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Active Enhancer Assessment by H3K27ac ChIP-seq Reveals Claudin-1 as a Biomarker for Radiation Resistance in Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) represents the third most common malignant tumor in the worldwide. Radiotherapy is the common therapeutic treatment for CRC, but radiation resistance is often encountered. ChIP-seq of Histone H3K27 acetylation (H3K27ac) has revealed enhancers that play an important role in CRC. This study examined the relationship between an active CRC enhancer and claudin-1 (CLDN1), and its effect on CRC radiation resistance.

Methods: The target CRC genes of active enhancers were obtained from public H3K27ac ChIP-seq, and the genes highly expressed in radio-resistant CRC were screened and intersected with enhancer-driven genes. The clinical roles of CLDN1 in radiation resistance were examined using the t-test, standard mean deviation (SMD), summary receiver operating characteristic curve and Kaplan-Meier curves. The co-expressed genes of CLDN1 were calculated using Pearson Correlation analysis, and Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes and Gene Set Variation Analysis (GSVA) analyses were used to examine the molecular mechanisms of CLDN1.

Results: Total 13 703 CRC genes were regulated by enhancers using 58 H3K27ac ChIP-seq. Claudin-1 (CLDN1) was enhancer-driven and notably up-regulated in CRC tissues compared to non-CRC controls, with a SMD of 3.45 (95 CI % = .56-4.35). CLDN1 expression was increased in radiation-resistant CRC with a SMD of .42 (95% CI = .16-.68) and an area under the curve of .74 (95% CI = .70-.77). The cell cycle and immune macrophage levels were the most significant pathways associated with CLDN1.

Conclusion: CLDN1 as an enhancer-regulated gene that can boost radiation resistance in patients with CRC.

Keywords

enhancer, ChIP-sequence, claudin-1 (CLDN1), radiation resistance, colorectal cancer

Introduction

Colorectal cancer (CRC) represents the third most common malignant tumor in the world and the second most common cause of cancer-related mortalities.[1] CRC is a multifactorial disease that is affected by genetic and environmental factors, but age, nutritional status, physical activity, and other changes also play major roles in CRC development.[2-7] Not only that, early diagnosis and treatment of CRC are difficult because CRC is asymptomatic in its early stages.[8] Currently, the effective

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therapeutic strategies for CRC include combinations of surgery, chemotherapy, molecular targeted therapy, and/or radiation.[9-11] Radiotherapy, which uses high-energy rays to irradiate tumors, is an important strategy for the treatment of CRC and can reduce local recurrence and improve patient survival.[12-14] Some clinical trials have shown that radiotherapy lowers the recurrence of CRC, even without the implementation of radical surgery.[15] However, radiotherapy cannot arrest CRC development in a few patients, while other patients with CRC have tumors that are insensitive or completely resistant to radiation.[16,17]

Some research evidence has shown that a number of molecules, known as enhancers or super-enhancers, can promote the onset and development in CRC.[18,19] Enhancers as a type of cis-acting element, playing important roles in transcriptional regulation, and transcription disorders of the genome in cancer might be caused by an imbalance of enhancer activity.[20,21] A large number of research studies indicated that enhancers or super-enhancers were important regulatory factors in CRC and that an abnormal status of an enhancer would promote the onset and development of CRC.[22-24] However, acetylation modification of the 27th lysine site in histone H3 (H3K27ac) is positively related to enhancer activity,[25,26] so the modification status of H3K27 could help us to identify enhancer activity in radio-treated CRC.[26-30] Hence, worldwide screening programs for enhancers associated with CRC radiotherapy are now being implemented to increase the treatment efficacy and to amplify the search for predictors of CRC treatment responses.

One possible candidate for enhancer regulation is claudin-1 (CLDN1), a member of the integrin family that encodes the tight junction protein that maintains intercellular junctions. CLDN1 functions as a carcinogenic factor and could promote various malignant tumors, including CRC, by promoting the epithelial-mesenchymal transition (EMT), by activation of transcription factors in the colonic epithelium, or by the anoikis pathways.[31-33] Ouban et al. indicated that CLDN1 was an initiating factor for early CRC and that a high expression of CLDN1 could be detected in early-stage CRC.[34] CLDN1 expression is boosted by promoters and enhancers in CRC,[35] indicating that an enhancer might affect CRC by regulating genes like CLDN1. However, no research has yet examined the molecular patterns of CLDN1 and enhancers in radiotherapy resistance.

The aim of the present study was to overcome the adverse events of radiotherapy and to increase its curative effect in CRC. High-throughput data from multiple laboratories were enrolled, and various statistical methods were used to clarify the molecular patterns and clinical value of enhancer-driven CLDN1 in CRC patients.

Methods

Collection of Enhancer-Related Genes in Colorectal Cancer With H3K27ac ChIP-Seq Data

In the current study, we aim to explore the relation of enhancers and radio-resistance in CRC. Previous studies have confirmed that H3K27ac is a widely expressed biomarker for active enhancers and an outstanding indicator of enhancer activity.[26-30] We probed the role of enhancers in CRC by screening the H3K27ac ChIP-seq data from the NCBI-Gene Expression Omnibus (GEO) and ENCODE databases. The retrieval strategies were ((colon) OR (rectal) OR (colorectal) OR (intestine) OR (tract)) AND (H3K27ac) AND ((cancer) OR (tumor) OR (carcinoma) OR (adenocarcinoma) OR (neoplasm) OR (malignant)). We ensured the accuracy of the ChIP-seq samples by setting the following exclusion criteria: ① not the human sample, ② treated-tissues or cell lines, and ③ not the CRC tissue or cell lines. The inclusion criteria were ① the CRC tissue or cell lines and ② *Homo sapiens* and ③ untreated-tissues or cell lines. The treatments of CRC tissue or cell lines included drug treatment, transfection and so on, which were not allowed.

All the ChIP-seq samples for CRC were included in the next step. The Cistrome database included the ChIP-seq data from NCBI-GEO and ENCODE,[36] so we used the Cistrome Data Browser to search the ChIP-seq samples and identify putative enhancer-regulated genes. To screen out the more valuable genes, the regulatory score of the related genes in each sample was set as 2, and the repeat times among all samples were set to more than twice. The bigwig format data were also downloaded to show the ChIP-seq peaks of the genes that combine with H3K27ac.

The High-Throughput Data of CLDN1 Expression in Colorectal Cancer

The Expression Data of CLDN1 in Colorectal Cancer and Radio-Treated Colorectal Cancer Tissue. The process for screening public data was based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses guideline, and the last screening of the public data was on January 10, 2021. Previous research has shown that CLDN1 is an oncogene in CRC,[31-34,37,38] but the latest expression profile of CLDN1 in CRC is still unavailable, given the rapid development of high-throughput data. Therefore, the high-throughput arrays and the high-throughput RNA-sequencing data of CRC were obtained from The Cancer Genome Atlas (TCGA), GEO, ArrayExpress, and Sequence Read Archive to assess the expression status of CLDN1 in CRC patients. The retrieval strategies were colorectal AND ((carcinoma) OR (cancer) OR (malignant)). Similarly, to obtain the expression data for radio-treated CRC, the keyword "radiotherapy" was added to the retrieval strategies.

Screening Criteria for High-Throughput Data for Colorectal Cancer and Radio-Treated Tissue. The availability of the included data was ensured by employing a series of inclusion criteria. For routine CRC data, the inclusion criteria were ① cohort study or case control study; ② *H sapiens*; ③ primary CRC tissues; ④ the control group was non-tumorous tissues or normal tissues; and ⑤ CRC cancer and non-cancerous tissue controls were not treated. The exclusion criteria were ① the data were

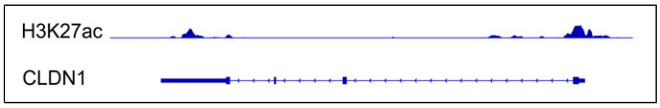

Figure 1. The binding site of H3K27ac and CLDN1 were explored by ChIP-seq. The active regions with H3K27ac were enriched in the promoter regions of CLDN1.

overlapping studies or duplicate data; ② animal samples; ③ CRC cell lines; ④ no control group; ⑤ the CRC tissues were treated; and ⑥ lack of CLDN1 expression information.

The inclusion criteria for radio-treated arrays and sequencing data were ① *H sapiens*; ② primary CRC tissue or CRC cell lines; ③ the CRC tissues were treated by radiation, and a non-treated group was present; and ④ CLDN1 expression was reported. The exclusion criteria were ① animal samples; ② the tissues were treated by other reagents or physical factors; ③ no control group; and ④ no report of CLDN1 expression.

Expression Matrix Handling for the High-Throughput Data

The processed arrays and sequencing data were downloaded according to the inclusion criteria. After extracting the expression profiles from each study, the different datasets in the same platform were removed to eliminate the batch effect using the combat algorithm in the sva package. The K-Nearest Neighbor algorithm was utilized to replenish missing values in the expression profiles. The log2 (x + 1) transformation was used for the non-normalized data to ensure a Gaussian distribution.

The clinicopathological features of the CRC patients, such as age, gender, tumor stage, tumor grade, radiation resistance, and prognostic information, were also extracted and arranged for each study to allow further analysis.

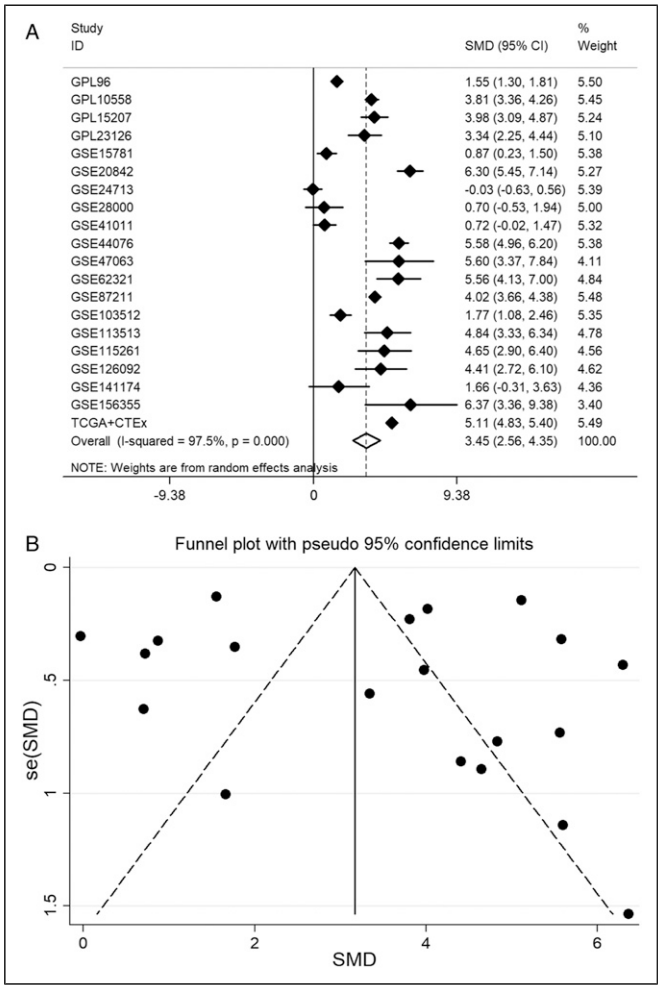

Figure 2. The Integrative analysis of CLDN1 expression in CRC. (A) The expression tendency of CLDN1 in CRC compared to non-tumorous colorectal tissue. (B) The publication bias was shown by funnel plot.

Comprehensive Expression of CLDN1 in Colorectal Cancer. A large number of studies had confirmed that CLDN1 could initiate the occurrence and development of CRC, but the latest comprehensive effects of CLDN1 in CRC patients were not known because of the continuous updating of high-throughput data. We obtained an overall review for CLDN1 in CRC by extracting the expression of CLDN1 from each study and then calculating the sample size, mean value, and standard deviation in the CRC and non-tumor groups for integrative analysis. STATA 12.0 was used to calculate the standard mean deviation (SMD) and 95% confidence interval (95% CI) to estimate the expression tendency of CLDN1 in the CRC and non-tumor groups. Funnel plots were used to assess publication bias in this integrative analysis.

At present, no research has searched for a relationship between CLDN1 and radiation resistance in CRC. Therefore, the expression data of CLDN1 in each radio-treated CRC study were extracted to determine whether CLDN1 was a radiation resistance-related gene. Student's t test and SPSS 22.0 were utilized to show the expression differences for CLDN1 in the radio-resistant group and radio-sensitive group, and the scatter diagrams with P values were constructed using Graphpad 5.0. We obtained an overall view of CLDN1 in radio-treated CRC by collecting the sample size, mean value, and standard deviation in each dataset for the calculation of the SMD. The Cochran Q test and I^2 test were used to evaluate the heterogeneity of the integrative model. The random effect model was used if high heterogeneity was noted (I^2>50% or P < .05); otherwise, the fixed-effect model was used.

We also explored the predictive power of CLDN1 for radiotherapy resistance by constructing the receiver operating characteristic (ROC) curves with Graphpad 5.0. The summary receiver operating characteristic (SROC) curve was calculated using the cut-off value (Youden's index) to delimit the best true positive, false positive, false negative, and true negative values for each dataset. The sensitivity and specificity of the SROC curve was also determined. For Student's t test, a P value less than .05 indicated a significant difference.

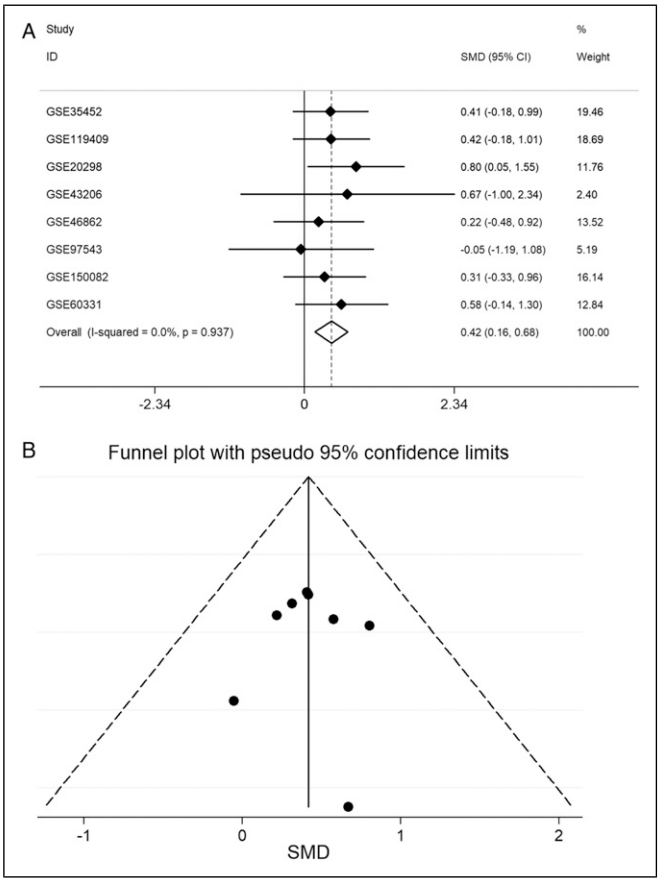

Figure 3. The Integrative analysis of CLDN1 expression in radio-treated CRC. (A) The expression of CLDN1 in radiation resistance group was higher than in radiation sensitivity group. (B) The publication bias was shown by funnel plot.

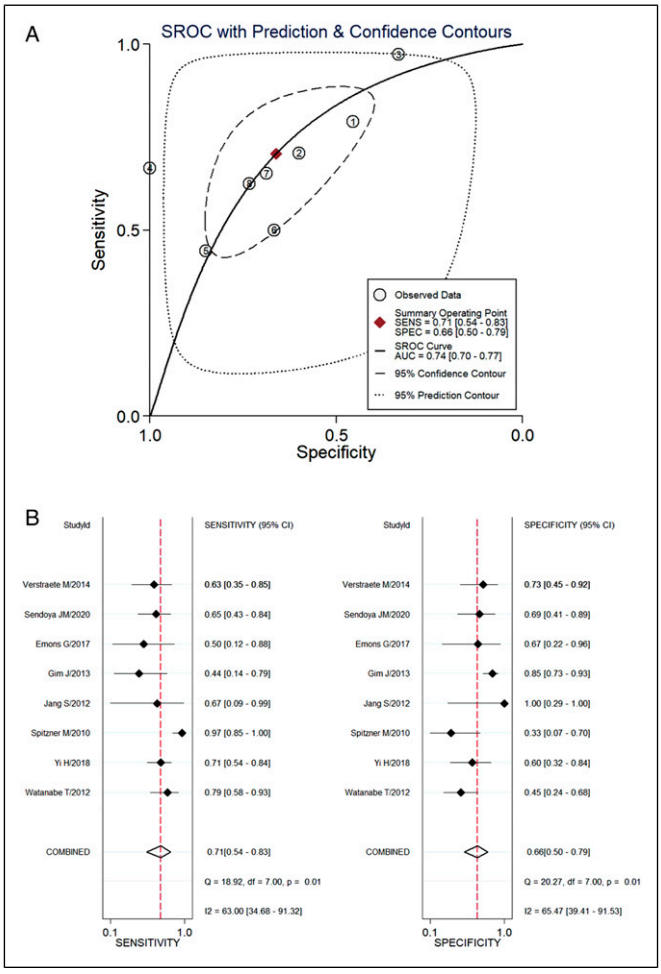

Figure 4. The judgment ability of CLDN1 between radio-resistance and radio-sensitivity in CRC. (A) SROC was used to assess the diagnosis capacity of CLDN1. (B) The forest plot of sensitivity and specificity in SROC.

Prognosis Analysis of CLDN1 in Radio-Treated Colorectal Cancer Patients. After calculating the expression difference and classification performance of CLDN1 in radio-resistance and sensitivity patients with CRC, a prognostic analysis was also performed. We acquired the prognostic value of CLDN1 in radio-treated CRC patients from the studies that provided usable prognostic information. Before starting the prognostic analysis, the CRC patients were divided into a high-CLDN1 expression group and a low-CLDN1 expression group, according to the median expression value of CLDN1. The clinical outcomes of these 2 groups were assessed by a hazard ratio calculated by Cox regression analysis. Kaplan–Meier curves were also generated, and the log-rank test was utilized to investigate the statistical difference between the 2 groups.

The Prospective Molecular Mechanism of CLDN1 in Radiation Resistance of Colorectal Cancer

In the above process, the clinical significances of CLDN1 in radio-treated patients with CRC were examined. Hence, we intended to explore how CLDN1 affect the radiotherapy resistance in CRC.

The Co-Expressed Genes of CLDN1 in Radio-Treated Colorectal Cancer. The role of CLDN1 in radiotherapy resistance was clarified by determining the co-expressed genes of CLDN1 in the radio-treated CRC datasets using Pearson correlation analyses. The genes with P values less than .05 were considered co-expressed genes. The co-expressed genes in each dataset were intersected to screen the significantly co-expressed genes of CLDN1. In addition, the molecular mechanism of enhancers in radiotherapy was determined by overlapping the CLDN1 co-expressed genes with enhancer-related genes.

Biological Function Analysis of CLDN1. The molecular mechanism of CLDN1 and its co-expressed genes were explored by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the online tool metascape (http://metascape.org/gp/index.html). The adjusted P (adj P) value was set to .05 to determine the statistical significance of the enrichment pathways. The bar plot and chord diagrams were prepared for GO analysis and KEGG

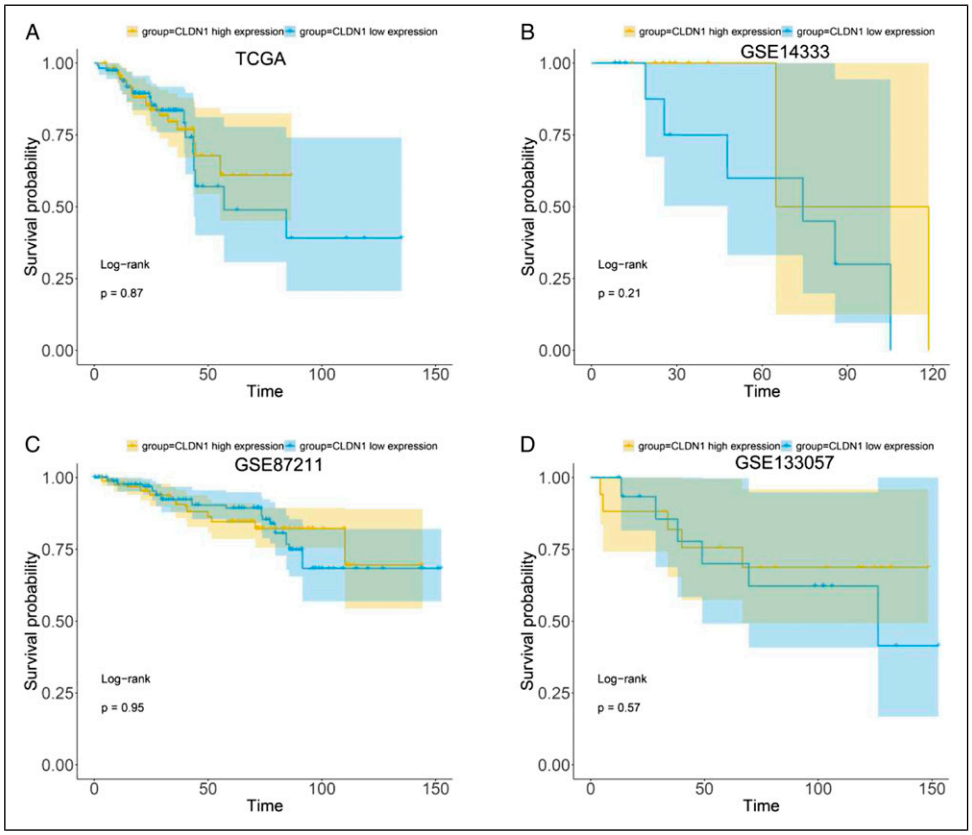

Figure 5. K-M curves and Cox regression were used to perform the prognostic value of CLDN1 for radio-treated CRC patients, whereas none of the datasets had statistical significance. (A) TCGA. (B) GSE14333. (C) GSE8721. (D) GSE133057.

pathway analysis using the GOplot and ggplot2 packages. We explored the molecular mechanism of CLDN1 and its co-expressed genes in radiation resistance using the included datasets from radio-treated patients and Gene Set Variation Analysis (GSVA). In GSVA, the radiation-resistant group was the experimental group and the radiation-sensitive group was the contrast group, and the KEGG set was used as the background gene set. A pathway with a log fold change (logFC) > .5 and adj $P < .05$ was considered a significant pathway.

The Relation Between CLDN1 and the Microenvironment of Colorectal Cancer. Events in the tumor microenvironment, such as immune infiltration, can affect the occurrence, development, and resistance of a tumor. In this study, the relationships between CLDN1 and immune infiltration in CRC were investigated at the tissue and cellular levels.

We searched for the relevance of CLDN1 and the microenvironment by calculating the correlation between CLDN1 and immune infiltration (including $CD8^+$ and $CD4^+$ T cells and macrophages) in CRC with the TIMER algorithm and Pearson correlation analysis with the TIMER2.0 database (http://timer.cistrome.org/). The effects of immune infiltration and CLDN1 on CRC patient outcomes were also explored by Cox regression analysis.

CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/) is a database that enrolls multiple single-cell RNA sequence (scRNA-seq) data for various cancers. The relationship between CLDN1 and cellular functions (angiogenesis, cell cycle, DNA damage, EMT, etc.) of CRC was assessed with the CancerSEA database.

Results

The Relevant Enhancer Genes in Colorectal Cancer

H3K27ac can distinguish active from inactive enhancers.[26-30] Here, a total of 58 ChIP sequencing datasets for CRC with H3K27ac were gathered according to the inclusion criteria. The potential genes in these samples, downloaded from Cistrome, were considered regulatory genes of enhancers or super-enhancers. In total, 13 703 likely enhancer-regulated genes were screened after pooling the genes in the 58 samples. CLDN1 was a significant enhancer-regulated gene. As shown in Figure 1, H3K27ac had good binding ability with CLDN1.

The High-Throughput RNA-Sequences and Microarrays of Colorectal Cancer

The High-Throughput Data of Colorectal Cancer Tissues. We intuitively displayed the expression of CLDN1 in CRC using

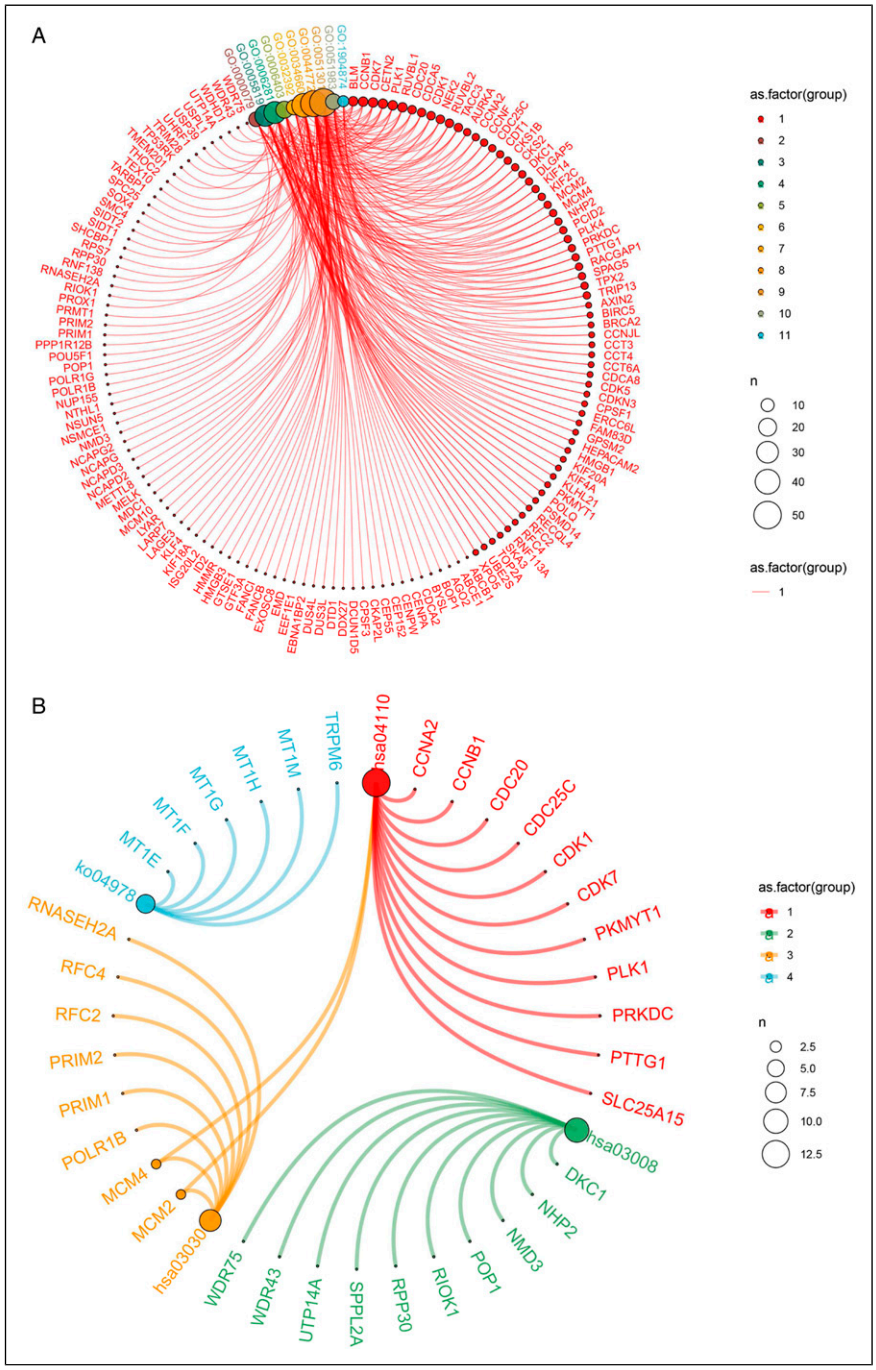

Figure 6. GO and KEGG analysis of CLDN1 in CRC. (A) GO analysis. The red points were the genes enriched in the GO terms, and the colorful points were the GO terms. (B) KEGG pathway analysis. Each color was corresponded to each part that was composed of a pathway and genes enriched in the pathway. Pathway hsa04110: Cell Cycle; hsa03030: DNA replication; hsa03008: Ribosome biogenesis in eukaryotes; ko04978: Mineral absorption.

the 58 CRC datasets from 20 high-throughput platforms, including 1446 tumor samples and 1084 non-tumorous samples, and screening them according to the inclusion criteria (Supplemental Figure 1). The sample size was sufficient for the integrative analysis. Eight radio-treated CRC array and sequencing datasets, including 157 radio-resistant samples and 146 radio-sensitive samples, were concurrently screened and processed (Supplemental Figure 2).

The Expression of CLDN1 in Colorectal Cancer Tissues. Previous research had shown higher expression of CLDN1 in CRC than in normal tissue; however, no firm conclusion was drawn

because of the need to update the most recent high-throughput arrays and the high-throughput RNA-sequencing data. We obtained an overall result by calculating the SMD of CLDN1 in CRC based on 1446 tumor samples and 1084 non-tumorous samples after removing the batch effect. This yielded an SMD value of 3.45 (95% CI = .56-4.35). A random effect model was used because of the high heterogeneity (I^2 = 97.5%, P = 0) (Figure 2A). The funnel plot showed no publication bias, and the highly expressed CLDN1 agreed with the previous research (Figure 2B).

The Expression of CLDN1 in Radio-Treated Colorectal Cancer. We intuitively displayed the expression tendency of CLDN1 in radio-treated data by constructing scatter diagrams for the 8 datasets (Supplemental Figure 3). We also performed an integrative analysis based on the 157 radio-resistant samples and 146 radio-sensitive samples in 8 radio-treated datasets and obtained an SMD of .42 (95% CI = .16-.68). No heterogeneity was evident in the model, so the fixed-effects model was used (Figure 3A). The funnel plot showed no publication bias (Figure 3B).

We evaluated the discernment capacity of CLDN1 to radioresistance by constructing the ROC curve for the 8 radio-treated CRC datasets (Supplemental Figure 4). The integrative results showed that the area under the curve of the SROC curve was .74 (95% CI = .70-.77), with a sensitivity and specificity (sensitivity = .71 and specificity = .66) (Figures 4A and B).

The Cox regression and Kaplan–Meier curves were used to reveal the prognostic value of CLDN1 in radio-treated CRC patients. In this study, a total of 634 radio-treated CRC patients with prognosis information were screened from the TCGA, GSE14333, GSE87211, and GSE133057 datasets. However, no statistical difference was detected (Figures 5A–D).

The Prospective Molecular Mechanism of CLDN1 in Radiotherapy of Colorectal Cancer

Co-Expressed Genes of CLDN1 in Radio-Treated Colorectal Cancer. A total of 450 co-expressed genes were considered significant CLDN1-related genes after overlapping the 8 datasets. In addition, 398 overlapping genes between CLDN1 co-expressed genes and enhancer-related genes were screened for enrichment analysis (Supplemental Figure 5).

The Signaling Pathways of CLDN1 in Radiotherapy of Colorectal Cancer. The GO and KEGG pathways were explored to determine the molecular mechanism of CLDN1 based on the overlapping genes. The top 3 terms in the GO analysis were cell division, mitotic cell cycle phase transition, and noncoding RNA (ncRNA) metabolic processes, which were sorted by the adjP value (Figure 6A). Only 4 pathways were significant in the KEGG analysis: the pathways with adjP from low to high were the cell cycle, DNA replication, ribosome biogenesis in eukaryotes, and mineral absorption (Figure 6B).

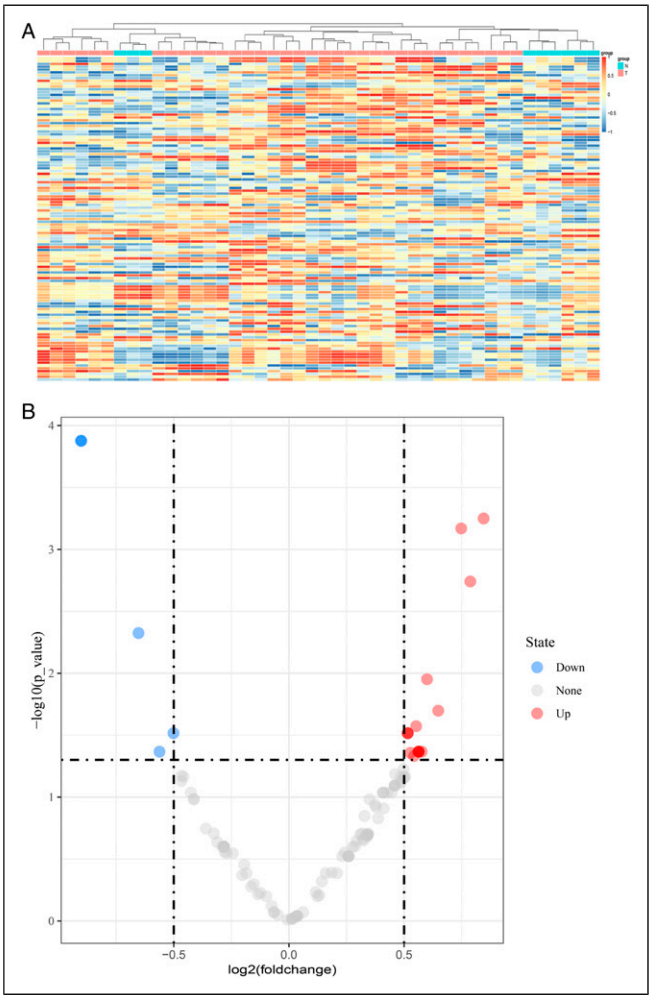

Figure 7. GSVA analysis of CLDN1 in radio-treated CRC. (A) GSVA analysis. The expression level of pathways in radio-treated CRC dataset was calculated and the result of GSVA analysis was shown by heat map. (B) Volcano plot was utilized to exhibit the differently expressed pathways; the red terms were the potential pathways which promoting the radio-resistant effect in CRC.

GSVA analysis based on the radio-treated datasets revealed only GSE20298 with a significant pathway. Heat maps and volcano plots were used to reflect the expression differences in the pathways in GSE20298 (Figures 7A and B). In the case of the resistant group as the experimental group, the pathways with logFC > .5 were the promoter factors in radiotherapy resistance. The pathways in GSVA were the glycosphingolipid biosynthesis lacto and neolacto series, N-glycan biosynthesis, and steroid biosynthesis.

Tumor Microenvironment Assessment of CLDN1 in Colorectal Cancer. We examined the correlation between CLDN1 and the microenvironment of CRC based on tissue and cellular aspects. CLDN1 was positively related to $CD8^+$ T cell, neutrophil, and macrophage levels in rectal cancer (READ) patients, and a high macrophage level indicated poor prognosis in READ patients

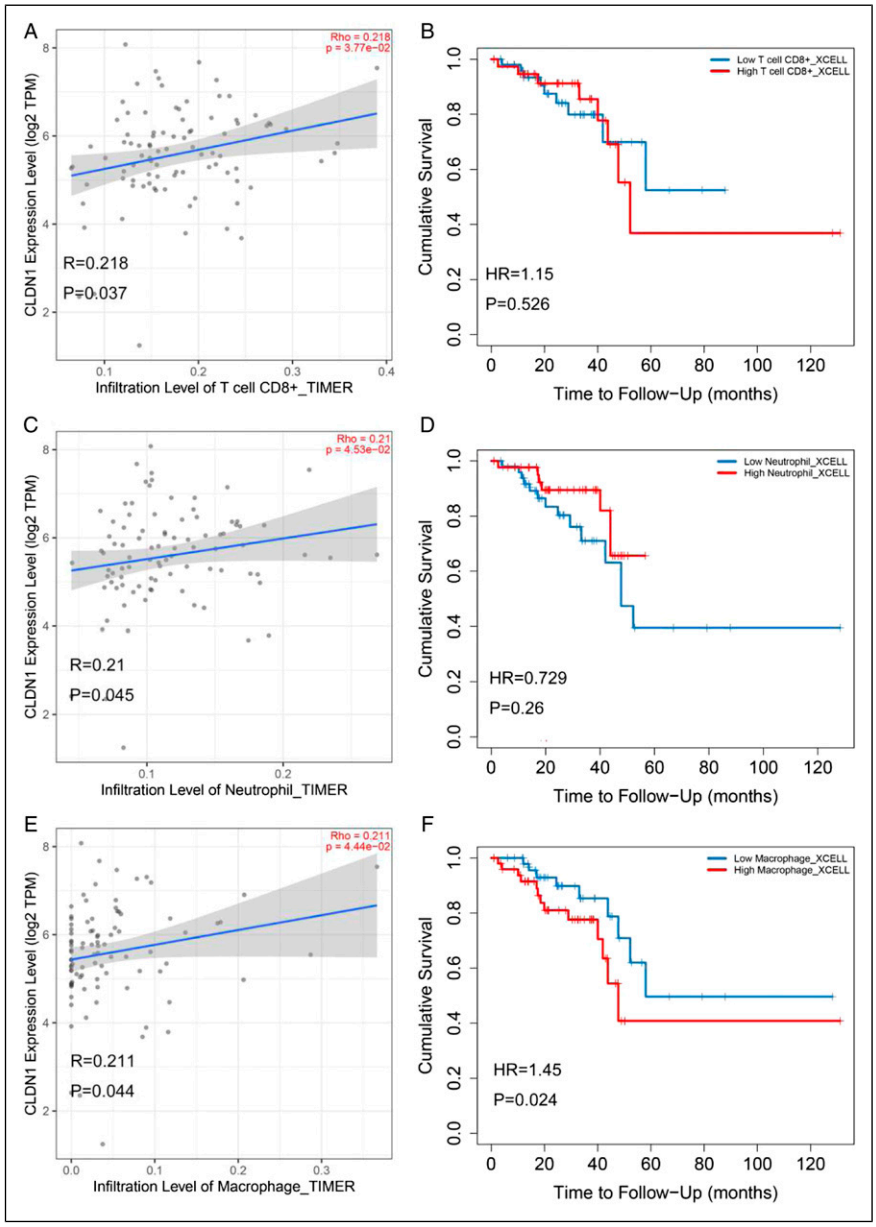

Figure 8. The correlations between CLDN1 and immune infiltration level in CRC patients were performed, as well as the outcomes of different immune environment. (A) CLDN1 was positively correlated to T cell $CD8^+$ level. (B) T cell CD8 + was not the risk factor for CRC patients. (C) CLDN1 was positively correlated to Neutrophil level. (D) Neutrophil level was not associated with the prognosis of CRC patients. (E) CLDN1 was positively correlated to Macrophage level. (F) High Macrophage level was a risk factor for CRC patients.

(Figures 7A–F and Figure 8). CLDN1 was negatively correlated with the EMT, according to the scRNA analysis (Supplemental Figure 6).

Discussion

The relationship between enhancers or super-enhancers and radiotherapy resistance in CRC is still unknown. In this study, the potential regulatory genes of enhancers were screened based on multiple ChIP-seq samples with the H3K27ac hallmark, and the clinical value and possible molecular mechanism of enhancer genes were also explored. CLDN1 was identified as a remarkable enhancer gene; therefore, high expression of CLDN1 could be considered a risk factor for radiation resistance in CRC patients. The high expression level of CLDN1 in CRC tissue was also now confirmed based on the latest high-throughput data.

A large number of studies have shown that enhancers play a key role in the onset and development of tumors, including CRC[22-24] and that H3K27ac is associated with a higher activation of transcription and therefore serves as an active enhancer mark.[26-30] Therefore, we explored the mechanism of

enhancers in CRC from 58 ChIP-seq samples of CRC with H3K27ac and obtained the enhancer-related genes from Cistrome. The genes that appeared more than twice in the 58 ChIP-seq samples were seen as credible enhancer genes, and CLDN1 appeared 4 times. Research on enhancers and CLDN1 in CRC has rarely been conducted, but the present study shows that CLDN1 is a potential enhancer-regulated gene based on the multiple ChIP-seq data.

The function of enhancers in CRC has been reported,[22-24] but research on enhancers and radiotherapy effects is still lacking. We explored the resistance effects of enhancers in CRC by screening and intersecting the enhancer-driven genes and differently expressed genes in radio-treated CRC datasets. Interestingly, the expression of CLDN1 was higher in 157 radio-resistant samples than in 146 radio-sensitive samples, according to the SMD model. Moreover, the SROC curve showed that CLDN1 expression could be considered a risk factor for a radio-resistant effect. However, the prognostic analysis showed that no correlation was detected between CLDN1 and radio-treated patients with CRC. The reason may be due to the lack of sufficient clinical data. So, according to the ChIP-seq data, SMD model, and SROC curve, CLDN1 can be seen as a pivot between an enhancer and a radiation effect, as the enhancers may strengthen the expression of CLDN1 to increase resistance to radiotherapy in patients with CRC.

CLDN1 is an oncogene in CRC, and it can regulate the occurrence and development of CRC by multiple biological effects on processes such as the EMT.[31] We obtained a more comprehensive biological mechanism by employing CLDN1 and its co-expressed genes to explore the molecular mechanism of enhancers in CRC radiation resistance. A total of 398 co-expressed genes of CLDN1 were calculated based on the data from radio-treated patients with CRC and intersected with the regulatory genes of the enhancer. KEGG analysis indicated the involvement of 4 pathways—the cell cycle, DNA replication, ribosome biogenesis in eukaryotes, and mineral absorption—in the potential molecular mechanism regulated by enhancers in CRC radiation resistance. Of these, the cell cycle is the most significant pathway. The GSVA analysis further indicated the biosynthesis-related pathways were the potential molecular mechanism in CRC radiation resistance. Previous studies have reported that CLDN1 can promote the onset and development of CRC by inhibiting cell apoptosis and promote the EMT pathway.[39] The EMT is also an important pathway affected by CLDN1, according to single-cell analysis. Both the cell cycle and a strong EMT effect could lead to radiation resistance in tumor cells.[40] Therefore, CLDN1 could be considered a booster of radiation resistance in CRC.

High expression of CLDN1 has also been reported in CRC tissue compared to normal tissue, but the latest expression tendency is unknown because of the new arrays and sequencing data. In this study, the public high-throughput data were enrolled, and the integrative analysis was calculated after removing the batch effect. Compared to non-tumorous colorectal tissue, the expression of CLDN1 is up-regulated in CRC. The heterogeneity of the results might be due to the following: ① too many datasets on different platforms and ② the influence of other factors in CRC patients, such as age, gender, and clinical stage. Nevertheless, CLDN1 could be seen as an oncogene in CRC because of the large sample size examined here.

Nevertheless, some limitations still exist in our study. The first is that the expression difference for CLDN1 in independent radio-treated data is not remarkable, although the integration analysis shows a significant difference. Other factors, such as tumor stage, tumor grade, or gender, are likely to change the expression of CLDN1 in CRC patients who undergo radiotherapy, but these clinical information details were not recorded in the selected studies. Even so, CLDN1 can be seen as a predictor of radiation resistance according to the SMD and SROC obtained here. Another limitation is that we need more in vivo or in vitro experiments to explore the molecular mechanism of CLDN1 in CRC radiation resistance.

Conclusion

According to the H3K27ac ChIP-seq and various high-throughput datasets, CLDN1 appears to function as an enhancer-regulated gene that acts as a biomarker in CRC patients with radiation resistance.

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Declaration of Conflicting Interests

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Supplemental material

Supplemental material for this article is available online.

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