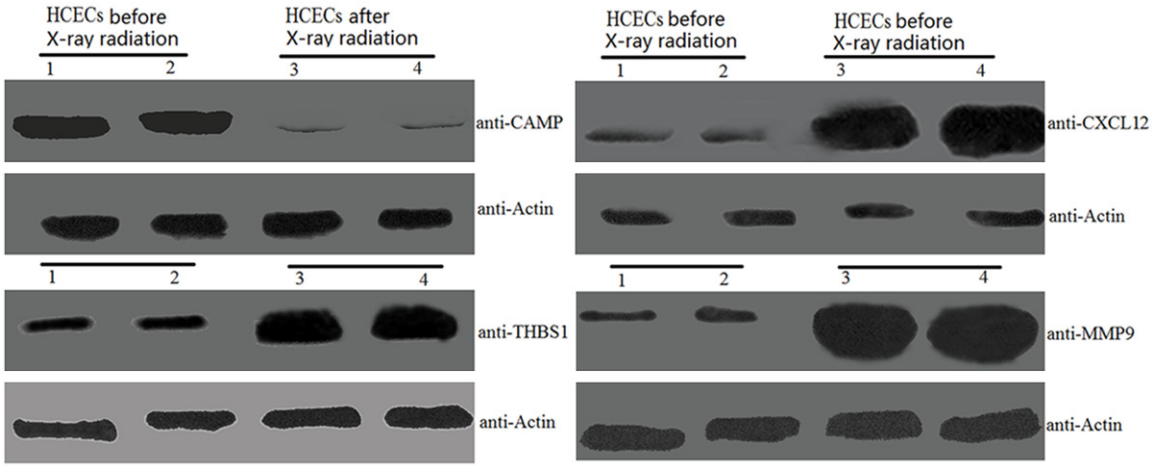

Figure 4. Western blot showing CAMP, THBS1, CXCL12 and MMP9 protein in HCECs before and 4 h after 400 cGy X-ray irradiation. Representative experiments are shown.

study would address the gene expression conversions after X-ray radiation in human cervical cancer vascular endothelial cells and provide novel treatment strategies. In our research, human cervical carcinoma-associated microvascular endothelial cells were extracted from six cervical epithelial cancers before and 4 h after 400 cGy X-ray irradiation, then cDNA microarray analysis and qPCR technology was used to identify gene alterations. Compared with the control group, 46 genes differentially expressed (≥2 fold) in primary cultured cervical microvascular endothelial cells. Some genes and gene clusters were presented in this research, which were associated with tumor angiogenesis, cell cycle regulation, inflammation and the immune response, cell growth, DNA synthesis and repair, chemokines, cell adhesion and so on. Above results provide an important theoretical basis for the combined treatment of antiangiogenesis and radiation therapy in human cervical carcinoma.

MAP17, which is a small (17-kDa) non-glycosylated membrane and Golgi apparatus protein, may decrease cell proliferation in vitro and tumor growth in vivo [9] and may play an important role in tumor biology [10]. MAP17 triggers a ROS-dependent and senescence-like response. Reactive oxygen species (ROS) generation in tumors and subsequent oxidative stress are at sub-lethal levels; further ROS increases might lead tumor cells to death [11-15]. Therefore, MAP17 could be a marker for increasing oxidative stress in tumor cells. MAP17 is overexpressed in many tumors of different origins. Overexpression MAP17 of tumor cells showed an increased tumoral phenotype associated with an increase in ROS. In addition, overexpression of MAP17 was further paralleled by a decrease in the tumorigenic capacity of carcinoma cells through reactive oxygen species (ROS) [12]. Guijarro MV et al found that MAP17 was expressed in 60% human mammary tumors while it was not expressed in normal or benign neoplasia. MAP17 level was strongly correlated with mammary tumoral progression and tumor stage [11]. Jaeger C's study demonstrated that MAP17 was expressed in keratinocytes and may be involved in epidermal physiology and pathology [16]. Furthermore, Guijarro and Leals data displayed at least in prostate and ovarian carcinomas, that overexpression of the protein strongly correlated with malignant progression [17]. Some data indicate that although MAP17 expression might bypass TNF (Tumor Necrosis Factor)-induced growth arrest, it is not the only determinant of this response [18]. Guijarro et al recorded that MAP17 protein activated phosphatidylinositol-3-kinase (AKT) through ROS, which can resist apoptosis induced by Myc in the absence of serum [19]. Current cervical carcinoma' treatment includes radiotherapy and brachytherapy plus cisplatin, which are ROS-inducing therapies [20, 21]. For cervical carcinomas, the percentage of overexpression in tumor samples is higher than 70%. Some researches indicated a high level of MAP17 was a good prognostic factor in cervical cancer treated with radiotherapy

and cisplatin [22]. In the present study, it was found that MAP17 showed high levels of expression in cervical cancer-derived endothelial cells following 400 cGy X-ray irradiation, compared with levels in control group (8.10 fold increases). This suggests that MAP17 was the main factor enhancing the curative effect of radiotherapy in cervical cancer-derived endothelial cells;however, further studies are needed about its clinical application in cervical endothelial cells.

Phosphatase and tensin homolog (PTEN) is a protein that, in humans, is encoded by the PTEN gene. The PTEN protein plays a key role in the regulation of the phosphatidylinositol-3-kinase (PI3K/AKT) signal conduction pathway, which, in turn, regulates proliferation, migration, and apoptosis [23]. The PI3K/AKT signaling pathway represents a major cell survival pathway and plays a critical role in oncogenesis and tumor cell growth [24]. PTEN is a negative factor for activation of AKT [25]. Functional studies demonstrate that PTEN is a highly effective tumor suppressor, but it is frequently mutated, deleted, or epigenetically silenced in various human cancers [26-29]. Since PTEN mutation and deletion can lead to abnormal AKT activation [30, 31]. Activation of AKT is resistant to irradiation therapy [32-36]. Upregulated PTEN expression suppressed AKT activity and increased radiation-induced apoptosis, resulting in enhancement of radiosensitivity in tumor cells [37]. A study investigated that PTEN expression in glioma cells had an effect on cell proliferation, cell cycle, sensitivity to ionizing radiation [38]. Zhang X et al detected that upregulated PTEN enhanced radiosensitivity of colorectal carcinoma cells [39]. Kim EJ's study showed that PTEN knockdown acquired radioresistance in non-small-cell lung cancer cells [40]. Lu D's result showed that PTEN expression correlated with incidence and progression of uterine cervical cancer [41]. Radiation therapy continues to be one of the most important treatment methods in advanced stages of cervical cancer. Local recurrence after radiation therapy is a pattern of treatment failure attributable to radioresistance of cancer cells. PTEN (6.04-fold) presented high expression state persistently in our present study and this implied that PTEN may be associated with radiation sensitivity of cervical carcinoma-derived endothelial cells. However, the specific mechanism linking radiosusceptibility has not been elucidated in cervical cancer.

Radiotherapy acts on malignant tumors by a cytotoxic effect, however, low-dose radiation may lead to neovascularization [42]. The chemokine significantly impacted carcinoma-associated vasculogenesis, carcinoma growth and metastasis. These chemokine-associated genes included CXCL1, CXCL12 and CCL2, which also significantly changed in our result. Study [43] showed that head and neck carcinomas cells had a continued upregulation pattern of CXCL1 and CXCL12 after X-ray irradiation. Finding from Chen M et al [44] presented that a single-dose whole-body γ-ray irradiation (8 Gy) activated the neuronal retina via upregulated expression of chemokines (CCL2 and CXCL12). Many researches [45-47] held that the radiation upregulated chemokines expression. Kryczek et al [48] demonstrated that inhibition of the CXCL12 axis may inhibit human spongioblastoma regrowth after radiotherapy. The results provide an important experimental basis for combining anti-vasculogenesis with radiation therapy in human cervical cancer. Nevertheless, CXCL1, CCL2 and CXCL12 expression as well as their relevant mechanisms in cervical carcinoma angiogenesis and radiotherapy is still rarely unknown.

MMPs play a crucial role in carcinoma vasculogenesis and tumor progression, at the same time, are modulators of the tumor microenvironment. MMP-9 overexpressed in cervical cancer-derived endothelial cells after radiation, and was closely associated with cervical cancer invasion, metastasis and vasculogenesis [49]. The decrease of MMP-9 expression was connected with reduced angiogenesis in radiotherapy for human nasopharyngeal carcinoma in mice [50]. In our study, MMP-9 showed high expression (7.30 fold) in cervical carcinoma-associated endothelial cells following irradiation. Finding from Rajput S and Kumar BN demonstrated that MMP2 was upregulated after radiotherapy, and thymoquinone pre-sensitization can recover the expression of the proteins back to control proteins expression [51]. Zhang W and Liu Y found S100A4 potently promoted Hep-2 invasion by increasing cell motility and MMP-9 production [52]. Therefore, MMPs inhibitor may be a new therapeutic target for cervical cancer.

THBS1 is the target gene of thrombospondin-1 (TSP1). TSP1 can inhibit angiogenesis in the tumor microenvironment [53] and plays a role in the regulation of tumor growth and metastasis [54]. TSP1 may inhibit cutaneous melanoma progression by suppressing tumor vessel formation [55]. Rofstad et al [56] showed that TSP1 could prevent a primary tumor from distantly disseminating micro-metastasis after radiotherapy, and improve the radiation sensitivity.Treatment with exogenous TSP1 before radiotherapy enhanced the antitumor effect of radiotherapy. The radiopotentiation TSP1 involved at least two distinctly different mechanisms: reducing the fraction of radiobiologically hypoxic parenchymal tumor cells and increasing the radiation sensitivity of the tumor microvasculature by promoting radiation-induced endothelial cell apoptosis [56]. In addition, Maxhimer et al [57] showed that blocking the TSP1 interactions may protect the surrounding tissue from radiolesion, at the same time, improve tumor radiosensitivity. In this study, THBS1 showed high expression in cervical carcinoma-associated endothelial cells following irradiation. This also confirmed that THBS1 was associated with enhancing the function of radiotherapy in cervical carcinoma-associated endothelial cells and its specific mechanisms need to be further elucidated.

In our study, we researched on the relevant signaling pathways involved in tumor angiogenesis, cell cycle regulation, inflammation and the immune response, DNA synthesis and repair, chemokines and cell adhesion, including the cytokine-cytokine receptor interaction, the chemokine signaling pathway and the p53 signaling pathway. Our data displayed that these pathways may be relevant to the change of cervical cancer-derived endothelial cells following radiotherapy. However, the functions of these pathways require further investigate in cervical cancer radiotherapy.

In conclusion, agrowing body of evidence has established that antiangiogenic treatment strategies offer a number of compelling advantages over conventional cancer therapies and have greater practical significance to control the tumor growth and metastasis. Our study has identified genes alterationsby 400 cGy X-ray radiation in human cervical cancer-derived endothelial cells. Radiation-induced conversional genes and gene-related pathways in microvascular endothelial cells may provide the theoretical basis for the combined treatment of anti-angiogenesis and radiation therapy in human cervical carcinoma. We recognize the limitations of our study. Our study is a kind of screening rather than detailed research. We need further investigation to define these identified genes in vitro and in vivo.

Disclosureof conflict of interest

None.

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