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Evaluation of the Effect of Radiotherapy on CCL5/miR-214 -3p/MALAT1 Genes Expression in Blood Samples of Breast Cancer Patients

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ABSTRACT

Current cancer therapies include chemotherapy, radiation therapy, immunotherapy, and surgery. Despite these treatment methods, a major point in cancer treatment is early detection. RNAs (mRNA, miRNAs, and LncRNA) can be used as markers to improve cancer diagnosis and treatment. This research examined how radiotherapy affected *CCL5, miR-214, and MALAT-1* gene expression in the immune pathway in peripheral blood samples from radiation therapy-treated breast cancer patients. Before and after radiotherapy, peripheral blood was collected from 15 patients in four steps. Blood samples were collected in an outpatient facility from 20 healthy female volunteers with no history of malignant or inflammatory conditions. RNA was extracted from the blood samples and cDNA was synthesized. *CCL5, miR-214*, and *MALAT-1* gene expression were determined by real-time polymerase chain reaction (RT-PCR). *CCL5* protein levels in the serum were determined in 80 samples (60 BC and 20 healthy controls) using Quantikine Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D Systems). The data were then statistically evaluated. There was a significant difference between *CCL5* levels in tumoral and adjacent normal blood samples ($p < 0.05$). The results also show that the level of gene expression and serum concentration of *CCL5* protein in different phases of radiotherapy is significantly different. On the other hand, the expression level of the *miR-214* gene was significantly decreased in patients compared to the control group, but this decrease was not significant for the *MALAT-1* gene ($p< 0.05$). Also, after each stage of radiotherapy, the expression level of these two genes showed a decrease, but in the fourth week after radiotherapy, this decrease was significant ($p< 0.05$). Radiotherapy is associated with a decrease in the expression of *miR-214* and *MALAT-1*, as a result, an increase in the expression of *CCL5*. An increase in the concentration of *CCL5* protein is accompanied by an increase in the level of monocytes, which ultimately causes the infiltration of macrophages and can ultimately cause cancer recurrence. It is suggested that these genes can probably be used as diagnostic and therapeutic radiotherapy markers in breast cancer.

Keywords: Breast cancer, radiotherapy, ELISA, *miR-214, CCL5, MALAT-1*

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Introduction

Inflammation is a characteristic of cancer that promotes the growth of the tumor. Both acute and chronic inflammation processes have been indicated to significantly affect the development of cancer. Recent findings on inflammation have demonstrated a link between inflammatory processes and the neoplastic transformation, tumor growth, and metastatic development, as well as the occurrence of recurrences. In particular, inflammatory processes mediated by the tumor microenvironment play a role in the progression of breast cancer (BC) (1).

C-C motif ligand 5 (*CCL5*) or regulated upon activation, normal T-cell expressed and secreted (RANTES) is a chemokine associated with aggressive BC (2). *CCL5* is a chemotactic cytokine extensively secreted by natural killer cells, epithelial cells, T cells, fibroblasts, and platelets (3). It is also secreted by specific tumor cells, including malignant melanoma (4), ovarian cancer (5), prostate cancer (6), and BC cells and, assists in the absorption and stimulation of these cells. In normal breast epithelium cells, the *CCL5* expression is lower, but in the primary breast tumor site, metastasis site, and local lymph nodes, it is higher (7). Therefore, the expression of *CCL5* during the process of malignant transformation of BC is necessary. Moreover, chemokine levels have a negative and positive correlation with patient prognosis and the density of tumor-associated macrophages, respectively. Hence, inhibition of chemokine receptors or their production could prevent the recruitment of macrophages by tumor cells (7).

Long noncoding RNAs (LncRNAs) are transcribed RNA molecules (greater than 200 nucleotides in length) that regulate various biological functions either directly or indirectly (8). Many lncRNAs play a role in human diseases and cancers by inducing cell cycle progression, invasion, and metastasis (9). Metastasis-associated lung adenocarcinoma transcript-1 (*MALAT-1*) is a preserved and well-characterized lncRNA responsible for various biological processes via a variety of mechanisms (10). Under hypoxia conditions, it is also important in inflammation, angiogenesis, and metastasis (11).

MALAT-1 expression has been suggested to enhance in the liver, cervix, BC, colorectal, renal, prostate, gastric, and other cancers. *MALAT-1* can mediate tumor cell proliferation, tumorigenesis, autophagy, metastasis, and drug-resistant phenotypes in cancer cells by targeting multiple transcription and growth factors, epigenetic changes, and hormones. However, the link between elevated *MALAT-1* expression levels and the inflammatory microenvironment in cancer is an issue that needs further investigation (12).

MicroRNA (miRNA) is another type of endogenous non-coding single-stranded RNA studied extensively as a circulating marker (13). These RNAs can down-regulate approximately one-third of genes by binding to the 3′-untranslated region of target messenger RNA (mRNA) and play a role in a wide range of biological processes such as cell proliferation, apoptosis, migration, and carcinogenesis. (14). Many miRNAs are also involved in BC carcinogenesis and development (15). miRNAs regulate mRNA expression by degrading or silencing these molecules, and lncRNAs activate and repress genes via transcriptional or post-translational mechanisms. miRNAs collaborate with lncRNAs to influence the transcriptome (16, 17).

The majority of miRNAs are expressed differentially in BC. *miR-214-3p* can hinder tumor growth and metastasis by inhibiting the expression of the leucine zipper tumor suppressor. While *miR-214-3p* decreases in BC, its mechanism of action and expression in different stages of radiotherapy are unknown. This mRNA is down-regulated in various human cancers, including BC, cervical pancreatic, and hepatocellular cancers,

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as well as rhabdomyosarcoma (18). It also impacts the radiotherapy response of non-small cell lung carcinoma (NSCLC) cells via the regulation of p38MAPK, apoptosis, and senescence. However, the mechanism and function of *miR-214-3p* in radiotherapy in BC are still obscure (19).

Radiotherapy is an essential and integral part of BC treatment. Based on two large meta-analyses, adjuvant radiotherapy declines local recurrence and ameliorates survival after breast-conserving surgery and mastectomy (20, 21). However, BC is growingly considered a heterogeneous disease, and radiotherapy cannot assist all patients in terms of survival and nor is the absolute advantage equal across risk groups (22). Even though local recurrence rates in BC reduces with advancements such as screening, pathognomonic examination, and modern medical therapy, identification of patients who may not take advantage of radiation therapy and avoid the morbidity and burden of adjuvant radiotherapy is crucial (23). As a beneficial tool, gene expression could allow clinicians to perform therapies considering the molecular characteristics of each tumor (24).

Materials and methods

Collection of blood samples

Only BC patients with no previous chemo- or radiotherapy were enrolled in the study. Following, five mL of peripheral blood was collected from 15 BC patients referred to Imam Khomeini Hospital in Tehran, Iran (2020-2021) before, and after the first, second, and fourth weeks of completion of their radiation therapy. Radiation therapy was administered to all patients using an Elekta Precise linear accelerator at a regular daily dose of 1.8 Gy (dose per fraction: 1.8 Gy). In treatment phases I, II, and III, the radiation treatment dose was 9, 9, and 18 Gy (36 Gy in the fourth week) for all patients (Table 1). Five days per week, radiation therapy was administered using a 3D- conformal protocol with 18 MV photon beams. Furthermore, no specific medication was prescribed to any of the patients during radiotherapy. Blood samples were collected in an outpatient facility from 20 healthy female volunteers with no history of malignant or inflammatory conditions. In addition, 3 mL of blood samples were collected in Vacutainer Serum Separator Tubes II, allowed to clot for 30 minutes, and centrifuged at 2000 rpm for 4 minutes. After that, the serum is stored at -80 °C until the assay. Tarbiat Modares University Ethics Committee approved the study based on a code of ethics (IR.MODARES.REC.1400.232).

Table 1. Details of radiotherapy doses in this study.

Phases of treatment	Number of sessions	Dose per fraction (Gy)	Dose in per phase (Gy)
Phase I	5	1.8	5*1.8= 9
Phase II	5	1.8	5*1.8= 9
Phase III	10	1.8	10*1.8=18
Total	20	1.8	20*1.8=36

RNA extraction

Total RNA was extracted from whole blood using the EZ-RNA Reagent (BioBasic, Canada) as directed by the manufacturer. RNA was extracted and stored at − 80 °C in 30 µL of RNase-free water. It should be noted that for all processes, the total RNA procedure was followed. The 260/280 and 260/230 absorbance ratios of all RNA samples were used to assess RNA purity using a Nanodrop (Thermo Fisher Scientific). For

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RNA, a 260/280 ratio of 2.0 and a 260/230 ratio of 2.0-2.2 were considered "pure".

Reverse transcription and quantitative real-time PCR (qRT-PCR)

M-MuLV enzyme (Sinaclone, Iran), random hexamers, and stem-loop primers were used to synthesize cDNAs according to the manufacturer's instructions. The StepOne Real-Time PCR system (Applied Biosystems) was applied to perform RT-qPCR with specific primers (Table 2). B2M and C/D box 48 small nucleolar RNA were used as internal controls to normalize mRNA and miRNA expression. To assess fold changes in cDNA, the relative quantification method ($2^{-\Delta\Delta Ct}$) was used. A PCR assay was conducted for each sample with two duplicates and repeated twice.

Table 2. Primer sequences.

Gene	Primer sequences	Tm(°C)
CCL5	F: 5′-GCTGTCATCCTCATTGCTACTG	62
	R: 5′-TGGTGTAGAAATACTCCTTGATGTG	63
MALAT-1	F: 5′- GAATTGCGTCATTTAAAGCCTAGTT	59
	R: 5′- GTTTCATCCTACCACTCCCAATTAAT	59
B2M	F: 5′- CCACTGAAAAAGATGAGTATGCCT	59
	R: 5′- CCAATCCAAATGCGGCATCTTCA	61
F-mir-214-3p	5′- ACACTCCAGCTGGGACAGCAGGCACAGACA	60
miR-214-3p STL	5′- CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGCCTG	61
F-U48	5′- GAGTGATGATGACCCCAGGTAA	62
U48 STL	5′-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACGGTCAG	61

Complete blood counts with differentials

Complete blood counts with differential measurements were made in normal and BC patients before and after a basic course of external beam radiation therapy. Clinical laboratory tests (complete blood count) were performed for participants at Imam Khomeini Hospital's Department of Laboratory Medicine. Within 4 hours of the blood draw, all samples were hand-delivered.

Chemokine detection in Serum Samples

Blood samples were collected from participants at four different times: 72 hours before the start of RT, one week after RT was completed, and two and four weeks after RT was completed. Also, blood samples were collected from normal women who did not have any diseases related to immunity and cancer. To collect serum samples, we followed our standard procedures: A 3 mL SSTII (serum separator tube) blood sampling tube was used to collect serum. The blood sampling tubes were centrifuged (1300 ×g, 10 minutes at 4°C) within 1 hour. The serum was then isolated and immediately frozen in aliquots at -20°C. The samples were kept at -80°C for an extended period. Circulating levels of *CCL5* were measured using Quantikine Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D Systems) in 80 samples (15 BC in four steps, 20 healthy controls). The Quantikine Human *CCL5*/*RANTES* ELISA kit (R&D Systems) was used to perform ELISA to determine circulating *CCL5* levels. At 450 nm, absorbance was measured. To correct plate imperfections, readings at 570 nm were subtracted from those at 450 nm. Every sample was evaluated twice. On each microplate, a new standard curve was created by diluting a known concentration standard. Using a logistic curve-fitting algorithm, the mean absorbance for the duplicate wells was applied to calculate the chemokine concentration for each sample. All absorbance values were within the linear portion of the standard curve

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after suitable dilution. Concentration levels read from the standard curve were multiplied by the dilution factor.

Statistical analysis

The results were reported in the form of descriptive statistics indicators in the form of numbers (percentage) for qualitative variables and means ± standard deviation (SD) for quantitative variables. To compare the expression of genes between two groups of patients and healthy, an independent t-test was used, and between two groups of control and patients over time, an analysis of variance of repeated measures (Repeated Measure ANOVA) was used. For data analysis, SPSS Version 19 software was used. A significant level was defined as a probability value of less than 5% ($p< 0.05$).

Results

Of 21 women screened for this study, 16 met our diagnostic and temporal inclusion criteria. Of these, 16 study subjects enrolled, 15 completed the pre-RT baseline and the 3-week post-RT observational study and 40.48 ± 7.3 years, and that of the control groups was 42.21±6.2. Most had estrogen (86.7 %) or progesterone (80.0) receptor-positive breast tumors, 86.7% had invasive ductal carcinoma, and 13.3% also had ductal carcinoma in situ and Invasive lobular carcinoma. *HER2/neu* overexpression was present in 33.3% of the participants' biopsied tissue samples. Patient clinicopathological details are shown in table 3.

Table 3. Patient tumor sample characteristics.

Characteristic	Detail	Frequency	Percentage
Type of breast	l/r	1	6.7
	left	5	33.33
	right	9	60.0
Estrogen receptors (ERs)	-	2	13.3
	+	13	86.7
progesterone receptor (PRs)	-	3	20.0
	+	12	80.0
HER2/Neu receptor	-	10	66.7
	+	5	33.3
Grade	1--3	1	6.7
	2	6	40.0
	2--3	3	20.0
	3	5	33.3
Sze	<=2 cm	4	26.7
	2-4 cm	7	46.7
	>4 cm	4	26.7
Stage	IA	2	13.3
	IIA	4	26.7
	IIB	3	20.0
	IIIA	5	33.3
	IIIC	1	6.7
Type	DCIS	1	6.7
	IDC	13	86.7
	ILC	1	6.7

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Analysis of gene expression

The peripheral blood sample was obtained from each of the 15 radiotherapy-treated BC patients before, and one, three, and four weeks after the start of treatment. Gene expression analysis was performed on the corresponding blood samples from BC patients and controls. The results were normalized to endogenous control genes and expressed in relative quantity. Expression of *CCL5* was significantly elevated in BC patients compared to controls ($p<0.05$, Figure 1A). But on the contrary, *miR-214-3p* expression was significantly decreased in tumor samples compared to normal (Fig. 1B). Also, no significant difference was found between *MALAT-1* gene expression in both groups (Figure 1C).

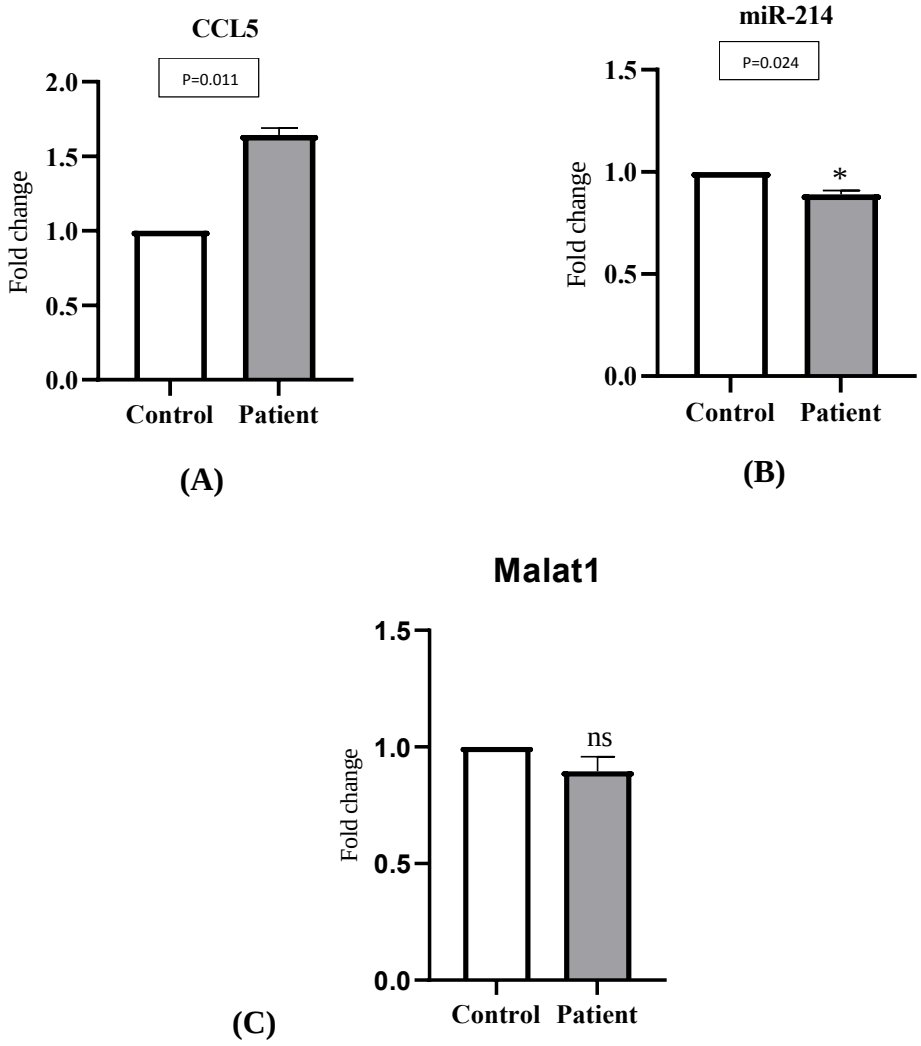

Fig.1. Expression of *CCL5, miR-214, and MALAT-1,* in breast cancer patients and controls. GraphPad Prism, independent T-test method**.** Expression of *CCL5* was significantly elevated in BC patients compared to controls ($p = 0.011$). But on the contrary, *miR-214-3p* expression was significantly decreased in tumor samples compared to normal ($p =0.024$). Also, no significant difference was found between *MALAT-1* gene expression in both groups ($p = 0.073$).

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CCL5, miR-214, and *MALAT-1* expression in samples from radiotherapy-treated BC patients

The level of *CCL5* expression has shown a significant increase in all steps of radiotherapy, so this increase in expression was higher in the fourth week after the start of radiotherapy compared to the previous weeks. Unlike *CCL5*, the expression level of *miR-214-3p* had a decreasing trend, which was not significant for the first week, but its expression level decreased significantly in the second and fourth weeks. Also, there was no significant difference in the expression of *MALAT-1* before radiotherapy and in the first and second weeks after radiotherapy, but its expression increased significantly in the fourth week after radiotherapy (Figure 2).

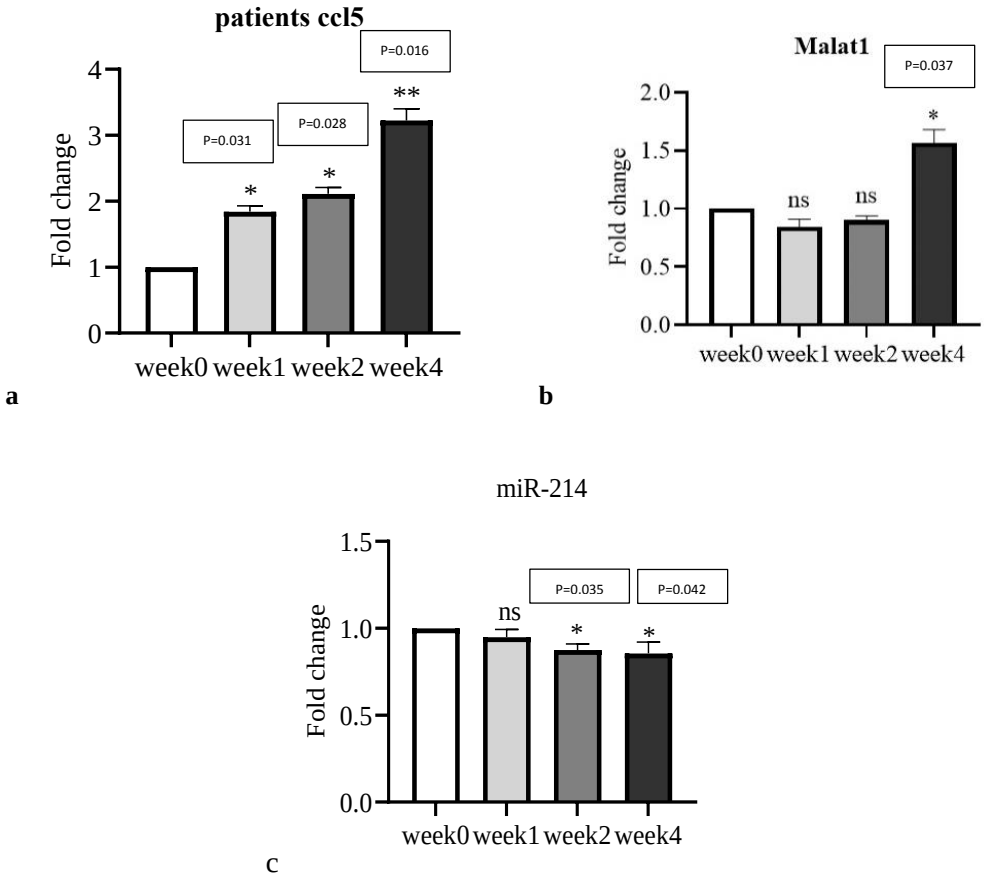

Fig.2. Fold change in expression of the genes *CCL5, miR-214, and* MALAT-1, before, and 1, 2, and 4 weeks after radiotherapy. GraphPad Prism, Paired T-test method. The level of CCL5 gene expression has increased over time with increasing radiation dose **(a)**. The expression level of MALAT-1in the first and second weeks after radiotherapy (after receiving 9 gy and 18 gy of radiation) has no significant difference compared to before the start of radiotherapy, but in the fourth week after radiotherapy (after receiving 36 gy of radiation), the expression level of MALAT-1 has had a significant increased **(b)**. The level of miR-214 gene expression in the first week after radiotherapy was not significantly different from that before the treatment, but in the second and fourth weeks, when the patients received 18 and 36 gy of radiation, respectively, the gene expression was significantly lower than before the start treatment **(c)**.

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CCL5 protein concentration

ELISA was used to assess the level of *CCL5* protein in the supernatant of normal adjacent and tumoral blood samples from BC patients. Two biological replicates were used for each case and control sample. Figure 3 shows the *CCL5* protein level in breast tumors and normal. There was a significant difference detected in *CCL5* serum levels of BC patients (Mean (SEM) of 80.68 (17.68) ng/mL) compared to healthy controls (49.04 (10.34) ng/mL). Also, as seen in figure 4, the concentration of *CCL5* protein has increased in each stage of radiotherapy in BC patients.

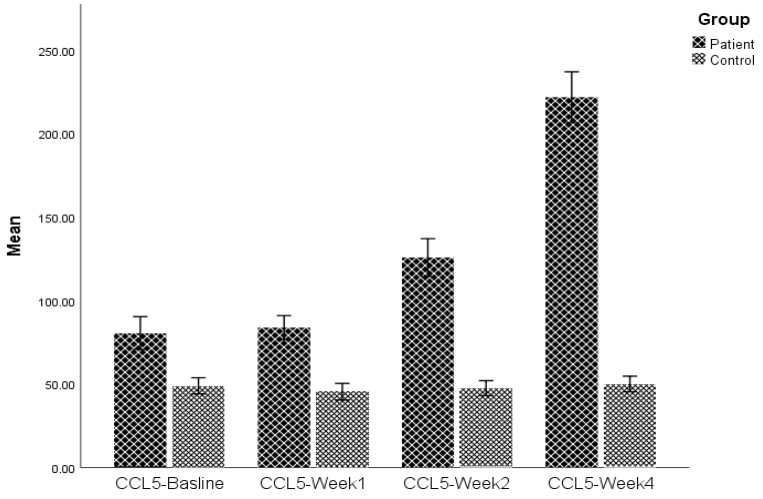

Fig.3. CCL5 chemokine level. CCL5 levels were evaluated by ELISA in the supernatant of normal adjacent and tumoral blood samples from BC patients. Each case and control sample were assayed in two biological replicates. The supernatant of the adjacent had a mean concentration of 49.04 pg/mL and the supernatant of the tumor had a mean concentration of 80.68 pg/mL. The mean concentration before radiotherapy: 80.68 pg/mL 83.87 pg/mL in Stage I, 125.87 pg/mL in Stage II, and 221.93 pg/mL in Stage III.

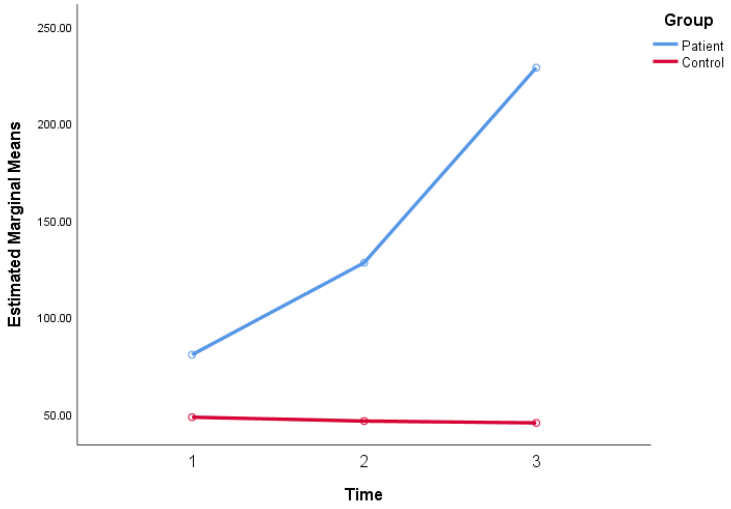

Fig.4. Changes in CCL5 chemokine levels in the patient and control groups studied four times.

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Changes in White Blood Cell and Differential Counts Associated with RT

Based on the results of the independent T-test, it was observed that the difference between the mean neutrophils in the fourth week between the patient and the control was statistically significant (p-value = 0.003). Also, the difference in the average level of monocytes in the first, second, and fourth weeks between the patient and control groups was statistically significant, so the average of these blood cells in the patient group was higher than the healthy group ($p<0.05$). Also, in the group of patients, after each stage of radiotherapy, the average level of monocytes increased significantly compared to the previous stage, but on the contrary, the increase in the level of lymphocytes, neutrophils, and eosinophils after each stage of radiotherapy was not significant (Figure 5).

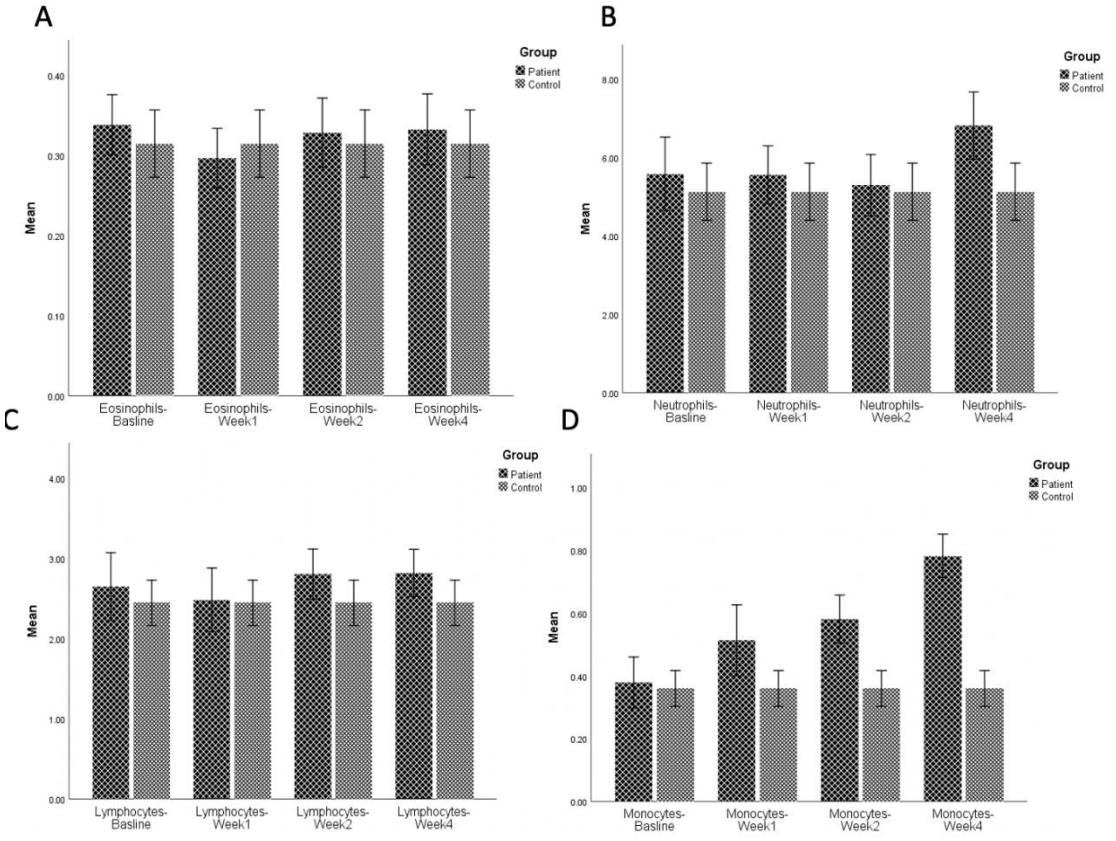

Fig.5. Pre- and post-radiotherapy white blood cell and differential count for eosinophils (A), neutrophils (B), lymphocytes (C), and monocytes (D), in breast cancer patients (n = 15) in four steps before and after radiotherapy and control (n=20).

Association of CCL5 chemokine level and white blood cell changes during treatment with RT

Table 4 shows the results of investigating the effect of increasing *CCL5* protein on the level of changes in white blood cells using a mixed-effects model. The results in this study showed that with the increase of *CCL5* protein during the treatment period, the levels of eosinophils, neutrophils, lymphocytes, and monocytes increased by 0.00004, 0.00749, 0.00147, and 0.00228 respectively, and only the changes of monocyte cells were statistically significant ($p<0.001$) (Table 4).

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Table 4. Association of CCL5 chemokine and White blood cell changes.

Parameter	Estimate	Std. Error	95 % CI	t	P-value
Eosinophils	0.00004	0.00016	(-0.00028 – 0.00036)	0.239	0.812
Neutrophils	0.00749	0.00326	(0.00095 – 0.01402)	2.294	0.025
Lymphocytes	0.00147	0.00139	(-0.00131 - 0.00425)	1.060	0.293
Monocytes	0.00228	0.00034	(0.0016 - 0.00297)	6.644	<0.001

Based on the regression analysis results, it can be seen that only the effect of *CCL5* protein on monocytes in the fourth week is statistically significant (p=0.031), so with an increase of one unit in the amount of *CCL5* protein, the level of monocytes increases by 0.003. The effect of the *CCL5* protein on other studied blood cells was not statistically significant (Table 5).

Table 5. Association of CCL5 chemokine and White blood cell changes during different weeks of treatment based on regression analysis.

Cell type	**time**	**B**	**Std. Error**	**t-value**	**P-value**
Eosinophils	Baseline	0.002	0.001	1.856	0.086
	Week1	-0.001	0.001	-0.764	0.459
	Week2	-0.002	0.001	-1.958	0.072
	Week4	-0.001	0.001	-0.704	0.494
Neutrophils	Baseline	-0.011	0.026	-0.417	0.684
	Week1	-0.003	0.028	-0.089	0.931
	Week2	0.013	0.019	0.690	0.503
	Week4	-0.014	0.015	-0.936	0.366
Lymphocytes	Baseline	0.004	0.012	0.328	0.748
	Week1	0.002	0.015	0.124	0.904
	Week2	-0.007	0.007	-1.005	0.333
	Week4	0.000	0.005	-0.029	0.978
Monocytes	Baseline	-0.001	0.002	-0.628	0.541
	Week1	0.006	0.004	1.355	0.199
	Week2	-0.001	0.002	-0.627	0.542
	Week4	0.003	0.001	2.414	0.031

Discussion

The microenvironment of a tumor is strongly correlated with cancer development, and the cells surrounding a tumor are necessary for tumor progression. *CCL5* is an essential protein that contributes to chronic inflammatory disorders and malignancies via the recruitment of inflammatory cells (25). *CCL5* is overexpressed in various cancer types and involved in different essential steps of cancer spread, i.e. proliferation, migration, invasion, angiogenesis, and metastatic colonization, after activating the CC chemokine receptors 1, 3, and 5 (26). In addition to cancer growth and progression, *CCL5* is associated with resistance to common treatments such as radiotherapy and conventional chemotherapy drugs (e.g. cisplatin

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and tamoxifen) (27). The elevated levels of *CCL5* have been found to be a biomarker for cancer and also for predicting prognosis and improvement of therapeutic approaches (28).

Based on the results of this study, a significant difference was detected in circulating the protein levels of *CCL5* between BC patients and healthy controls, but this difference was insignificant in Hartmann et al.'s investigation (29). The present study also discovered that the concentration of *CCL5* was higher in the tumor than in normal tissue, which supports the findings of Niwa et al. and Eissa et al. (30, 31). In our study, the *CCL5* expression level and protein concentration increased in each stage of radiotherapy, but the expression levels of *MiR-214-3p* decreased.

CCL5 is a key chemotactic factor for macrophages in the microenvironment of tumors (32). While the exact role of *CCL5* in tumor biology is unclear, this cytokine is known to be important for triggering and amplifying the antitumor host response and also inducing appropriate immune responses against tumors (33). *CCL5* expression by BC causes the migration of monocyte to the tumor site, increased production and recruitment of proinflammatory cytokines, and recruitment and activation of inflammatory cells, which could contribute to metastasis formation (34). Macrophages abundantly exist in the tumor microenvironment. Although effective in decreasing primary tumors, radiotherapy may enhance the infiltration of macrophages into tumor sites, thereby accelerating tumor progression (35, 36).

In our study, a significant correlation was observed between the *CCL5* concentration and blood monocyte level in patients with BC. Moreover, with the elevation of CCL5 concentration and the received doses of radiotherapy, the level of blood monocytes was enhanced in patients. In cancer cells, RT increased the 12-LOX expression levels. Furthermore, the expression of *CCL5* elevated via the AKT/NF-B pathway. *CCL5* enhances the recruitment of macrophages to tumor tissues and also their polarization to the immunosuppressive M2 subtype, thereby facilitating metastasis (37). Radiation also increases the migration of macrophages in NSCLC models. This behavior is caused by *CCL5* because its inhibition remarkedly lessens the number of migrated macrophages. Further analysis has demonstrated the overexpression of *CCL5* by IL-6, which is prevention-impaired macrophage migration (38). Evidence has also indicated that the expression of *CCL5* is much higher in parents than in radioresistant tumor tissue. This finding highlights the function of *CCL5* in $CD8^+$ T cells and macrophage infiltration (39). Walens and associates implied that macrophages provide residual BC cells in mice with the protein collagen that they need to grow. In the experiments, mice with HER2-positive BC were given targeted cancer therapy. Following treatment, *CCL5* levels increased and remained high in residual tumors in mice (40).

CCL5 levels were also found to be high in residual BC tumors in women, suggesting that high levels of this protein may reduce the period between tumor treatment and relapse, as *CCL5* tends to attract macrophages, which deposit collagen in residual tumor tissues. Collagen helps promote tumor growth because recurrent tumors enhance collagen levels, and BC patients with high levels of collagen in their tumors typically have poor outcomes. Therapeutic interventions that inhibit the *CCL5* release or macrophages from supplying collagen to residual tumor cells could contribute to the prevention of recurrence. Therefore, the increased expression levels of *CCL5* may result in recurrence following treatment in BC patients. As a result, based on our findings, radiotherapy can elevate the *CCL5* expression and also the number of

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monocytes. The outcome is the infiltration of macrophages. Of note, when considering radiotherapy or other therapies associated with increased *CCL5* levels, cancer recurrence should be taken into account.

MiRNAs, in addition to being diagnostic biomarkers, can be used as biomarkers for the treatment of BC and its radiation resistance. An earlier study also indicated that miRNAs can be used as biomarkers for the prediction of treatment responses (41). By suppressing target genes or degrading mRNA, miRNAs take a key role in macrophage infiltration, cell proliferation, differentiation, apoptosis, and other biological functions in normal cells. Differentially expressed miRNAs may interfere with the proliferation and differentiation of normal cells, which often results in the development of various cancers, as well as cardiovascular and immune disorders. Alterations in miRNA expression have been denoted to have a crucial role in tumorigenesis and development. *miR-214-3p* induced apoptosis in BC cells by targeting and sensitizing the *RFWD2-p53* cascade to doxorubicin. The *miR-214-3p* can also contribute to the development of BC by targeting *CCL5*. Analyzing some samples of early oral squamous cell carcinoma has reflected the significant role of *miR-214-3p* in prognosis. In general, miRNAs play an essential role in the malignant proliferation of BC and may have a role in the success of BC radiation therapy (42, 43).

In the present study, we observed the down regulation of *miR-214-3p* in BC patients. We also noticed a decreased expression level of *miR-214-3p* with each stage of radiotherapy and an increased level of *CCL5* gene expression. Liu et al. reported that *miR-214-3p* could suppress breast tumors. Therefore, it could be concluded that *miR-214-3p* suppresses BC, as well (44). In our analysis, we found that the *miR-214-3p* expression level was lower in BC patients than in the control group, which indicates the tumor suppressor role of *miR-214-3p*. Gu et al. have stated that the overexpression of *miR-214-3p* is considerably associated with different cancers (45). Based on bioinformatics studies, *CCL5* is a target for *miR-214-3p*; therefore, reduction of *miR-214-3p* expression can elevate the level of *CCL5*, which could be a *miR-214-3p* downstream target gene. *miR-214-3p* down regulation in BC cells increased cell proliferation but prevented apoptosis, which suggests that the down-regulation of *miR-214-3p* can contribute to BC development. In this study, we observed that the *miR-214-3p* expression decreased over time with radiotherapy, while the *CCL5* expression increased.

Overexpression of the lncRNA *MALAT-1* is a characteristic of human oncogenesis. *MALAT-1* promotes tumor metastasis, the formation of solid tumors, and the development of hematologic malignancies (46) and is involved in multiple human cancers, including NSCLC, hepatocellular carcinoma, and bladder cancer (47, 48). Our study explored that *MALAT-1* expression is increased in BC, which supports the findings of Lin et al. and Ying et al. (47, 48) but contradicts those of Kim et al. (49). *MALAT-1* overexpression blocks and *MALAT-1* deficiency induces BC metastasis; the effect of *MALAT-1* deficiency can be reversed by reintroducing this lncRNA. The abundance and aberrant expression of *MALAT-1* in many cancers display its role in cancer development (50). Based on MTT and Transwell assays, *MALAT-1* promotes proliferation and migration in LoVo and HCT116 cells (51, 52).

Xu et al. (53) have reported that *MALAT-1* has a significant biological motif located at the 3′ ends of *MALAT-1* (6918–8441 nt), and this motif has a crucial role in the biological processes of cell proliferation, migration, and invasion. Guo et al. have revealed the involvement of *MALAT-1* in cervical tumorigenesis, cell cycle progression, and invasion through regulating gene expression of caspase-3, -8, *Bax*, *Bcl-2*, and *Bcl-*

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xL (54). The findings of our study exhibited a higher expression of *MALAT-1* in BC patients than in normal women, which affirms previous research and emphasizes the function of *MALAT-1* in BC. Our findings also disclosed that *MALAT-1* expression increased over time with radiotherapy. While *MALAT-1* expression can have a role in metastasis development, radiotherapy can have a detrimental impact on the prognosis of the disease. As a result, *MALAT-1* expression can serve as a biomarker for cancer. According to a meta-analysis, *MALAT-1* overexpression has been associated with weak clinical outcomes in cancer (55). Therefore, *MALAT-1* could be used as a potential predictor of tumor metastasis and prognosis after radiotherapy.

In summary, trials of breast irradiation after BC continue to support the evidence that irradiation significantly reduces the expression of *MALAT-1* and *miR-2143p*, while increasing the expression of *CCL5*. We show that radiotherapy has an effect on the *CCL5*, *MALAT-1*, and *miR-2143p* axis of human BC with increased immunity-associated signaling and that this could be a novel treatment approach for overcoming the therapeutic ionizing radiation in tumors associated with this signaling network. The expression of these genes could be used to determine the efficacy of radiotherapy. More studies with larger sample sizes, we believe, are required to validate this hypothesis.

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References

1. Piotrowski I, Kulcenty K, Suchorska W. Interplay between inflammation and cancer. Rep Pract Oncol Radiother. 2020;25(3): 422-7.

2. Velasco-Velazquez M, Pestell RG. The CCL5/CCR5 axis promotes metastasis in basal breast cancer. Oncoimmunology. 2013;2(4):e23660.

3. Donlon TA, Krensky AM, Wallace MR, Collins FS, Lovett M, Clayberger C. Localization of a human T-cell-specific gene, RANTES (D17S136E), to chromosome 17q11.2-q12. Genomics. 1990;6(3):548-53.

4. Mrowietz U, Schwenk U, Maune S, Bartels J, Kupper M, Fichtner I, et al. The chemokine RANTES is secreted by human melanoma cells and is associated with enhanced tumour formation in nude mice. Br J Cancer. 1999;79(7-8):1025-31.

5. Wertel I, Tarkowski R, Bednarek W, Kotarski J. Relationship between RANTES and dendritic cells in ovarian cancer patients. Front Biosci (Elite Ed). 2011;3(1):227-32.

6. Vaday GG, Peehl DM, Kadam PA, Lawrence DM. Expression of CCL5 (RANTES) and CCR5 in prostate cancer. Prostate. 2006;66(2):124-34.

7. Soria G, Ben-Baruch A. The inflammatory chemokines CCL2 and CCL5 in breast cancer. Cancer Lett. 2008;267(2):271-85.

8. Fang Y, Fullwood MJ. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. Genomics Proteomics Bioinformatics. 2016;14(1):42-54.

9. Do H, Kim W. Roles of Oncogenic Long Non-coding RNAs in Cancer Development. Genomics Inform. 2018;16(4):e18.

10. Sun L, Zhang P, Lu W. lncRNA MALAT1 Regulates Mouse Granulosa Cell Apoptosis and 17beta-Estradiol Synthesis via Regulating miR-205/CREB1 Axis. Biomed Res Int. 2021;2021:6671814.

[Downloaded from ijmcmed.org on 2024-10-12]

[DOI: 10.22088/IJMCM.BUMS.11.3.244]

11. Jiang X, Wang J, Deng X, Xiong F, Zhang S, Gong Z, et al. The role of microenvironment in tumor angiogenesis. J Exp Clin Cancer Res. 2020;39(1):204.

12. Xu WW, Jin J, Wu XY, Ren QL, Farzaneh M. MALAT1-related signaling pathways in colorectal cancer. Cancer Cell Int. 2022;22(1):126.

13. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466(7308):835-40.

14. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97.

15. Sakurai M, Masuda M, Miki Y, Hirakawa H, Suzuki T, Sasano H. Correlation of miRNA expression profiling in surgical pathology materials, with Ki-67, HER2, ER and PR in breast cancer patients. Int J Biol Markers. 2015;30(2):e190-9.

16. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet. 2016;17(1):47-62.

17. Yoon JH, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol. 2014;34:9-14.

18. Shan D, Shang Y, Hu T. MicroRNA-411 Inhibits Cervical Cancer Progression by Directly Targeting STAT3. Oncol Res. 2019;27(3):349-58.

19. Salim H, Akbar NS, Zong D, Vaculova AH, Lewensohn R, Moshfegh A, et al. miRNA-214 modulates radiotherapy response of non-small cell lung cancer cells through regulation of p38MAPK, apoptosis and senescence. Br J Cancer. 2012;107(8):1361-73.

20. Early Breast Cancer Trialists' Collaborative G, Darby S, McGale P, Correa C, Taylor C, Arriagada R, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet. 2011;378(9804):1707-16.

21. Ebctcg, McGale P, Taylor C, Correa C, Cutter D, Duane F, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. Lancet. 2014;383(9935):2127-35.

22. Arvold ND, Taghian AG, Niemierko A, Abi Raad RF, Sreedhara M, Nguyen PL, et al. Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol. 2011;29(29):3885-91.

23. Recht A, Comen EA, Fine RE, Fleming GF, Hardenbergh PH, Ho AY, et al. Postmastectomy Radiotherapy: An American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology Focused Guideline Update. Pract Radiat Oncol. 2016;6(6):e219-e34.

24. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415(6871):530-6.

25. Tsai M-J, Chang W-A, Huang M-S, Kuo P-L. Tumor microenvironment: a new treatment target for cancer. Int Sch Res Notices. 2014;2014.

26. Trellakis S, Bruderek K, Dumitru CA, Gholaman H, Gu X, Bankfalvi A, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. Int J Cancer. 2011;129(9):2183-93.

27. Yi EH, Lee CS, Lee JK, Lee YJ, Shin MK, Cho CH, et al. STAT3-RANTES autocrine signaling is essential for tamoxifen resistance in human breast cancer cells. Mol Cancer Res. 2013;11(1):31-42.

28. Moran CJ, Arenberg DA, Huang CC, Giordano TJ, Thomas DG, Misek DE, et al. RANTES expression is a predictor of survival in stage I lung adenocarcinoma. Clin Cancer Res. 2002;8(12):3803-12.

[Downloaded from ijmcmed.org on 2024-10-12]

[DOI: 10.22088/IJMCM.BUMS.11.3.244]

29. Hartmann MC, Dwyer RM, Costello M, Potter SM, Curran C, Hennessy E, et al. Relationship between CCL5 and transforming growth factor-beta1 (TGFbeta1) in breast cancer. Eur J Cancer. 2011;47(11):1669-75.

30. Niwa Y, Akamatsu H, Niwa H, Sumi H, Ozaki Y, Abe A. Correlation of tissue and plasma RANTES levels with disease course in patients with breast or cervical cancer. Clin Cancer Res. 2001;7(2):285-9.

31. Eissa SA, Zaki SA, El-Maghraby SM, Kadry DY. Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. J Egypt Natl Canc Inst. 2005;17(1):51-5.

32. Borsig L, Wolf MJ, Roblek M, Lorentzen A, Heikenwalder M. Inflammatory chemokines and metastasis--tracing the accessory. Oncogene. 2014;33(25):3217-24.

33. Nesbeth Y, Scarlett U, Cubillos-Ruiz J, Martinez D, Engle X, Turk MJ, et al. CCL5-mediated endogenous antitumor immunity elicited by adoptively transferred lymphocytes and dendritic cell depletion. Cancer Res. 2009;69(15):6331-8.

34. Connolly KA, Belt BA, Figueroa NM, Murthy A, Patel A, Kim M, et al. Increasing the efficacy of radiotherapy by modulating the CCR2/CCR5 chemokine axes. Oncotarget. 2016;7(52):86522-35.

35. Timaner M, Bril R, Kaidar-Person O, Rachman-Tzemah C, Alishekevitz D, Kotsofruk R, et al. Dequalinium blocks macrophage-induced metastasis following local radiation. Oncotarget. 2015;6(29):27537-54.

36. Okubo M, Kioi M, Nakashima H, Sugiura K, Mitsudo K, Aoki I, et al. M2-polarized macrophages contribute to neovasculogenesis, leading to relapse of oral cancer following radiation. Sci Rep. 2016;6:27548.

37. Mi S, Qu Y, Chen X, Wen Z, Chen P, Cheng Y. Radiotherapy Increases 12-LOX and CCL5 Levels in Esophageal Cancer Cells and Promotes Cancer Metastasis via THP-1-Derived Macrophages. Onco Targets Ther. 2020;13:7719-33.

38. Wang X, Yang X, Tsai Y, Yang L, Chuang KH, Keng PC, et al. IL-6 Mediates Macrophage Infiltration after Irradiation via Up-regulation of CCL2/CCL5 in Non-small Cell Lung Cancer. Radiat Res. 2017;187(1):50-9.

39. Chen HY, Xu L, Li LF, Liu XX, Gao JX, Bai YR. Inhibiting the CD8(+) T cell infiltration in the tumor microenvironment after radiotherapy is an important mechanism of radioresistance. Sci Rep. 2018;8(1):11934.

40. Walens A, DiMarco AV, Lupo R, Kroger BR, Damrauer JS, Alvarez JV. CCL5 promotes breast cancer recurrence through macrophage recruitment in residual tumors. Elife. 2019;8.

41. Wang L, Jiang J, Chen Y, Jia Q, Chu Q. The roles of CC chemokines in response to radiation. Radiat Oncol. 2022;17(1):63.

42. Zhang J, Su B, Gong C, Xi Q, Chao T. miR-214 promotes apoptosis and sensitizes breast cancer cells to doxorubicin by targeting the RFWD2-p53 cascade. Biochem Biophys Res Commun. 2016;478(1):337-42.

43. Zhang F, Wang B, Long H, Yu J, Li F, Hou H, et al. Decreased miR-124-3p Expression Prompted Breast Cancer Cell Progression Mainly by Targeting Beclin-1. Clin Lab. 2016;62(6):1139-45.

44. Liu B, Tian Y, Li F, Zhao Z, Jiang X, Zhai C, et al. Tumor-suppressing roles of miR-214 and miR-218 in breast cancer. Oncol Rep. 2016;35(6):3178-84.

45. Gu F, Li L, Yuan QF, Li C, Li ZH. Down-regulation of survivin enhances paclitaxel-induced Hela cell apoptosis. Eur Rev Med Pharmacol Sci. 2017;21(15):3504-9.

46. Wang J, Xu AM, Zhang JY, He XM, Pan YS, Cheng G, et al. Prognostic significance of long non-coding RNA MALAT-1 in various human carcinomas: a meta-analysis. Genet Mol Res. 2016;15(1).

47. Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene. 2007;26(6):851-8.

48. Ying L, Chen Q, Wang Y, Zhou Z, Huang Y, Qiu F. Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition. Mol Biosyst. 2012;8(9):2289-94.

[Downloaded from ijmcmed.org on 2024-10-12]

[DOI: 10.22088/IJMCM.BUMS.11.3.244]

49. Kim J, Piao HL, Kim BJ, Yao F, Han Z, Wang Y, et al. Long noncoding RNA MALAT1 suppresses breast cancer metastasis. Nat Genet. 2018;50(12):1705-15.

50.Gutschner T, Hammerle M, Diederichs S. MALAT1 -- a paradigm for long noncoding RNA function in cancer. J Mol Med (Berl). 2013;91(7):791-801.

51. Wilusz JE, Freier SM, Spector DL. 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. Cell. 2008;135(5):919-32.

52. Ji Q, Liu X, Fu X, Zhang L, Sui H, Zhou L, et al. Resveratrol inhibits invasion and metastasis of colorectal cancer cells via MALAT1 mediated Wnt/beta-catenin signal pathway. PLoS One. 2013;8(11):e78700.

53. Xu C, Yang M, Tian J, Wang X, Li Z. MALAT-1: a long non-coding RNA and its important 3' end functional motif in colorectal cancer metastasis. Int J Oncol. 2011;39(1):169-75.

54. Guo F, Li Y, Liu Y, Wang J, Li Y, Li G. Inhibition of metastasis-associated lung adenocarcinoma transcript 1 in CaSki human cervical cancer cells suppresses cell proliferation and invasion. Acta Biochim Biophys Sin (Shanghai). 2010;42(3):224-9.

55. Schmidt LH, Spieker T, Koschmieder S, Schaffers S, Humberg J, Jungen D, et al. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. J Thorac Oncol. 2011;6(12):1984-92.

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