

Research on CCR7, CD209 and CD19 as Novel Immune Markers for Prognosis of Cervical Cancer after Radiotherapy

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1 **Research on CCR7, CD209 and CD19 as Novel Immune Markers for**
2 **Prognosis of Cervical Cancer after Radiotherapy**

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Abstract

Background: One of the most prevalent malignant neoplasms among women worldwide is Cervical cancer (CC). Radiotherapy is the primary treatment modality for this disease, yet its efficacy is contingent upon a multitude of factors. It is therefore imperative to identify reliable biomarkers for radiotherapy in the treatment of CC. Currently, there are still relatively few detection methods available to assess the sensitivity to radiotherapy. This study aims to analyze the changes in peripheral blood immune cells before and after radiotherapy for locally advanced cervical cancer, and to identify biomarkers related to radiotherapy sensitivity and prognosis.

Methods: Peripheral blood samples from 15 patients with locally advanced cervical squamous cell carcinoma (FIGO stage IIB-IVA) before and after radiotherapy were collected for transcriptome sequencing to screen differentially expressed genes before and after radiotherapy. The prognostic value of biomarkers was verified in combination with the TCGA-CESC database, and the molecular regulatory mechanism was analyzed.

Results: CCR7, CD209 and CD19 were determined as key radiotherapy markers through machine learning combined with ROC curve (AUC of the training set > 0.7). The expressions of CCR7 and CD19 were significantly decreased after radiotherapy, while CD209 was significantly increased ($p < 0.05$). The expression of CCR7 was positively correlated with that of PD-1 ($r = 0.43$), and the prognosis of the group with low expression of CCR7 was better ($p < 0.05$). The significant markers verified by qPCR were CCR7 and CD209 ($P < 0.05$).

Conclusion: Our study indicates that CCR7, CD209, and CD19 can serve as novel

immune markers for cervical cancer radiotherapy. CCR7 not only predicts the prognosis of cervical cancer patients but also represents a potential drug target for inhibiting the migration of tumor cells.

Keywords: Cervical cancer; Radiotherapy biomarkers; Bioinformatics; Immune infiltration

1. Introduction

Cervical cancer (CC) is the most common malignant tumor in gynecology, with global incidence and mortality rates ranking fourth among female tumors^[1]. According to statistics from the World Health Organization, in 2020, there were approximately 604,000 new cases and 342,000 deaths worldwide. The incidence and mortality rates in developing countries are much higher than those in developed countries. In China, approximately 110,000 new cases and 60,000 deaths were reported^[2]. HPV (Human Papillomavirus) infection is the main factor causing CC lesions. Approximately 90% of CC patients can detect HPV viruses^[3,4]. HPV is a double-stranded circular DNA virus. When persistent infection occurs and immunity declines, it can integrate into the host genome, causing alterations in key genes and leading to abnormal immune conditions in the tumor microenvironment. In recent years, the treatment methods for CC have become more diverse, and the diagnostic and therapeutic levels have continued to improve. However, globally, 37% of CC patients are diagnosed at the local advanced stage, and in developing countries including China, this proportion exceeds 50%^[5]. This poses a serious threat to women's health. The treatment for locally advanced CC mainly involves combined radiotherapy and chemotherapy, immunotherapy, targeted therapy, etc. However, the prognosis of patients varies significantly under the same radiotherapy regimen, and some even develop radiotherapy resistance, highlighting the importance of

individualized treatment. At present, a variety of predictive biomarkers for radiotherapy sensitivity of CC have been identified, such as serum ICAM-1, VCAM-1, and PAX1^[6,7]. However, the research results are based on a specific cohort and may not be directly applicable to all patients with CC. The heterogeneity of CC and the complexity of treatment responses deserve further study, and it is necessary to explore more valuable biomarkers that can predict therapeutic effects.

Studies have found that the tumor immune microenvironment (TME) has a significant impact on the occurrence, development, and treatment of CC. TME is the environment in which tumor cells survive and is a complex collection including stromal cells, immune-related cells, vascular system, extracellular matrix, and numerous cytokines secreted by them, regulating anti-tumor immune responses through multiple pathways^[8]. Immunotherapy and radiotherapy can change the TME, and various immune-related cells enter the TME through peripheral blood circulation to exert their functions. Peripheral blood has a certain impact on the TME, but currently, there are few studies observing the effect of radiotherapy on immune cells in CC from the in vivo level, and the mechanism has not been fully elucidated. However, the mechanism of radiotherapy on immune cells in the TME of CC remains unclear, and there is a lack of in-depth research at the in vivo level.

This study analyzed the peripheral blood immune cells in CC patients receiving radiotherapy. By integrating transcriptome sequencing data and various bioinformatics analysis methods, it systematically identified radiotherapy biomarkers from multiple dimensions, further understanding the changes of immune cells during radiotherapy, and searching for markers with significant differences and related to the prognosis of patients. It also delved into the biological pathways and immune microenvironment involved, providing a better basis for combining immunotherapy with radiotherapy.

98 **1 Research Materials and Methods**

99 **1.1 General Information**

100 A total of 15 patients with locally advanced CC who received radical
101 chemoradiotherapy in the Second Affiliated Hospital of Xi 'an Jiaotong University
102 from February 2025 to May 2025 were collected. All patients had signed relevant
103 consent forms. The patients ranged in age from 39 to 73 years, with a median age of
104 54 years, were all patients with squamous cell carcinoma, and all patients received
105 pelvic intensity modulated radiation therapy (IMRT).

106 **1.2 Inclusion criteria**

107 Inclusion criteria for CC patients: (1) Age: 18-70 years old; (2) Generally good
108 condition, initial anti-tumor therapy, KPS $\geq$ 70; (3) Histological examination (biopsy)
109 confirmed cervical squamous cell carcinoma by pathology of our hospital; (4)
110 Cervical HPV test indicated a positive result; (5) FIGO stages ranged from IIB to IVA;
111 Exclusion criteria: (1) Combined with other tumors or infectious diseases related to
112 the immune system; (2) Received surgery, radiotherapy or other systemic treatments
113 before radiotherapy.

114 **1.3 Therapy method**

115 Three dimensional intensity modulated pelvic radiotherapy and intracavitary
116 brachytherapy with pelvic radiotherapy dose 45-50.4 Gy, 1.8-2.0Gy/f, with pelvic
117 lymph node metastasis, paraaortic lymph node metastasis and retroperitoneal lymph

node metastasis. The dose of local lesion was 60Gy, and the total dose of intracavity irradiation was 30-40 Gy. The chemotherapy regimen was paclitaxel cisplatin (paclitaxel 135-175 mg/m², cisplatin 60-80mg/m², intravenous infusion, q21-28d).

1.4 Specimen collection

Collect 15mL of peripheral venous blood (from the elbow vein) from patients with cervical squamous cell carcinoma using EDTA anticoagulation tubes. Collect two blood samples from each patient: 1) before radiotherapy; 2) 15-20 times after pelvic radiotherapy. The operation should avoid contamination, and the anticoagulation tubes should be clearly marked with the patient's name and sample number.

2. Research process

2.1 Collection of the sample and data

The research protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Transcriptome sequencing was performed on blood samples from 15 patients with CC before and after radiotherapy. Prior to the collection of samples, each participant provided informed consent.

2.2 Extraction of the RNA and library construction

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The extraction was performed according to the manufacturer's instructions. A NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA) was used to determine the amount and purity of RNA in each sample. Cleaved RNA fragments were then reverse

transcribed into cDNA using SuperScript™ II Reverse Transcriptase (Invitrogen, Cat. 1896649, USA). After a series of PCR amplification procedures for the ligated products, 2x150 bp paired-end sequencing (PE150) was performed on an Illumina Novaseq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China).

2.3 Data source and processing

Moreover, the RNA-seq data with cervical squamous cell carcinoma (CESC), which constitute the training set (TCGA-CESC), were downloaded from The Cancer Genome Atlas Program (TCGA) database (<http://cancergenome.nih.gov/>). The data set comprises a sum of 291 samples with survival information. The data underwent filtration using the sequencing results filtering tool trim galore (v 0.6.6) and the adapter trimming tool cutadapt (v 3.4 with Python v 3.9.4). The FastQC software (v 0.11.5) and the MultiQC software (v 1.10.1) were used for quality control. The following steps were taken to process the data: deleted reads containing aptamers, polyA and polyG, reads containing more than 5% unknown nucleotides (N) and reads containing more than 20% poor quality bases. A total of Gbp of purified pairwise reads were then generated. The Hisat2+FeatureCounts processes were employed to identify clean reads in the human reference genome GRCh38 (hg38), with a comparison rate exceeding 80% available for subsequent analysis. Subsequently, sequence comparisons were conducted utilising the human genome as a reference sequence. The reads were assembled and quantified by StringTie, and the location and depth distribution of read coverage was visualised by plotting the mapped reads.

2.4 Differential expression analysis

The DESeq2 (v 1.38.0)^[9] was attempted to examine differently expressed genes (DEGs) between pre-RT and post-RT groups in mRNA-seq with the threshold set to p value < 0.05 and |Log₂ fold change (FC)| > 2. Furthermore, ggplot2 (v 3.3.6) package^[10] was used to plot the volcano map for the top 10 DEGs with the largest variation. The heatmap of DEGs were drawn by ComplexHeatmap (v 2.14.0) package^[11].

2.5 Enrichment analysis of the DEGs and construction of a network of protein-protein interaction (PPI)

Further functional studies were required in order to elucidate the potential role of these DEGs in relevant biological processes or disease states. Biological functions and pathways for DEGs were analysed using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, as implemented in the ClusterProfiler package. (v 3.18.1) package^[12]. Using STRING (<https://string-db.org>) (interaction score > 0.4), a PPI network was constructed for DEGs. The network was finally visualised using Cytoscape (v 3.10.0)^[13]. Subsequently, genes with interactions within the PPI network were identified as hub genes for further analysis.

2.6 Identification of diagnostic genes

In order to obtain diagnostic genes for CC, based on hub genes expression matrix, candidate gene 1 and 2 were obtained through the Least absolute shrinkage and selection operator (Lasso) and Boruta algorithms, which were conducted by the glmnet (v 4.1-6) package^[14] and the Boruta (v 8.0.0) package^[15], respectively. The two sets of candidate genes were taken to intersect and visualized by ggvenn (v 0.1.9) package^[16], resulting in the identification of the signature genes. The genes exhibiting consistent expression trends in the transcriptome sequencing and TCGA-CESC of pre-RT and post-RT samples by the R language pROC (v 1.18.0) package^[17] and then

diagnostic genes were screened based on the Receiver operating characteristic (ROC) curves (AUC > 0.7).

2.7 Differences expression and correlation analysis of CC radiotherapy biomarkers

The Wilcoxon test was performed on the differences expression of diagnostic genes in the pre-RT and post-RT samples of transcriptome sequencing data to further assess the expression of diagnostic genes. Ultimately, genes with differential expression were defined as CC radiotherapy biomarkers ($p < 0.05$). In order to observe the correlation between biomarkers of CC radiotherapy, Spearman correlation analysis was performed by the psych (v 2.2.9) package^[18] ($p < 0.05$) in transcriptome sequencing samples.

2.8 Gene Set Enrichment Analysis (GSEA) of CC radiotherapy biomarkers

In this study, gene set of the human KEGG pathway was selected as reference gene sets, based on the Molecular Signature Database (MSigDB) (<https://www.gsea-msigdb.org/gsea/msigdb>). Spearman correlation analysis of biomarkers with all genes in all samples used by the psych (v 2.2.9) package and ranked according to the correlation coefficient. Then, biomarkers were analyzed for GSEA enrichment by the Clusterprofiler (v 3.18.1) package^[19].

2.9 Analysis of immune infiltrating cells

In the following study, the infiltration of immune cells among all blood samples were calculated using the CIBERSORT algorithm and analysed by Wilcoxon test. Subsequently, a Spearman was employed to explore correlations in transcriptome sequencing samples between biomarkers and cells, with the results presented in a

visual format ($|\text{correlation coefficient (cor)}| > 0.3, p < 0.05$).

2.10 Prognostic value of biomarkers

In order to investigate the correlation between biomarkers and patient survival rates, samples were divided into two expression groups based on the median expression level of each biomarker in TCGA-CESC. The relationship between the differences expression of these biomarkers and the survival rates were examined by Kaplan-Meier (KM) survival curves in the survminer (v 0.4.9) package^[20].

2.11 Regulatory network construction

In order to ascertain the molecular regulatory mechanisms of the biomarkers, the ChEA3 database (<https://ngdc.cncb.ac.cn/databasecommons/database/id/6810>) was employed to predict transcription factors (TFs) for the biomarkers, while the miRTar Basedatabase(https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php) was utilized to predict the upstream microRNAs (miRNAs) of the biomarkers. Subsequently, the TF-biomarkers-miRNAs network was visualized.

2.12 Statistical analysis of data

The Cytoscape 3.8.2 platform and R 4.2.3 software were used for all statistical analyses. A Student's t-test or Wilcoxon test was employed to ascertain whether there were any statistically significant differences, p-value < 0.05 was indicated significant result.

3 Analysis result

3.1 Enrichment and PPI analysis in 575 DEGs

A total of 575 DEGs were obtained between pre-RT and post-RT group in this

study ($p < 0.05$, $|\text{Log}_2\text{FC}| > 2$), including 451 up-regulated and 124 down-regulated in post-RT groups, then results were shown in volcano map and heatmap (**Figure 1A and 1B**). A total of 72 Biological Process (BP) entries, 35 Cellular Component (CC) entries and 16 Molecular Function (MF) entries were enriched by 575 DEGs. In the BP assessment, they were mostly engaged in regulation of inflammatory response. DEGs were mainly enriched in CC of cell component pathways such as external side of plasma membrane. DEGs were mainly enriched in MF of metal ion transmembrane transporter activity (**Figure 1C**). Besides, total of 40 KEGG pathways were enriched and significantly enriched in cytokine-cytokine receptor interaction ($p.\text{value} < 0.05$) (**Figure 1D**). Then a sum of 25 nodes were shown in a PPI network diagram (**Figure 1E**). Ultimately, the 25 hub genes were obtained, to be employed for subsequent analyses.

3.2 Lasso and Boruta algorithms for diagnostic genes

The Lasso algorithm was employed to analyse the 25 hub genes, which were identified through the extraction of the aforementioned genes from the PPI network ($\lambda_{\min} = 0.0264$). A sum of 5 candidate genes 1 were obtained, which were CCR7, CD209, SIGLEC1, MMP9 and CD19 (**Figure 2A and 2B**). In addition, according to the results of Boruta algorithm, a total of 11 candidate genes 2 were obtained (**Figure 2C**). A total of 3 genes as signature genes (CCR7, CD209 and CD19) were taken from the intersection of the genes obtained from the above two methods (**Figure 2D**). As shown in the ROC plot, the AUC values for CCR7, CD209 and CD19 in the transcriptome sequencing and TCGA-CESC were greater than 0.7,

indicating that these three genes had good diagnostic value (**Figure 2E**). Therefore, the CCR7, CD209 and CD19 genes were defined as diagnostic genes for CC disease in this study.

3.3 CCR7, CD209 and CD19 gene defined as CC radiotherapy biomarkers

To further evaluate the expression results of CCR7, CD209 and CD19 in different samples, box plots demonstrated that expression of diagnostic genes in the pre-RT and post-RT samples of transcriptome sequencing data showed differences ($p < 0.05$). The CCR7 and CD19 genes were expressed at higher levels in the pre-RT, while the CD209 gene demonstrated elevated expression in the post-RT group (**Figure 3A**). Therefore, the CCR7, CD209 and CD19 genes were defined as CC radiotherapy biomarkers in this study. To gain further insight into the relationship between biomarkers, CD19 showed positive correlation with CCR7 based on Spearman analysis ($\text{cor} = 0.59, p < 0.001$) (**Figure 3B**).

3.4 GSEA enrichment analysis in biomarkers

The CCR7, CD209 and CD19 genes were analysed for GSEA enrichment ($p\text{-value} < 0.05$) using transcriptome sequencing data. The results demonstrated a significant enrichment of the top 5 pathways. The results demonstrated that CCR7 and CD19 were enriched in 22 and 15 pathways, respectively, with both biomarkers exhibiting enrichment in the ribosome pathway (**Figure 4A and 4B**). The CD209 was enriched in 4 pathways, such as olfactory transduction (**Figure 4C**).

3.5 Analysis of biomarkers with immune infiltrating cells

To gain further insight into immune cell infiltration, a comprehensive heat map

was constructed, illustrating the presence of 22 distinct immune infiltrating cells ($p < 0.05$) (**Figure 5A**). The box plot results demonstrated notable discrepancies of B cells naive, neutrophils, resting-memory CD4 T cells and CD8 T cells among all samples ($p < 0.05$) (**Figure 5B**). Furthermore, CCR7 showed positive correlation with B cells naive ($\text{cor} = 0.63$), resting-memory CD4 T cells ($\text{cor} = 0.77$) and CD8 T cells ($\text{cor} = 0.61$) ($p < 0.001$). It is noteworthy that both the CCR7 and CD19 genes exhibited a negative correlation with neutrophils ($p < 0.05$), and the CD209 gene exhibited a negative correlation with B cells naive ($\text{cor} = -0.35$, $p < 0.05$) (**Figure 5C**).

3.6 CCR7 gene showed significant difference between two expression group

In order to investigate the prognostic value of biomarkers, KM curves and log-rank tests demonstrated that the prognostic outcomes of the CCR7 gene on TCGA-CESC disease samples exhibited a difference among two expression groups and the results demonstrated survival was lower in patients with high expression ($p < 0.05$) (**Figure 6A**). Nevertheless, between the two expression group, the CD19 and CD209 gene had no significant difference in survival ($p > 0.05$) (**Figure 6B and 6C**).

3.7 Constructing a TF-biomarker-miRNAs network to explore molecular regulatory mechanisms

In order to investigate the molecular regulatory mechanisms of 3 biomarkers, the miRNAs-biomarkers-TF network was constructed. A whole of 57 interacting miRNAs were predicted by the 3 biomarkers, such as hsa-miR-1273a, hsa-miR-3670 and hsa-miR-1252-3p. Due to the large number of TFs, the genes corresponding to the top 100 scores were selected for network construction, and a total of 161 TFs were found,

such as RELA, TERF2 and PAX5 (**Figure 7**).

3.8 Immune checkpoint analysis

In order to understand the expression of immune checkpoint after and before radiotherapy in patients, 24 immune checkpoint genes were obtained from previous studies: CD86, TNFSF18, PDCD1, CD200, CD40LG, CD40, TMIGD2, CD70, LAIR1, CD44, PDCD1LG2, TNFRSF4, NRP1, TNFRSF18, CD276, HAVCR2, CD160, CD48, TNFSF4, ADORA2A, TNFRSF14, CD28, CD200R1, VTCN1 were intermixed with the genes in our training set to obtain the expression levels of related genes. Then, the difference of the expression of immune checkpoint molecules in training concentration was compared between the groups after and before radiotherapy. Wilcoxon test was performed on the expression of immune checkpoint molecules between the groups after and before radiotherapy, and the P-value of significance was calculated. The molecular expression of significantly different immune checkpoints was mapped between the groups after and before radiotherapy. As shown in **Figure8**, the 11 immune checkpoint molecules CD200, CD276, CD28, CD40, CD40LG, CD48, CD70, PDCD1 (PD-1), TMIGD2, TNFRSF18, and TNFRSF4 showed significant differences between the post-radiotherapy and pre-radiotherapy groups. In order to explore the relationship between radiotherapy markers and differential immune checkpoint, Spearman correlation analysis was conducted on radiotherapy markers and differential immune checkpoint using the 'psych' R package. We found that CCR7 had the strongest correlation with CD28 (cor

= 0.769, $P < 0.05$), CD19 had the strongest correlation with CD28 ($\text{cor} = 0.693$, $P < 0.05$), and CD209 had the strongest correlation with LAIR1 ($\text{cor} = 0.651$, $P < 0.05$). Moreover, CCR7, CD19, and CD209 were all significantly correlated with PD-1 (Figure8-9) .

3.9 Expression validation analysis

Expression validation analysis revealed differences in biomarkers (CCR7, CD209 and CD19) in pre-RT and post-RT groups. The results showed that the expression of CCR7 and CD19 in pre-RT group notably elevated compared to the post-RT group, while CD209 had higher expression in post-RT group. It is worth noting that, CCR7 and CD209 showed significant difference in two groups ($p < 0.05$, **Figure10,Table1**) , whereas CD19 showed no difference expression in two groups . Thees above findings suggested that the 3 biomarkers play key roles in elucidating the pathogenesis of CC.

4. Discussion

In the anti-tumor treatment of CC, TME has an inhibitory effect, mainly through down-regulating the activation of cytotoxic CD8+ T cells and NK cells, increasing the infiltration of immunosuppressive Treg cells and M2-type macrophages, and up-regulating the expression of PD-L1 in tumor cells^[21, 22].Studies have shown that the tumor microenvironment (TME) plays a significant role in radioresistance in CC, promoting tumor cell immune escape, with hypoxia being the main influencing factor^[23]. Radiotherapy also affects various molecules and cells in the TME. In this study, we performed transcriptome sequencing of blood samples from CC patients undergoing radiotherapy. Through LASSO, Boruta algorithms, and ROC analysis, we

identified three significant radiotherapy response biomarkers during radiotherapy for CC, namely: CCR7, CD209, and CD19. We also explored the biological pathways, differential expression, and molecular regulatory networks of these three biomarkers, as well as their potential role involved in radiotherapy response mechanisms.

Chemokine receptor 7 (CCR7) plays a crucial role in guiding cells into lymphoid organs and within them, and is essential for the acquisition of immunity and tolerance. CCR7 is significant in the establishment of thymic structure and function and the homing of regulatory T cells to lymph nodes. This receptor is also a key regulator of cancer cell migration and dendritic cell movement^[24]. CCL21 and CCL19 are both ligands of CCR7, and stimulating CCR7 can enhance G protein activation, cell migration, ERK 1/2 signaling pathways, and calcium mobilization, strongly driving the chemotactic migration of tumor cells expressing CCR7^[25,26]. In CC, studies have shown that the expression of CCR7 in cancer tissues is significantly higher than that in normal tissues, and it is closely related to lymphatic vessel invasion (LVI) and lymph node metastasis ^[27,28]. TCGA data further confirmed that high expression of CCR7 was significantly associated with lymphatic metastasis in patients with cervical squamous cell carcinoma and could serve as an independent prognostic factor ^[28]. This study verified through qPCR that CCR7 is significant for the prognosis of CC, suggesting that CCR7 can not only serve as a predictive marker for the radiotherapy response of CC but also may become a potential therapeutic target for inhibiting tumor migration.

This study found that CCR7 was significantly reduced after radiotherapy compared to before radiotherapy. In TCGA-CESC disease samples, the prognosis of the CCR7 low-expression group was better ($p < 0.05$). Therefore, CCR7 can not only predict the prognosis of CC patients but also serve as a drug target to inhibit tumor

cell migration. Currently, drugs targeting CCR7 inhibitors are still under research, including Navarixin and Cmp2105, which are currently undergoing clinical trials to test their preventive metastatic activity^[29,30]. This study also found that both CD4⁺T cells and CD8⁺T cells in the peripheral blood of patients significantly decreased after radiotherapy, suggesting that radiotherapy, while killing tumors, may also cause functional inhibition of T lymphocytes...The expression of CCR7 is positively correlated with PD-1, and both decrease after radiotherapy. However, in some patients, the expression of PD-L1 increases, reflecting the complex regulatory effect of radiotherapy on immune checkpoint molecules. Radiotherapy can induce immunosuppression through multiple pathways: including the gradual depletion of T cells after early activation, the release of TGF- β and the increase of MDSCs, as well as the upregulation of PD-L1 expression through the ATM/ATR/Chk1 pathway and interferon - γ ^[31-33]. These findings provide a theoretical basis for the combination of radiotherapy and immunotherapy. For instance, the KEYNOTE-A18 study demonstrated that pembrolizumab combined with radiotherapy and chemotherapy could significantly improve progression-free survival (HR = 0.70, p = 0.0020), and it was approved by the FDA for use in stage III-IV cervical cancer in 2024^[34]. However, high-dose radiotherapy may also cause immunosuppression by reducing the expression of co-stimulatory molecules and promoting the secretion of TGF- β 1^[35]. Therefore, in combined therapy, it is necessary to precisely control the timing and dosage of radiotherapy and immunotherapy to maximize the synergistic anti-tumor effect.

The CD19 protein is a type I transmembrane glycoprotein on the surface of B cells, with a relative molecular weight of 95 ku. It belongs to the immunoglobulin superfamily (IgSF) and has a single transmembrane region, an intracellular C-terminal,

and an extracellular N-terminal. It is a biomarker for B-cell lymphomas and follicular dendritic cells, and is not expressed or is expressed at a low level in other lineages or other tissues. Therefore, it is the best target for B-cell malignancies including acute lymphoblastic leukemia and lymphomas^[36,37]. Research on CD19 in solid tumors is relatively scarce. In the peripheral blood of patients with HPV16-positive CC, the number of CD19 + B lymphocytes is significantly higher than that of HPV-negative patients, which may be related to the high expression of HPV oncogenic antigens on tumor cells^[38]. This suggests that CD19 may serve as an immune marker for HPV-related CC. More importantly, CD19+ B cells can differentiate into regulatory B cells (Breg) that produce IL-10. These Breg cells promote the differentiation of regulatory T cells (Treg) and inhibit the activity of CD8⁺ T cells, thereby establishing an immunosuppressive state in the cervical cancer microenvironment^[39,40]. CD19+ Breg cells promote tumor progression through immunosuppression. All CC patients in this study were HPV-positive. CD19 was highly expressed before treatment and significantly decreased after radiotherapy. The expression level of CD19 may be positively correlated with the degree of tumor immune infiltration. In the future, CD19, as a B-cell marker, may become a breakthrough in immunotherapy, but its mechanism in radiotherapy still needs further exploration.

CD209 is also known as DC-SIGN and belongs to the C-type lectin superfamily. It is mainly enriched in dendritic cells (DCs) and tumor-associated macrophages (TAMs) in the tumor microenvironment of CC^[41]. The study has shown that CD209 can bind to Lewis antigens (highly expressed in cancer), promoting the activation of T

cells and thereby initiating a series of immune responses^[41]. However, the role of CD209 in tumor immunity is controversial and varies by cancer type: in lung cancer, high expression of CD209 is associated with better survival rates and activation of CD8⁺ T cells, suggesting its potential involvement in anti-tumor immunity; however, in colorectal cancer, it was observed to have the opposite trend of promoting tumor progression^[42]. This functional difference may depend on the cell type expressing CD209 (such as pro-inflammatory DC and inhibitory TAM) and specific microenvironmental signals.. There have been no relevant studies on CC. In this study, it was found for the first time that CD209 significantly increased after radiotherapy. The cervical tumors of all patients significantly shrank after treatment, indicating that radiotherapy can activate the CD209 signaling pathway and increase the expression of CD209, which may have anti-cancer effects. Therefore, the dual roles of CD209 in promoting tumor formation and resisting tumor growth need to be further clarified through spatial transcriptomic techniques in order to precisely analyze the expression cell subgroups and functional states of this protein.

To deeply explore the upstream regulatory mechanisms of the three key radiotherapy biomarkers, CCR7, CD209, and CD19, the analysis results show that these three genes are at the center of a highly complex regulatory network. Key factors such as RELA (p65), TERF2, and PAX5 were screened out. RELA (p65) is a core component of the NF-κB signaling pathway. Radiotherapy activates NF-κB through mechanisms such as DNA damage, which may directly up-regulate or down-regulate the expression of these markers, thereby affecting the survival of tumor

cells and the remodeling of the immune microenvironment^[43]. PAX5 is a "master control factor" for B-cell specific development and differentiation^[44]. It may directly regulate the expression of CD19, providing strong upstream molecular evidence for CD19 as a B-cell related immune response marker^[45]. TERF2 is an important component of the telomere protection protein complex^[46]. The DNA damage response (DDR) caused by radiotherapy may indirectly regulate the expression of a series of downstream genes by affecting telomere-related proteins such as TERF2. Therefore, rapidly adjusting the protein expression levels of CCR7, CD209, and CD19 after radiotherapy can precisely control the final immune effect. The functions of many miRNAs in cancer remain unclear, which provides a new direction for future research.

The occurrence and development of CC involve complex biological processes. This study identified CCR7, CD209 and CD19 as novel immune-related biomarkers associated with the radiotherapy response and prognosis of cervical cancer. The expression of CCR7 was significantly decreased after radiotherapy and was correlated with improved patient survival, suggesting that it may not only serve as a prognostic marker but also as a therapeutic target for inhibiting tumor cell migration. The downregulation of CD19 after radiotherapy might reflect the B-cell-mediated immune regulatory mechanism in the tumor microenvironment. Meanwhile, the increase of CD209 after treatment might indicate its role in anti-tumor immune activation. These markers, especially CCR7 and CD209, were further verified by qPCR, highlighting their clinical relevance. Despite the limitations of this study, such as the limited sample size, its results reveal the significant potential of peripheral blood immune

indicators in evaluating the radiotherapy response of CC, providing new targets and theoretical basis for the future development of combined radiotherapy and immunotherapy strategies. Subsequent studies will focus on in-depth validation of the expression and function of these markers in the local tumor microenvironment.

Ethics approval and consent to participate

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Second Affiliated Hospital of Xi 'an Jiaotong University (protocol code: 2024042, date of approval:2024.4.28). Written informed consent has been obtained from the patient.

Consent for publication

All patients have provided written informed consent, and their detailed information, mainly including treatment plans and blood test results, has been released under a shared license.

Availability of data and materials

The datasets generated and analysed during the current study are available in the NCBI repository, The database link is:
<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1467262?reviewer=a99tp3q425t64mrksut9o1js1d>

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Author contributions

Dan Li: The Corresponding author is responsible for ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors, and that all authors receive the submission and all substantive correspondence with editors, as well as the full reviews, verifying that all data, figures and materials (including reagents).

Dan Li is responsible for designing and writing the project, analysis and statistics.

Yali Wang is responsible for the design and guidance of research projects.

Pengtao Yang is responsible for case enrollment.

Xinyue Cui is responsible for data collection.

Lei An is responsible for data collection

Yuanqing Huang is responsible for data analysis

Jiyuan Pan is responsible for case enrollment.

Liyuan Zhang is responsible for case enrollment.

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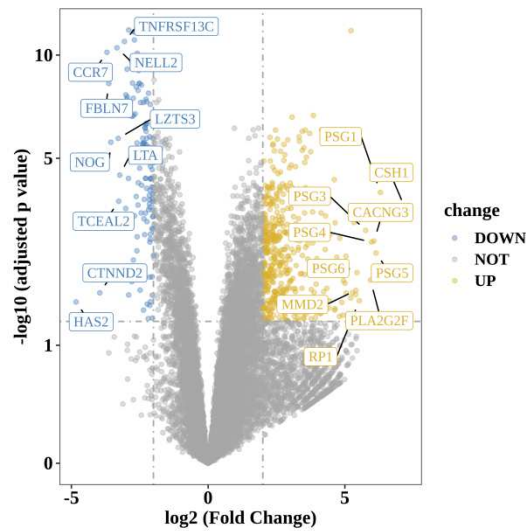


Figure 1A DEGs Volcano map

The horizontal axis represents the fold change (Post-RT/Pre-RT, logarithm to the base 2), and the vertical axis indicates the confidence level of significant tests ($-\log_{10}(\text{adj.P.Value})$). Each point in the figure represents a gene. Yellow and blue points represent significantly differentially expressed genes. Yellow points indicate upregulated gene expression, while blue points indicate downregulated gene expression. Gray points represent genes without significant differences.

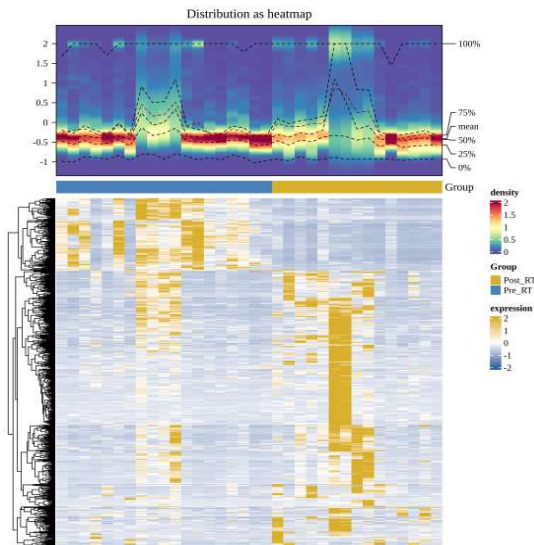
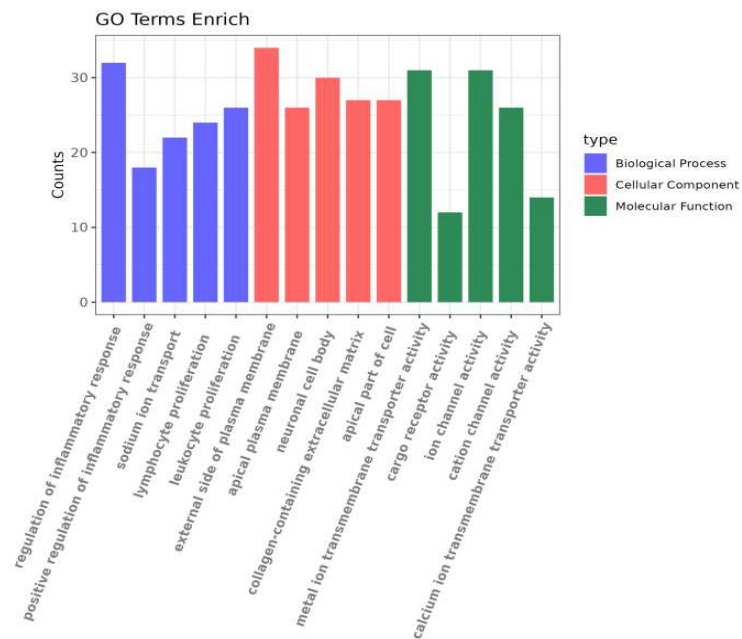


Figure 1B DEGs Heatmap

Each small square represents a gene, and its color indicates the expression level of the gene. The higher the expression level, the yellower the color; the lower the expression level, the bluer the color. The first row of color bars in the figure indicates the sample groups, with blue representing Pre-RT and yellow representing Post-RT. Each row represents the expression level of each gene in different samples, and each column represents the expression level of all differentially expressed genes in each sample.

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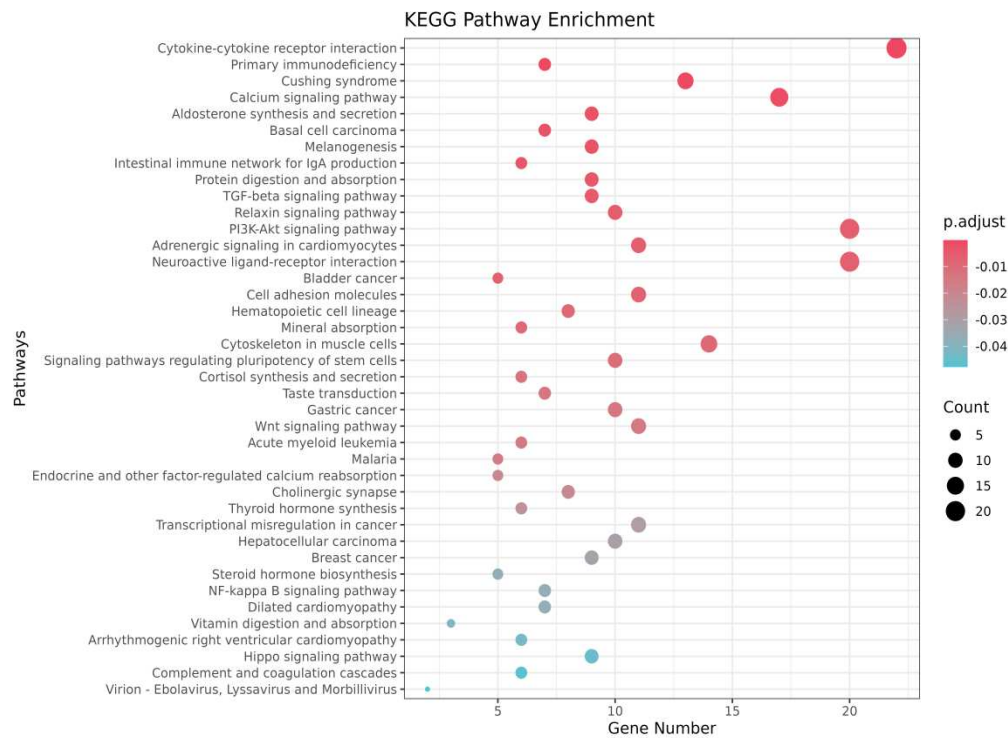
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670 Different colors represent the three parts of the GO analysis, BP, CC and MF, each showing the top 5 items.

671 **Figure 1C DEGs Gene GO enrichment analysis**

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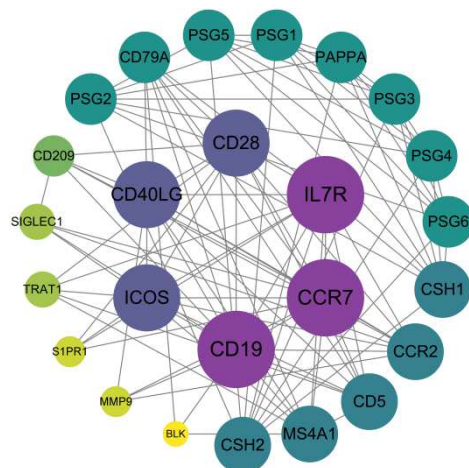
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675 **Figure 1D DEGs Gene KEGG enrichment analysis**

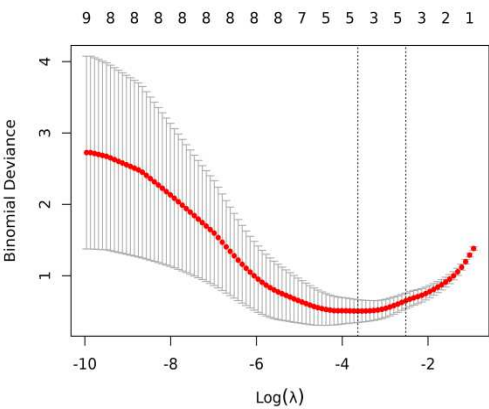
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Figure 1E PPI network diagram



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Figure 2A Lasso Regression analysis cross-validation

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The abscissa represents the logarithm of lambdas, and the ordinate represents the model error. From the above

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graph, it can be seen that the optimal value of lambda is at the lowest point of the red curve, that is, $\text{log}(\lambda)$ is

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-3.635394, where the model error is the lowest, and the corresponding 5 genes are not penalized to 0.

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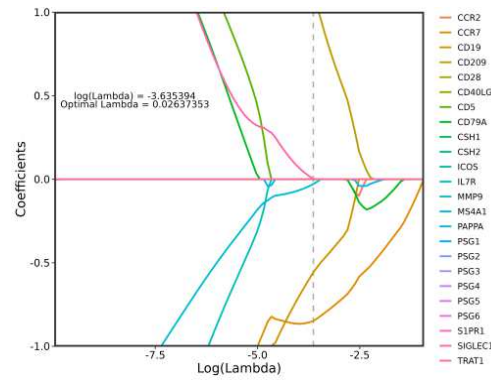
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The abscissa represents the logarithm of lambdas, and the ordinate represents the variable coefficients. As lambdas increase, the variable coefficients approach 0. When the optimal lambda is reached, the excluded coefficients equal 0 for the variables.

Figure 2B Lasso Regression analysis screening results

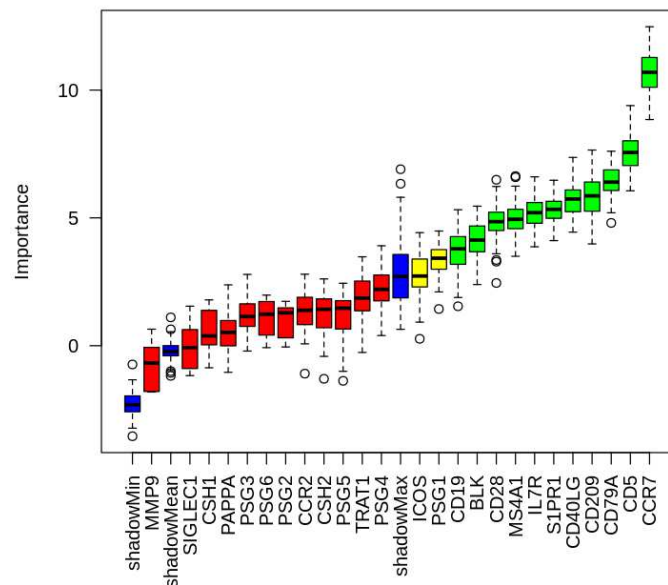
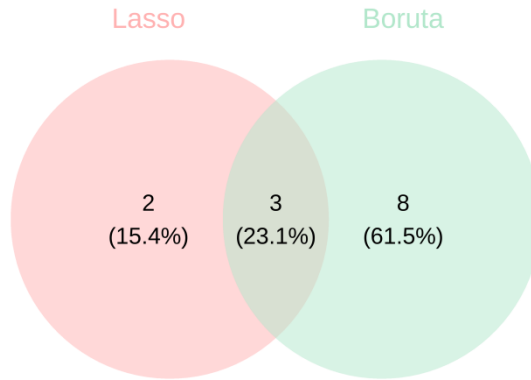


Figure 2C Boruta analysis screening results

Blue box: Minimum, average and maximum Z values of the shadow attribute; Red: Rejected features; Yellow: Features to be confirmed; Green box: Confirmed features.



In the figure, the pink areas represent the genes confirmed by Lasso regression, and the blue areas represent the genes confirmed by Boruta.

Figure 2D Key characteristic genes

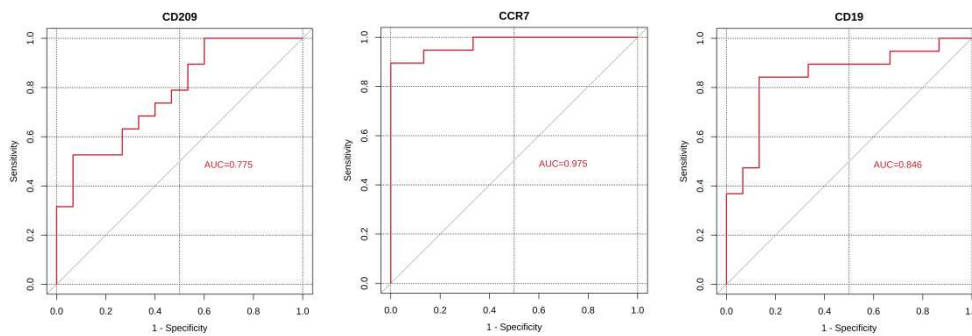


Figure 2E ROC curve of key genes

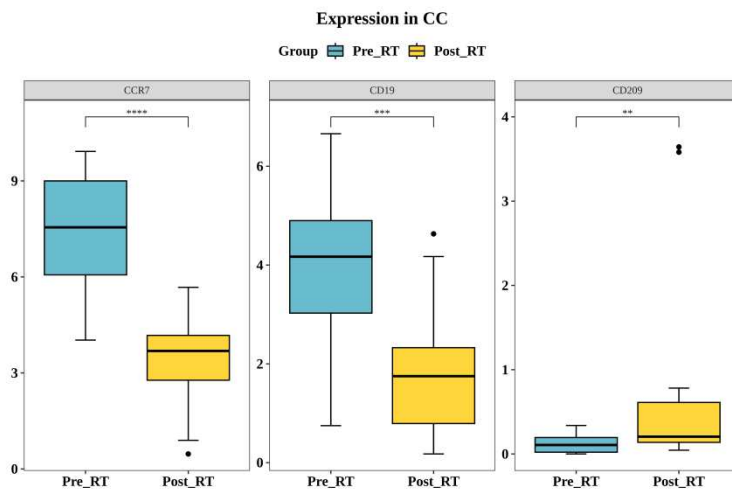


Figure 3A Analysis of gene expression levels

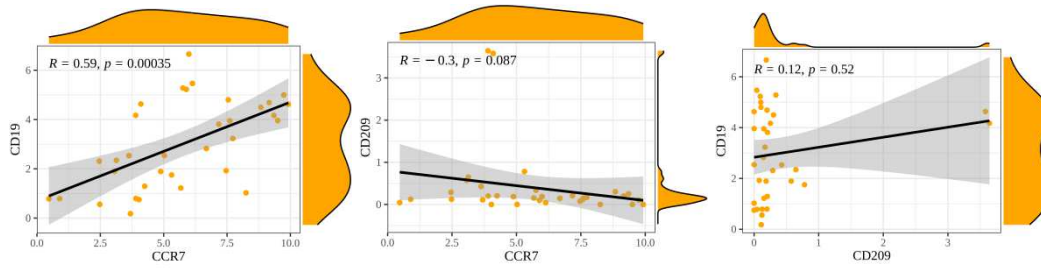


Figure 3B correlation analysis

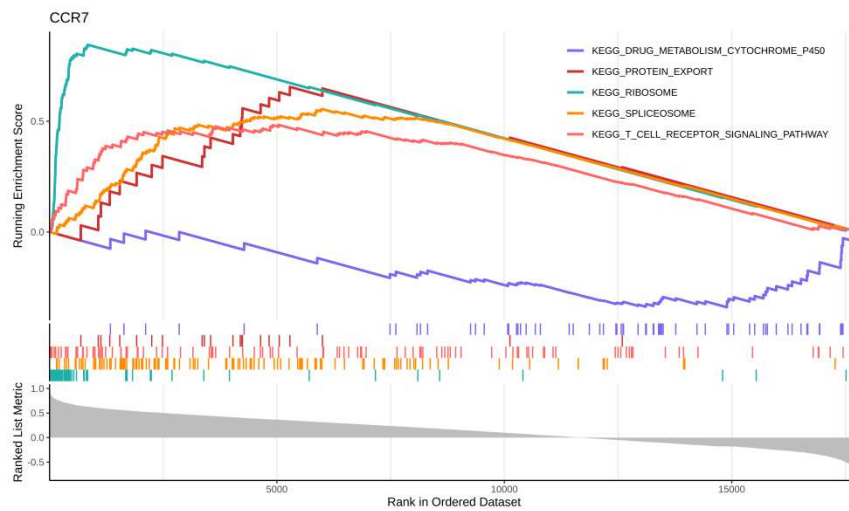


Figure 4A CCR7 enrichment analysis

The first part: The top five lines are the line graphs of the Gene Enrichment Score. The vertical axis represents the corresponding Running ES. There is a peak in the line graph, and this peak is the Enrichment Score of this gene set. The genes before the peak are the core genes of this gene set. The horizontal axis represents each gene in this gene set, corresponding to the vertical lines similar to barcodes in the second part. The second part: The part similar to barcodes represents Hits, and each vertical line corresponds to a gene in this gene set. The third part: It is the distribution graph of the rank values of all genes. The vertical axis represents the ranked list metric, that is, the value of the gene's ranking quantity.

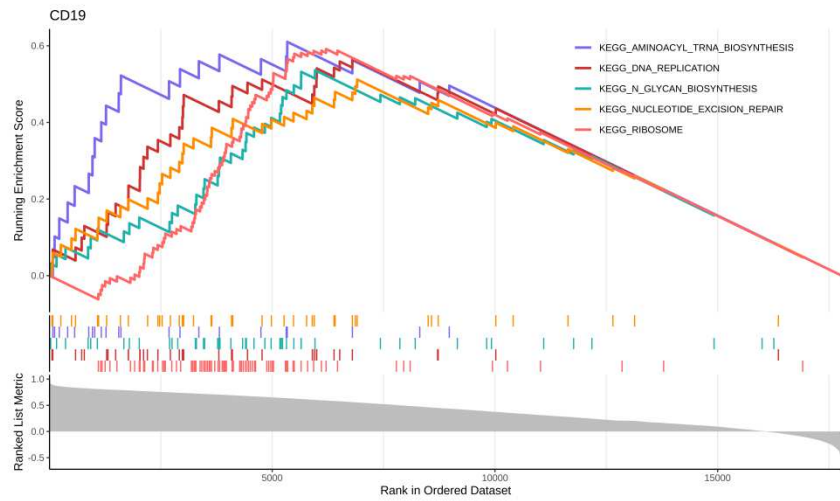


Figure 4B CD19 enrichment analysis

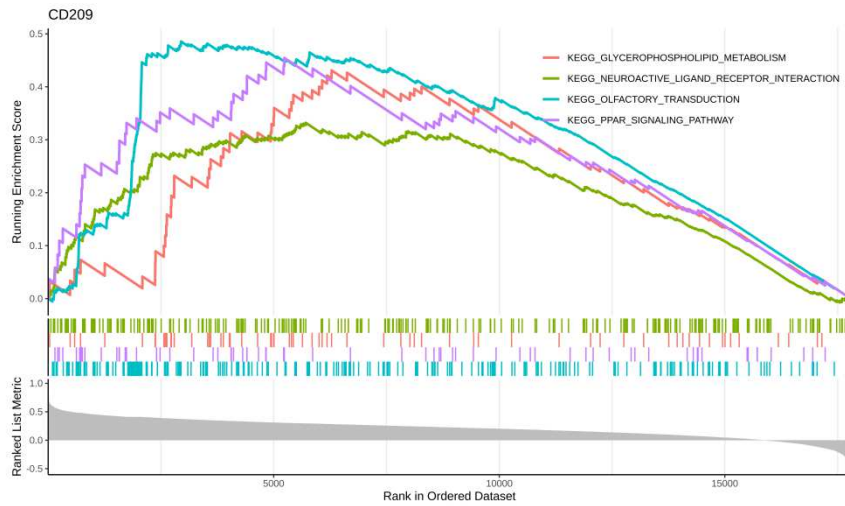


Figure 4C CD209 enrichment analysis

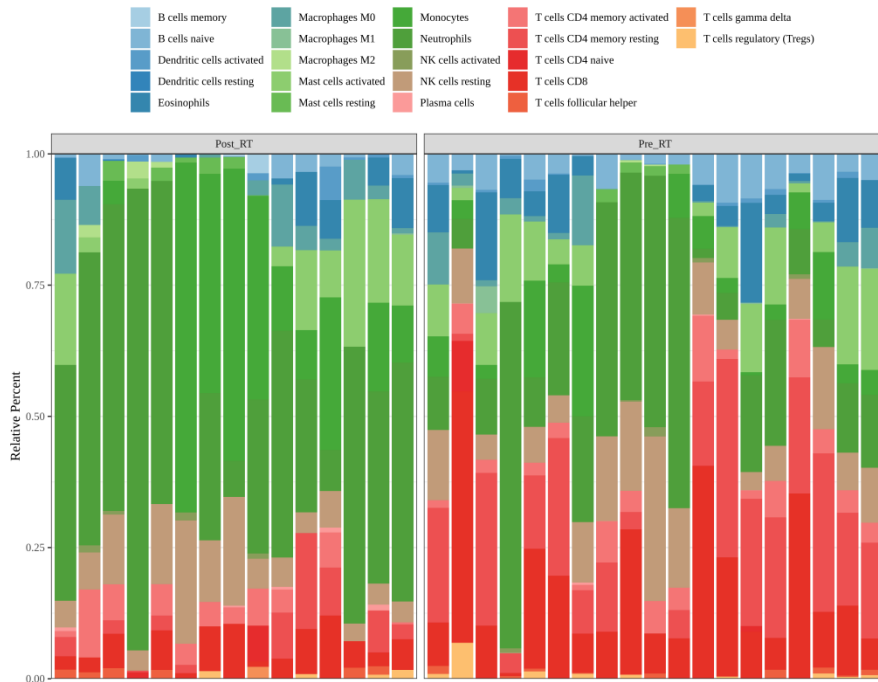


Figure 5A The infiltration of immune cells between the groups after and before radiotherapy

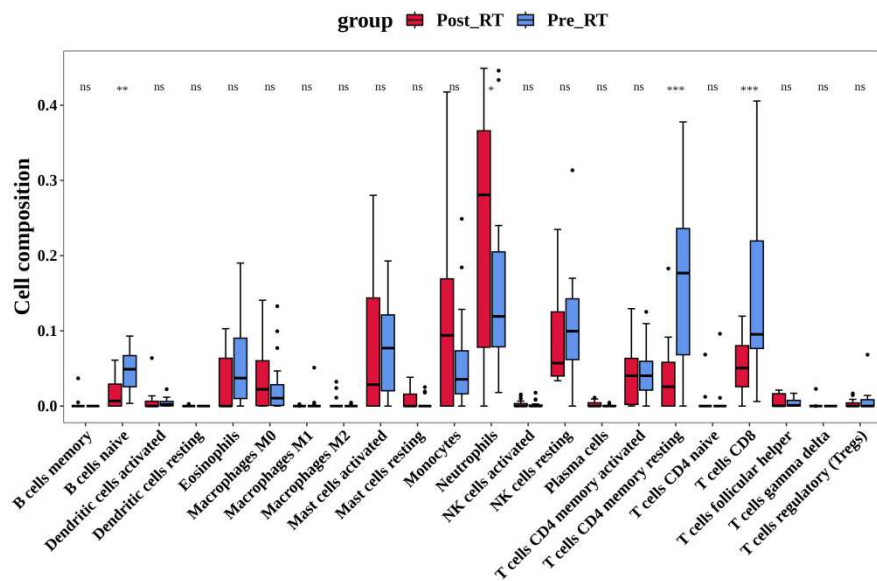


Figure 5B Differences in immune cells between groups after and before radiotherapy

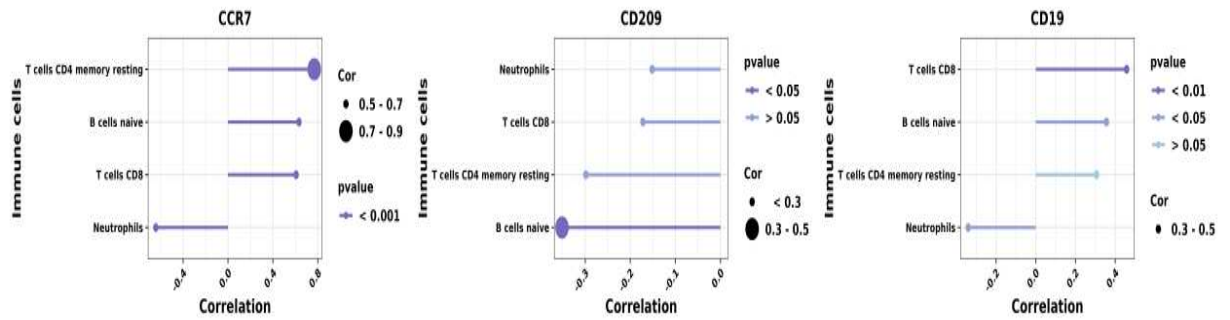


Figure 5C Correlation between radiotherapy markers and differential immune cells

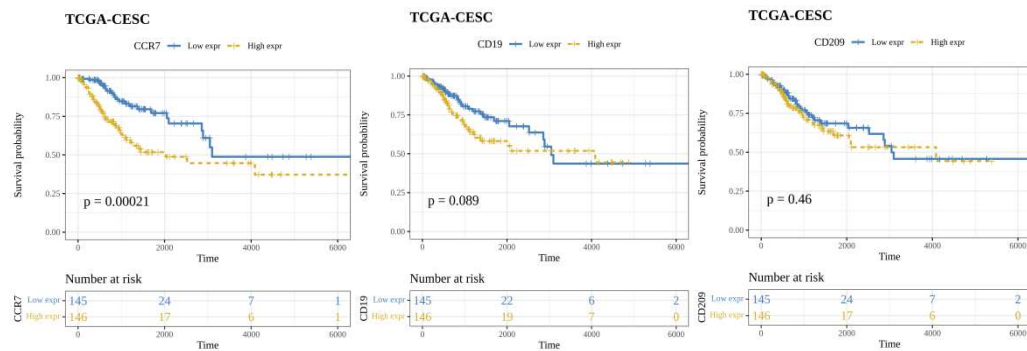


Figure 6 Analysis of the prognostic value of radiotherapy markers

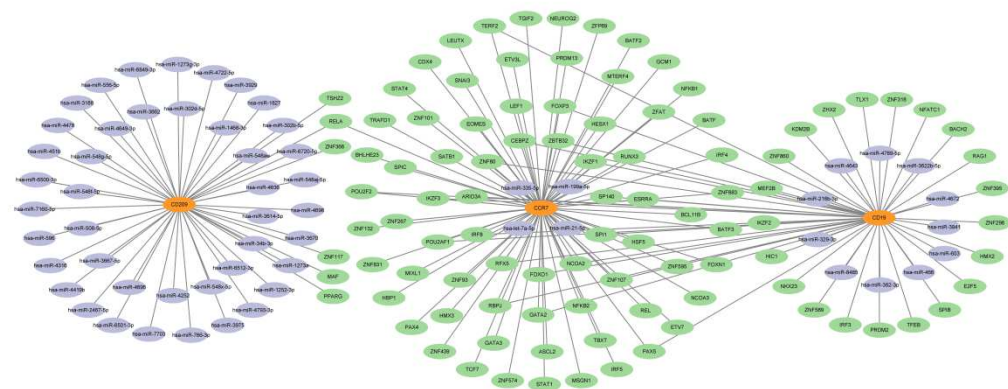


Figure 7 miRNAs-biomarkers-TF network

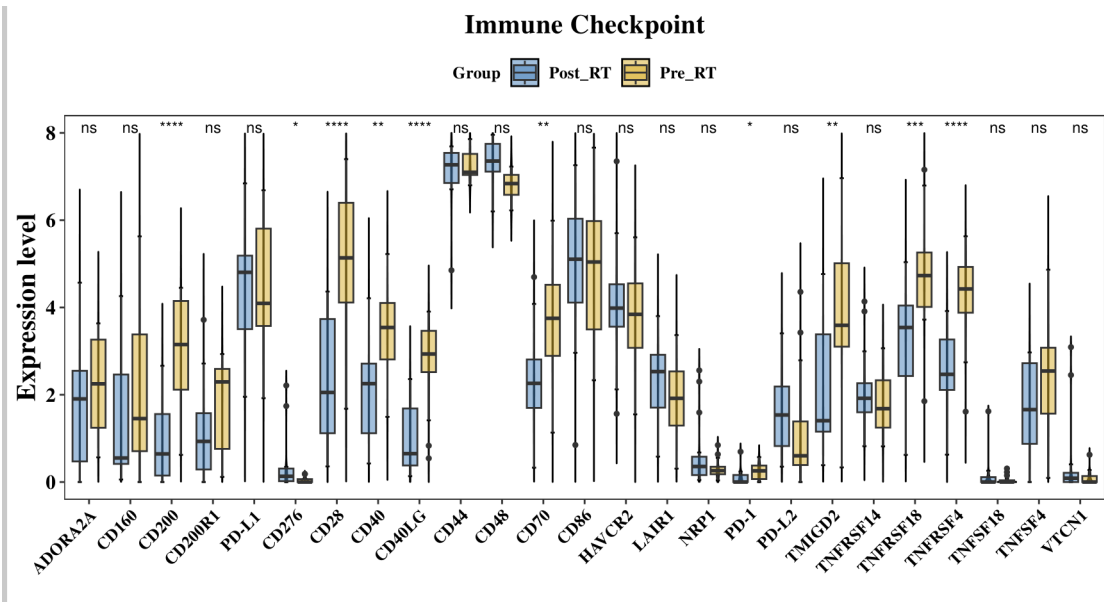


Figure 8 Box diagram

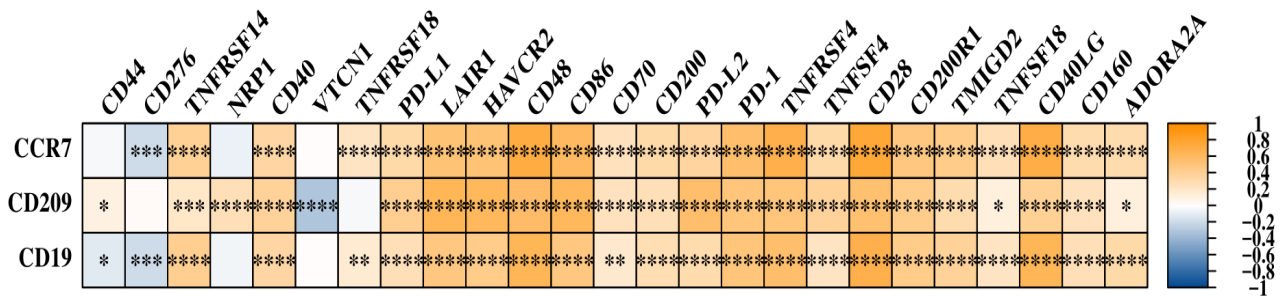


Figure 9 Spearman correlation analysis

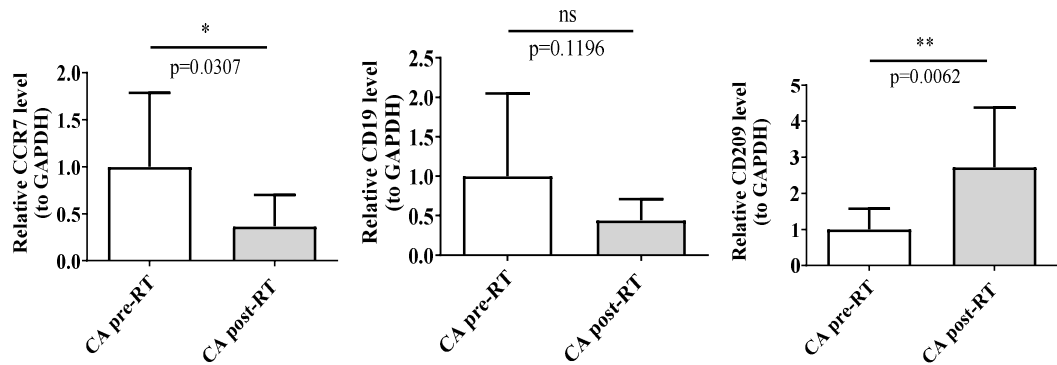


Figure10 Comparison of expression differences

Table1 QPCR verification result

	CA pre-RT	CA post-RT	p
CCR7	1±0.7875	0.3659±0.3339	0.0307
CD19	1±1.0475	0.4414±0.2673	0.1196
CD209	1±0.5763	2.7193±1.6564	0.0062