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Low Tumor Infiltrating Mast Cell Density Reveals Prognostic Benefit in Cervical Carcinoma

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Abstract

Objectives: Research on the role of mast cells (MCs) in cervical tumor immunity is more limited. Therefore, our study aimed to evaluate the prognostic value of MCs and their correlation with the immune microenvironment of cervical carcinoma (CC). **Methods:** The Cancer Genome Atlas (TCGA) data was utilized to obtain the degree of immune infiltration of MCs in CC. Meanwhile, this study retrospectively collected patient clinical characteristic data and tissue specimens to further verify the relevant conclusions. Mast cell density (MCD) was measured by the CIBERSORT algorithm in TCGA data and immunohistochemical staining of tryptase in CC tissues. Finally, differentially expressed genes (DEGs) of TCGA data were performed using "limma" packages and key gene modules were identified using the MCODE application in Cytoscape. **Results:** The results showed MCs were diffusely distributed in CC tissues. Moreover, we found that low tumor-infiltrating MCD was beneficial for overall survival (OS) in the TCGA cohort. Consistent conclusions were also obtained in a clinical cohort. In addition, a total of 305 DEGs were analyzed between the high tumor-infiltrating MCD and low tumor-infiltrating MCD group. Seven key modules, a total of 34 genes, were screened through the MCODE plug-in, which was mainly related to inflammatory response and immune response and closely correlated with cytokines including CSF2, CCL20, IL1A, IL1B, and CXCL8. **Conclusion:** In short, high tumor-infiltration MCs in CC tissue was associated with worse OS in patients. Furthermore, MCs were closely related to cytokines in the tumor microenvironment, suggesting that they collectively played a role in the immune response of the tumor. Therefore, MCD may be a potential prognostic indicator and immunotherapy target of CC.

Keywords

cervical carcinoma, mast cell, CIBERSORT, prognosis, tumor microenvironment

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Abbreviations

MC, mast cell; CC, cervical carcinoma; TCGA, The Cancer Genome Atlas; MCD, mast cell density; DEGs, differentially expressed genes; OS, overall survival; ROC, receiver operating characteristic curve; TME, tumor microenvironment; FIGO, International Federation of Gynecology and Obstetrics; IHC, immunohistochemistry; GO, Gene Ontology; PPI, protein–protein interaction network; BP, biological process, NF-kB, nuclear factor kB; VEGF, vascular endothelial growth factor; FGF-2, fibroblast growth factor; MMP, matrix metalloproteinases; MUCs, mucins.

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Introduction

Death due to cancers is a growing threat to human survival.[1] Cervical carcinoma (CC) is one of the leading causes of cancer death in women. Globally, CC accounts for almost 12% of all female cancers, making it the fourth most common female cancer in the world.[2,3] According to the 2018 Global Cancer Statistics report, the incidence and mortality of CC accounted for 6.6% and 7.5% of all female cancer patients, respectively.[4] As a highly heterogeneous disease, tumor microenvironment (TME) plays an important role in the occurrence, development, and prognosis of CC. Normal TME plays a central role in maintaining tissue homeostasis and serves as a barrier to tumorigenesis, while abnormal TME plays an opposite role.[5] Tumor-infiltrating immune cells, as an important component of TME, play a promote or anti-tumor function by influencing the host immune status and biological behavior.[6] Therefore, a better understanding of the TME is beneficial to the management of CC.

Mast cells (MCs), as innate immune cells, have been generally recognized for their roles in allergy and inflammation.[7] MCs are widely distributed in all tissues and are highly pluripotent cells that secrete numerous vasoactive and pro-inflammatory mediators,[8] proteases, cytokines, and chemokines.[7] However, the function of MCs in regulating TME has remained largely unknown.

As a source of pro-tumor and anti-tumor factors, MCs have dual roles in tumors, while in rare cases, they may be simple indolent bystanders.[9] On the one hand, in some tumors, MCs are always favorable for tumor development and are significantly associated with poor prognosis;[10,11] on the other hand, in some tumors, MCs always seem to exert anti-tumor effects and confer a survival advantage.[12] In CC, several studies have investigated the relationship between MCs and development with conflicting conclusions. Graham et al reported that the number of MCs decreased with tumor progression,[13] whereas Benitez-Bribesca et al, Wilkins et al, and Ferrandina et al supported that MCs infiltration increased with cancer development and was often associated with worse prognosis.[14–17] Therefore, studies that can reveal the relationship between mast cell density (MCD) and CC prognosis are urgently needed.

Here, we performed an immune infiltration study on The Cancer Genome Atlas (TCGA) cohort using the CIBERSORT algorithm. Meanwhile, the prognostic relationship between MCD and CC patients was also evaluated by collecting clinicopathological data and immunohistochemical staining of tissue specimens with MCs tryptase. Subsequently, differentially expressed genes (DEGs) analysis was conducted on TCGA data through the "Limma" package in R software. Finally, the MCODE plug-in in Cytoscape software was utilized to explore DEGs to observe the relationship between MCs and key genes. In brief, this study reduced the controversy of MCs in CC, suggesting that MCD was a potential prognostic biomarker and therapeutic target in CC.

Materials and Methods

TCGA Cohort Acquisition

The transcriptome profiling data of 253 patients with cervical squamous cell carcinoma were obtained from the TCGA database (https://portal.gdc.cancer.gov/cart). We finally analyzed the cases of 222 patients through sorting. Moreover, when we acquired the mRNA data of CC patients, duplicate values were deleted, and the "limma" package in R software was used to correct it into the corresponding input file. TCGA, as a publicly published database, requires neither the ethics committee's approval nor the patient's informed consent to obtain information.

CIBERSORT

CIBERSORT is an algorithm based on support vector machine regression that can calculate the cell composition of the tissue from the gene expression profile using the deconvolution method.[18] In our study, CIBERSORT was applied to TCGA data to obtain the estimated proportion of immune cell types in each tumor sample by using LM22, which was regarded as a reference expression signature for 100 permutations.

Clinical Patients Data Collection

Clinical data and paraffin specimens of CC patients who underwent radical primary tumor resections without prior treatment in Xinjiang Medical University Affiliated Tumor Hospital (Xinjiang, China) were collected retrospectively. Cases that received chemotherapy or radiotherapy before surgery and had autoimmune diseases were excluded. After excluding patients with incomplete information, 43 patients were eventually involved. All the cases of the study were confirmed to be CC by surgery and pathology. And the cancer was staged

according to the recommendations of the International Federation of Obstetrics and Gynecology (FIGO).

Immunohistochemistry

Formalin-fixed paraffin-embedded surgical specimens were used for the immunohistochemistry (IHC) study. The main steps of IHC were as follows: section drying, xylene dewaxing, graded alcohol hydration, phosphate buffered saline washing, antigen retrieval (microwave oven for 15 min), endogenous peroxidase inactivation, goat serum blocking, primary antibody overnight, secondary antibody incubation, diaminobenzidine staining, hematoxylin counterstaining, etc. The primary antibody was composed of rabbit anti-human polyclonal mast cell tryptase (diluted 1:100, Beijing Boiynthesis Biotechnology Co). Positive staining was assessed by computer-aided image analysis and Image J software. The evaluation of MC infiltration and density was determined from 3 random areas by 2 independent pathologists who were blinded to the pathology and clinical status of the patients.

Differentially Expressed Genes Analysis

R software (version 4.1.1) was utilized to standardize data preprocessing and ID conversion. DEGs analysis on microarray data from the TCGA repository was screened by the "Limma" package.[19] At least a 2-fold change was set and the adjusted *p*-value was .05. The results were visualized as a volcano plot and a heat map using "ggplot2" and "Pheatmap" packages of R software. Besides, Uniprot, a website (https://www.uniprot.org/) that can provide the comprehensive and high-quality resource of freely accessible protein sequence and functional information, was used for the unified standardization of DEGs.

Protein–Protein Interaction Network Creation

We analyzed the protein–protein interaction (PPI) of DEGs via STRING (http://string-db.org/) online database. The interaction with a combined score > 0.7 was selected as significant. An interactive network of component-target was visualized by Cytoscape software (version 3.7.1). Furthermore, MCODE, a density-based nonoverlapping clustering algorithm plug-in of Cytoscape software, was performed to establish a PPI network for identifying key gene modules. The scoring parameters for cluster analysis followed the default parameters of the application.

Gene Ontology Analysis

DAVID 6.8 (https://david.ncifcrf.gov/home.jsp) is the website that can clarify the biological functions of genes.[20] We performed gene ontology (GO) pathway enrichment analysis. *P*-value <.05 was considered to be statistically different.

Correlation Analysis Between MCs and MCODE Module of Genes

The correlation between MCs and key gene modules obtained from the MCODE plug-in was further learned to determine potential functions. Use the "circlize" package in R to perform the correlation analysis on the top 2 gene-rich clusters. Furthermore, in R software, the package of "ggcorrplot" and "ggthemes" was utilized to analyze the relationship between MCs infiltration and gene-rich clusters.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (version 26.0), GraphPad Prism (version 8.0.1), and R software (version 4.1.1). A two-sided $P < .05$ was regarded as statistically significant. Cutoff values for the MCs were calculated by receiver operating characteristic curve (ROC) analysis for grouping. Kaplan-Meier analysis and the Log-rank test were used to evaluate the relationship between MCD and overall survival (OS). Pearson's chi-square test or Fisher's exact test was used to assess the association between clinicopathological features and MCD.

Results

Relationship Between MCD and Clinical Characteristics of CC Patients

CIBERSORT algorithm was used to obtain the composition of 22 kinds of immune cells in the TCGA cohort of CC (n = 222) (Supplemental Table 1). It can be intuitively seen that macrophages and T cells accounted for the main part (Supplemental Figure 1). We then integrated 22 types of immune cells with patient clinical data to explore implications for patient outcomes. In short, MCs, CD4T cells, and NK cells were associated with statistically significant prognosis in CC patients (all $P < .05$, Supplemental Table 2).

To be more specific, Kaplan-Meier survival analyses and Log-rank tests were utilized to evaluate the prognostic value of MCD in CC. It was found that high tumor-infiltrating MCD was related to the poor prognosis of the patients ($p = .0003$, Figure 1A). We further carried out validation in a clinical patient. In the clinical patient cohort, we also gained a negative relationship between MCD and patient prognosis ($p = .0475$, Figure 1B). Cutoff value was set at 12.42% to define the high and low tumor-infiltrating MCs (Supplemental Table 3). A total of 43 CC patients were included in this study. Detailed descriptions of patients' demographic and clinicopathological characteristics were shown in Table 1. By using immunohistochemical staining of tryptase to identify MCs, MCs were dispersed in tumor tissues at different densities (Figure 2). Subsequently, the relationship between MCD and clinical features was accessed (Figure 3, Supplemental Table 3). Except for age ($p = .008$), other clinical variables were not related to the tumor-infiltrating MCs in CC tissue.

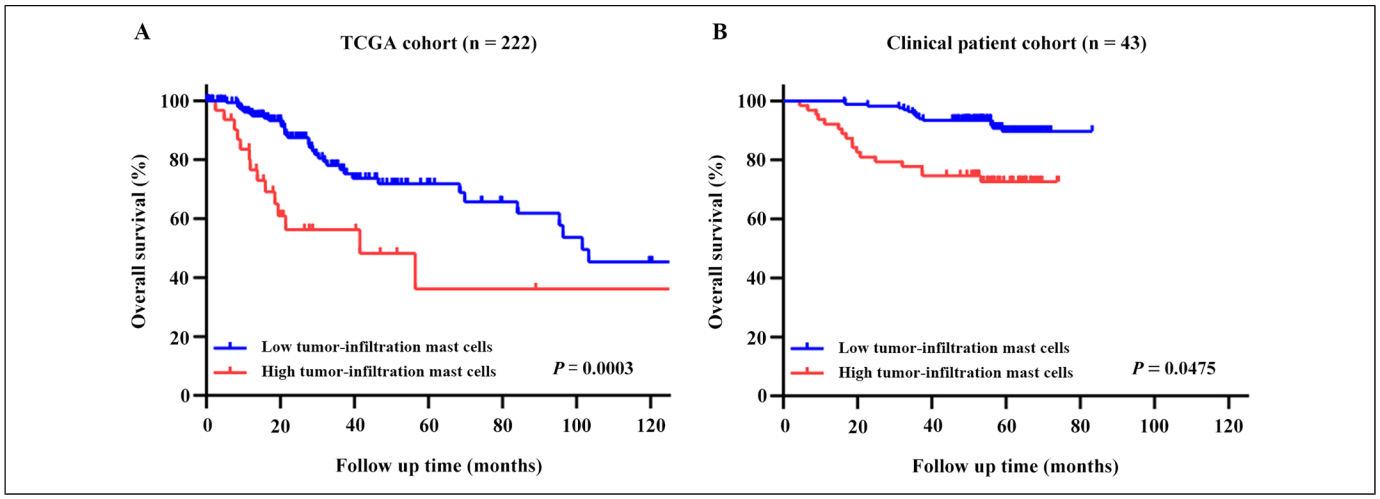

Figure 1. Kaplan-Meier analysis of overall survival in patients with cervical carcinoma. The results showed that the high tumor-infiltrating MCD group was associated with a worse prognosis for the patients. TCGA cohort (n = 222, $P = .0003$) (A). Clinical patient cohort (n = 43, $P = .0475$) (B).

Table 1. Clinical Characteristics of 43 Patients With Cervical Carcinoma.

Parameter	*N* (%) or mean (s.d.)
Total number of patients enrolled	43
Age at first diagnosis (years)	
≤58	32 (74.4%)
>58	11 (25.6%)
Ethnicity	
Han	24 (55.8%)
Others	19 (44.2%)
Differentiation	
Poor	9 (20.9%)
Middle/High	34 (79.1%)
Tumor size	
≤ 4 cm	31 (72.1%)
> 4 cm	12 (27.9%)
Lymph node metastasis	
No	31 (72.1%)
Yes	12 (27.9%)
FIGO stage	
IA ~ IIA2	37 (86.0%)
≥ IIB	6 (14.0%)
MCD	
Low	31 (72.1%)
High	12 (27.9%)

Identification of DEGs of TCGA Data

By performing DEGs analysis on the microarray data of TCGA using the "limma" package, we identified numerous genes that were up-regulated or down-regulated between high and low tumor-infiltrating MCD groups. We got 305 DEGs in total (Table 2), of which 278 genes were up-regulated and 27 genes were down-regulated. The volcano graph showed that significantly DEGs involved MUC6 and MUC5AC, as well as focused on several important cytokines and chemokines including CSF2, IL-1A, IL-1B, CXCL8, CCL20, etc (Figure 4).

PPI Analysis of DEGs

The DEGs between high tumor-infiltrating and low tumor-infiltrating MCD were submitted to the STRING online database, acquiring a PPI network consisting of 300 nodes and 164 edges. The results were visualized through Cytoscape software (Figure 5A). Furthermore, we also conducted MCODE analysis on DEGs, established PPI networks, and obtained 7 key gene modules, a total of 34 key genes (Table 3). To learn more about the potential biological mechanism related to the network, we screened the top 2 gene clusters with the highest clustering score (Figure 5B and C).

Functional Enrichment Analysis of the First 2 Key Gene Modules

Functional enrichment of the first 2 key gene modules was performed using DAVID 6.8. Biological process (BP) became the focus in GO functional enrichment analysis. The results showed that they were mainly related to inflammatory response and the immune response (Table 4).

Correlation Analysis

There was a certain intensity of association between the genes of the top 2 gene enrichment groups, principally focusing on MUC6 and MUC13. MUC6 was primarily related to CSF2, CXCL8, and IL1B. MUC13 was mainly associated with CSF2, CXCL8, and CCL20 (Figure 6A). In addition, the correlation coefficient and *p*-value were also obtained (Figure 6B, Supplemental Table 4). The results showed that MCs were moderately associated with CXCL8 ($P = 0$, $Cor = 0.57$), IL1A ($P = 0$, $Cor = 0.56$), and IL1B ($P = 0$, $Cor = 0.6$), were low correlated with CSF2 ($P = 0$, $Cor = 0.36$), and CCL20 ($P = 0$, $Cor = 0.39$) compared to other genes. Taken together, MCs may interact with cytokines in the TME, which lead to a combined effect in tumor progression.

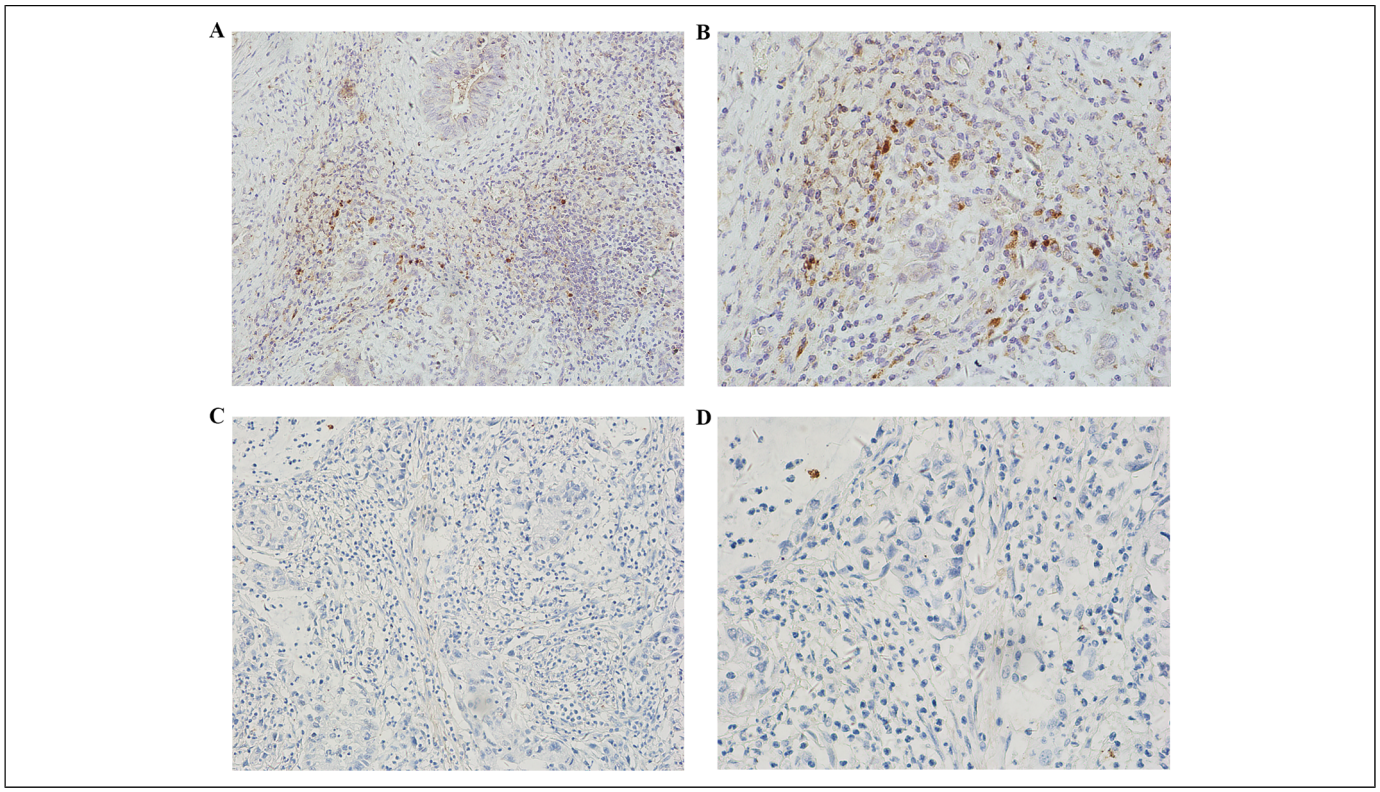

Figure 2. Representative images of tryptase^{+} mast cells staining in cervical carcinoma. Mast cells were dispersed in tumor tissues at different densities. Cervical carcinoma tissues with high mast cell infiltration were presented in 200× (A), 400× (B). Cervical carcinoma tissues with low mast cell infiltration were shown in 200× (C), 400× (D).

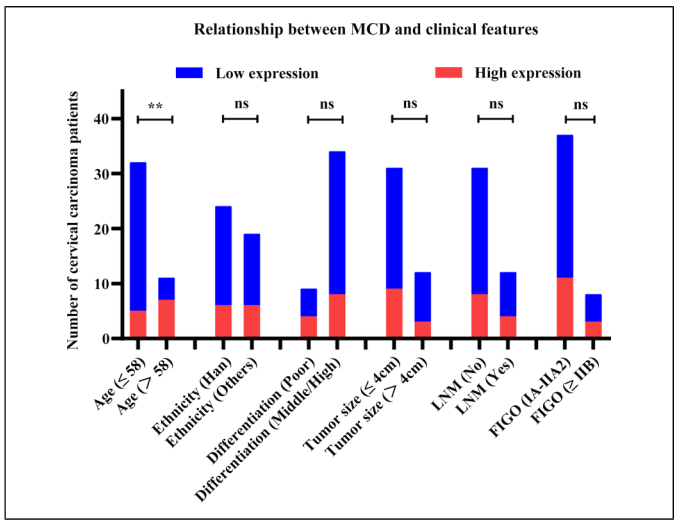

Figure 3. Relationship between mast cell density and clinical features. Except for age ($P = .008$), other clinical variables were not related to the expression of mast cells in cervical carcinoma tissue.

Discussion

Even with the improvement of early screening and diagnosis of CC, it is still one of the diseases threatening female health. The role of MCs in allergic and inflammatory responses has long been identified and well described.[21,22] In recent years, with the increasing research on MCs in cancer, its biological functions and mechanisms of action have been continuously revealed. However, studies on MCs and the prognosis of different cancers have reached different conclusions.[23–25] Similarly, previous studies also observed the anti- and pro-tumor effects of MCs in CC.[13–17] Our study aimed to explore and clarify this question.

MCs were inflammatory mediators that tended to congregate in the tumor periphery. The number of tumor-infiltrating MCs was related to increased microvessel density in the tumor, enhanced tumor growth and invasion, and poor clinical prognosis.[26,27] MCs release molecules like vascular endothelial growth factor (VEGF), tryptase, fibroblast growth factor (FGF-2), which can initiate tumor angiogenesis, and molecules like matrix metalloproteinases (MMP-9 and MMP-2) which can enable tumor niche remodeling, migration and invasiveness collectively resulting in cancer progression.[24] However, there is still controversy on the density of MC and the prognosis of CC patients. Here, externally published TCGA data was utilized to evaluate the prognostic value of MCs infiltration. The results showed that high tumor-infiltrating MCD was associated with worse OS. Furthermore, the conclusion was also verified in a clinical patient cohort. It was concluded that MCs were diffusely distributed in CC tissue using IHC, which suggested that MCD was involved in the development of CC. These

Table 2. The Common DEGs Included 278 Upregulated and 27 Downregulated Genes.

DEGs	Genes
Upregulated	TTR, PON1, TSPAN8, UGT2B11, ITIH1, UBE2U, SPATA21, SLC22A7, CEACAM16, ANKFN1, PAX2, PLA2G2A, CFHR4, DMRTB1, CSAG2, SST, FOLR1, SOX1, HS3ST4, CSAG3, NTS, CDH12, MT4, ADH1C, HTR2C, PIGR, AADACL2, FRRS1L, GPR12, UGT2B4, ALB, ZBBX, SLC18A3, PCDHGB1, RPRM, MAGEA6, C5orf49, CTAG2, KLHL34, FABP1, SPINK13, KRT24, DYNAP, PPP1R14D, INSL6, ORM2, ITLN1, GJB1, MRGPRE, PRAP1, KRT77, MAGEA10, IGFALS, KRT3, FGA, NTSR2, SCGB1D2, GNAT3, HSD3B1, CLEC2L, MAGEA12, AR, SRARP, CCKBR, SULT1C2, PRSS56, APOH, TRIM43, KCNJ18, FAM69C, TCEAL2, FAM228A, KLHL14, ELF5, CCL15, PIWIL2, CNGA3, USH1C, DRGX, NMUR1, ODAM, MAATS1, SIGLEC8, AMBN, CYP2F1, KNG1, PMP2, PENK, ALPI, TCHH, NOL4, KIR3DL2, C4BPB, ZIC1, KRTAP3-1, COL9A1, B3GAT1, UPK1B, SCGB2A2, CRISP2, BRINP2, FOXI2, TMEM72, WFDC6, CD8B, SERPINI2, ECEL1, GSTA3, MUC5B, SLC6A20, SERPINB12, SLC6A19, EPS8L3, DIRAS2, PTH2R, TLX1, ASTN1, IGF1, STATH, CSAG1, FDCSP, F11, OTC, SNTN, MAGEA3, SLCO1B1, FXYD4, PIWIL1, DPP10, MYH6, CRABP1, PRAC2, DEFA5, MTRNR2L1, SCUBE1, C20orf85, GPR50, MKRN3, DEFA6, HAND1, APOA5, KCNJ16, BMP5, TOX3, FABP4, INSL4, GABRG3, LHFPL4, XAGE2, TKTL1, AL583836.1, CCDC33, MAGEC2, ANKS4B, CHAT, CGA, C4BPA, TESC, NOBOX, SMIM31, OTX2, SYT5, FGF4, UGT2A1, LYPD8, CLDN8, SLC7A3, MUC5AC, CCDC190, C10orf82, ORM1, PAH, EDN3, ZNF492, CDX2, CTNND2, SLCO1A2, MOGAT2, C6, PLA2G2D, HSPB3, C19orf81, KCNB2, CXorf67, SPATA19, C6orf10, GMNC, CALCA, PCK1, MUC6, GC, STXBP6, CYP2C9, LRRC3B, AQP4, MAPK4, SYCE1, MAGEB2, PPP1R1B, GATA4, VIPR2, MIA, SLCO1B3, FAM216B, SOX14, HEPACAM2, PKDCC, CD8B2, ALDH1A1, HGD, TCERG1L, HMX1, AFM, POU6F2, MYOD1, EPHA7, MORN5, UGT1A9, TRIM72, CAPN8, SH3GL2, LIX1, KRT28, SLC28A2, MAGEC1, NRAP, UGT1A10, C7orf77, ASCL1, PCP4, HS3ST5, ALPP, KRT2, LRRTM1, SLITRK5, SYT4, PNLIPRP3, DACH2, SULT2A1, CA8, KIF1A, ETNPPL, PLD5, VIL1, KRT79, SHH, SAGE1, FAM81B, UGT2A3, RNF182, PCDHB2, TMEM179, MUC13, SCGB2A1, DDIT4L, APOA2, SLC9C2, PAK5, RNF186, REG1A, SERPINA4, TRIM71, CHRND, CCDC198, STAC2, TCF23, MAGEB17, DRC1, OR3A2, KCNK9, KRT36, MAGEA1, OR7D2, TCHHL1, CFTR, TMEM229A, CST5, FOXN4
Downregulated	OR2T8, CCL20, HMGA2, IL1A, SLITRK6, CSF2, NXPH2, LRRN4, KRTAP2-3, NKX2-2, IL1B, FAM163A, PLPPR5, MMP3, PDPN, PSG6, FAM71A, MMP10, NPY, HRH2, HAS2, FGF5, TFPI2, CXCL8, CGB8, CDH4, MMP1

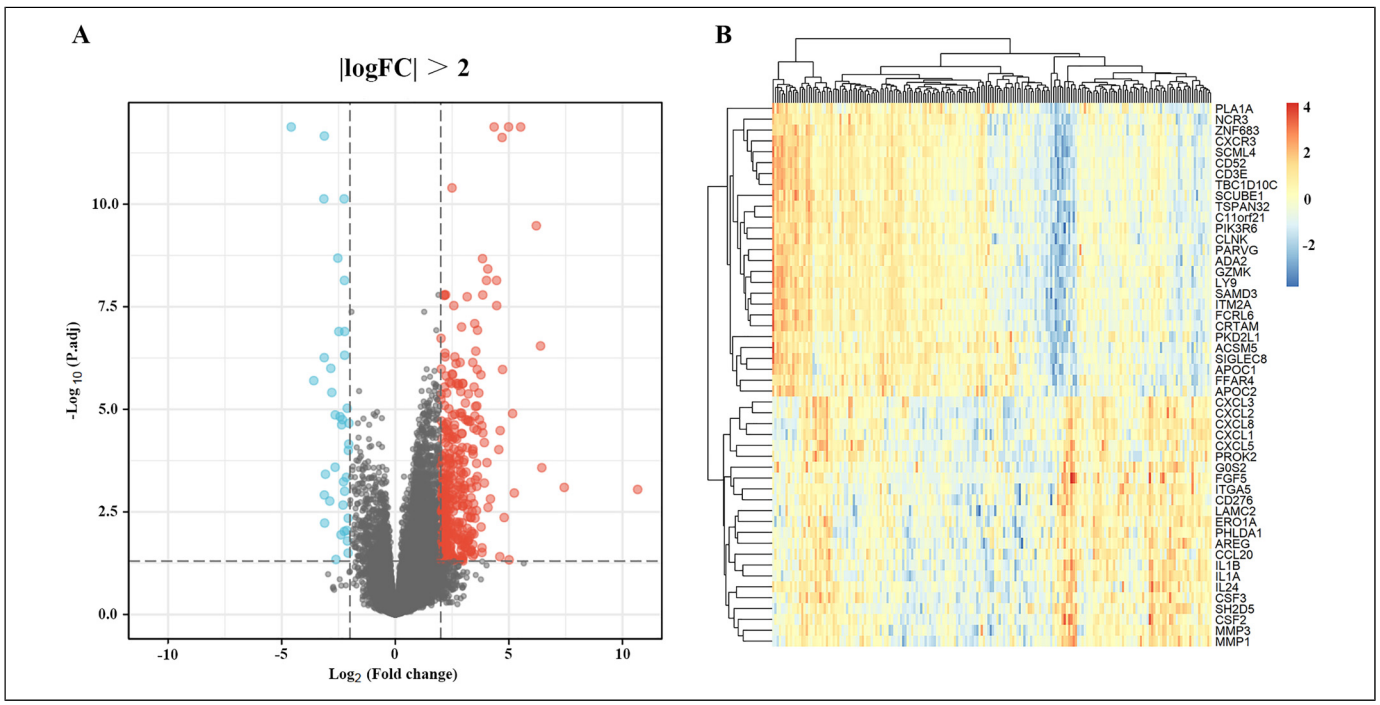

Figure 4. Differentially expressed genes analysis of TCGA data. 305 DEGs were obtained in total, of which 278 genes were up-regulated and 27 genes were down-regulated. Volcano graph (A). A heat map of the top 50 DEGs (B).

conclusions all suggested that MCs were potential biomarkers for the prognosis of CC.

Subsequently, we screened a total of 305 DEGs between the high tumor-infiltrating MCD and low tumor-infiltrating MCD group, of which 278 were up-regulated genes and 27 were down-regulated genes. STRING online database and Cytoscape software were used to construct a PPI network map of DEGs, and 34 key genes were screened through the

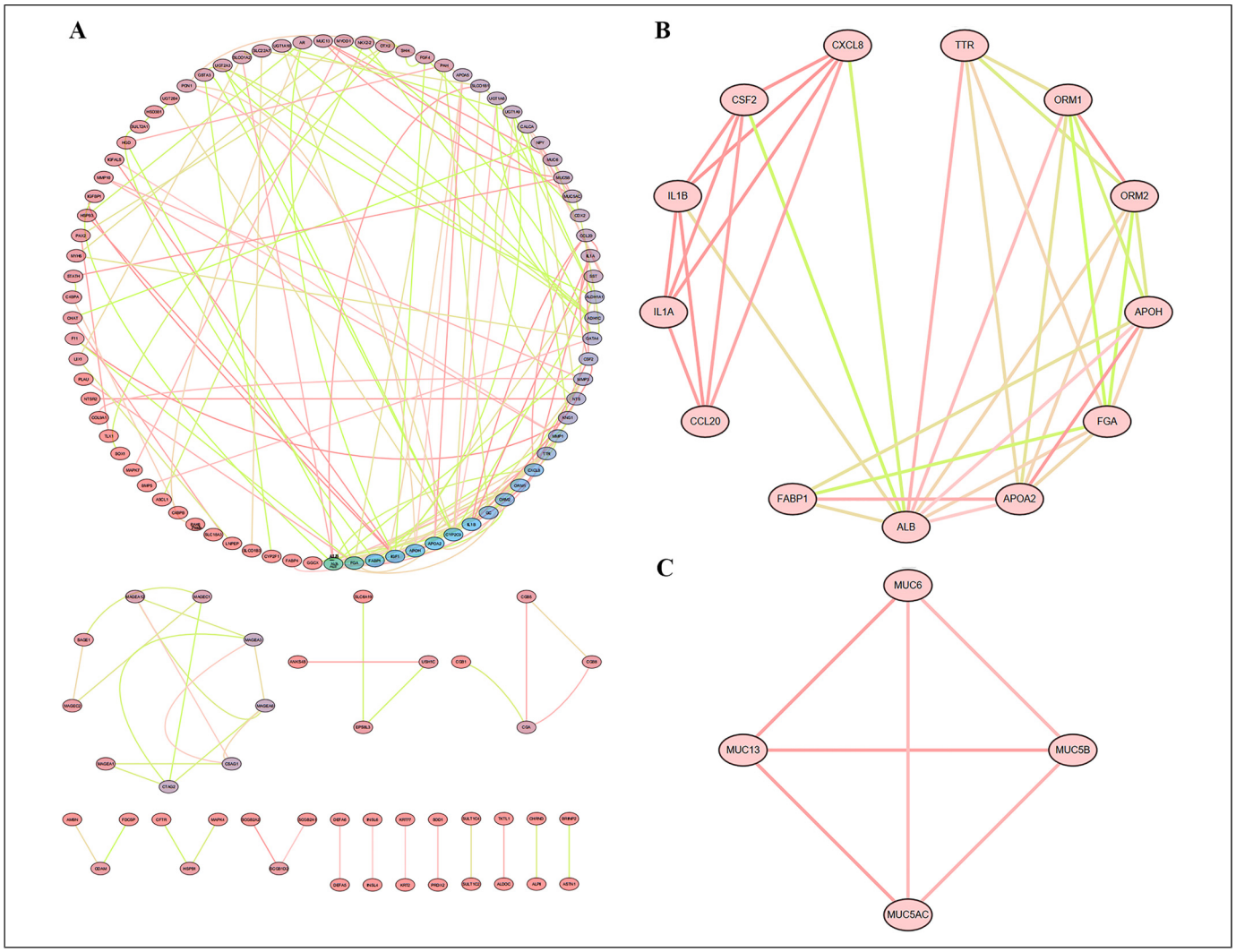

Figure 5. Complex and modular analysis of protein–protein interaction network for DEGs. 305 DEGs were filtered into the protein–protein interaction network and visualized by Cytoscape (A). The top 2 protein–protein interaction networks were analyzed in MCODE (B–C).

Table 3. Results for 7 Key Gene Clusters.

Cluster	Score (Density*#Nodes)	Nodes	Edges	Node IDs
1	6.167	13	37	IL1B, ORM1, TTR, IL1A, CCL20, APOH, ALB, APOA2, FGA, ORM2, CXCL8, CSF2, FABP1
2	4	4	6	MUC6, MUC5B, MUC13, MUC5AC
3	4	4	6	MAGEA6, CSAG1, MAGEA12, MAGEA3
4	4	4	6	NTS, CALCA, NPY, SST
5	3	3	3	MAGEC2, MAGEC1, SAGE1
6	3	3	3	MYH6, GATA4, MYOD1
7	3	3	3	CGB8, CGA, CGB5

MCODE plug-in. One of the first 2 key gene modules were some members of the mucins (MUCs) family. MUCs constituted a family of glycoproteins expressed by epithelial cells, and the expression of MUC changed with tumor progression in a variety of cancers.[28] Recent studies have shown that the increase of MUC13 was strongly associated with increased tumor grade and poor prognosis in carcinoma.[29,30] On the one hand, MUC13 promoted the activation of the nuclear factor kB (NF-kB) signaling resulting in the activation of downstream target genes, thereby facilitating cancer cell proliferation and

Table 4. Enrichment Analysis of the Top 2 MCODE Genes Function.

MCODE	GO	Description	Log10 (*P*)
MCODE-1	GO:0002576	Platelet degranulation	6.36×10^{-07}
MCODE-1	GO:0006954	Inflammatory response	1.10×10^{-04}
MCODE-1	GO:0006955	Immune response	1.64×10^{-04}
MCODE-1	GO:2001240	Negative regulation of extrinsic apoptotic signaling pathway in absence of ligand	3.07×10^{-04}
MCODE-1	GO:0030593	Neutrophil chemotaxis	9.80×10^{-04}
MCODE-2	GO:0016266	O-glycan processing	4.34×10^{-08}
MCODE-2	GO:0030277	Maintenance of gastrointestinal epithelium	2.14×10^{-03}

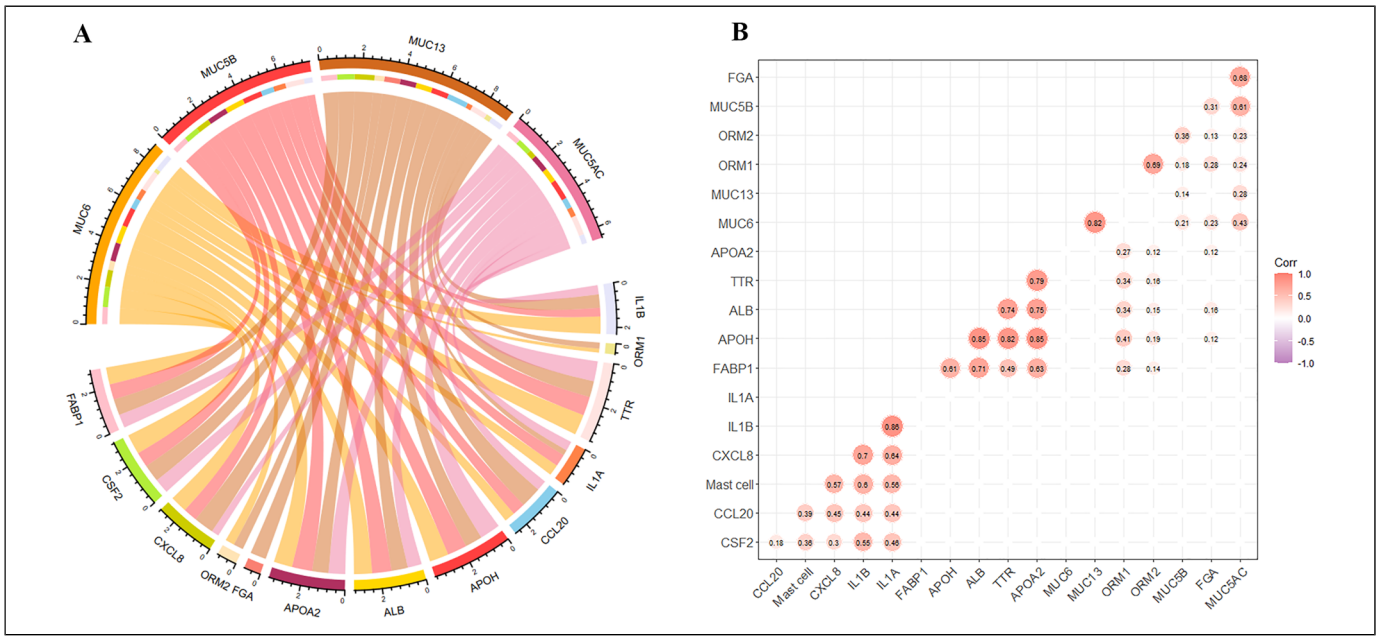

Figure 6. Correlation between mast cells and the top 2 gene enrichment modules. Mast cells were significantly correlated with CXCL8, IL1A, IL1B, CSF2, and CCL20. Comparison between the first 2 gene enrichment modules (A). Correlation analysis between mast cells and genes or among genes. The circles shown in the figure were all statistically significant, while the nonstatistically significant correlations were hidden. The values in the circles represented correlation coefficients (B).

blocking apoptosis.[29] On the other hand, overexpression of MUC13 can lead to tumor progression by regulating human epidermal growth factor receptor 2 (HER2) receptor tyrosine kinase activity.[31] MUC5AC and MUC6, as secreted gel-forming mucins, have been shown to have tumor-promoting effects in several malignant tumors, and high expression of MUC5AC and MUC6 in tumor tissue was significantly associated with poor survival.[32–35] In addition, MUC5AC was associated with epithelial-mesenchymal transition.[33] The interaction of MUC5AC with integrin β4 led to the activation of focal adhesion kinase (FAK) at Y397, which further promoted tumor metastasis. Finally, for MUC5B, its down-regulation may profoundly alter the proliferation, migration, and invasion of cancer cells through the Wnt/β-catenin pathway.[36] In our study, MUC13, MUC5AC, MUC6, and MUC5B in the MUC family were the key genes screened out. These genes have always been shown to promote tumorigenesis and progression of cancer. However, we did not thoroughly study their mechanism of action in CC in this paper. Whether MUC13, MUC5AC, MUC6, and MUC5B also show negative effects in CC needs to be clarified and verified in future studies.

The first 2 key gene modules were not only related to the MUCs family but also connected with cytokines, which were primarily involved in inflammation and immune responses. And through further correlation analysis, it was found that MCs had a certain correlation with CSF2, CCL20, IL1B, IL1A, and CXCL8 in the key gene module. Lee et al and Xu et al reported that the overexpression of CSF2 was implicated with advanced tumor status, more aggressive clinical course, and worse prognosis in cancer.[37,38] In fact, MCs can produce and release CSF2 factor to promote tumor growth.[39] The expression level of CCL20 in the CC tissues was significantly higher than in nontumor tissues and normal control tissues, and was correlated with advancing the FIGO stage.[40] It can also exert its effect by recruiting T Helper 17 Cells (Th17), an independent T-cell subset with protumorigenic properties, in the cervical TME.[41,42] This suggested that MCs were involved in tumorigenesis and progression of tumors in TME

together with other well-known immune cells and cytokines. This provided new insights on immunotherapy for CC.

In addition, IL1A and IL1B were 2 distinct genes encoding IL-1α and IL-1β, respectively, which served as pro-inflammatory factors.[43] The role of IL-1α and IL-1β has been validated in tumorigenesis, tumor invasiveness, and metastasis.[44–46] Meanwhile, IL-1α, as an important molecular marker for prognosis, was also an independent predictor of OS in CC patients.[44] IL1β reduced apoptosis mainly by altering B-cell lymphoma-2/BCL2-Associated X (BCL-2/BAX) protein ratio and increasing p53 mutation, contributing to the development and progression of CC.[46] Moreover, studies have shown that CXCL8 expression was increased in CC and cell lines compared to normal cervical tissue and cervical epithelial cell lines.[47–49] There was also significant correlation with clinical stage, histological grade, and distant metastasis. And high CXCL8 expression was an independent poor prognostic parameter for CC patients.[33] Our study suggested that MCs were positively associated with IL1A, IL1B, and CXCL8. Therefore, it is not difficult to deduce that high tumor-infiltrating MCs and these cytokines can produce the same poor clinical prognosis.

Eventually, it is worth mentioning that previous studies have shown that MCs play an indelible role in tumor therapy. There was a potential to suppress neo-vascularization through decreasing MC degranulation and reducing MC numbers, leading to a delay in the tumor growth.[26] It has already proved to be a rational and effective additional therapeutic strategy for tumors negatively affected by MCs. But there are still some limitations that cannot be ignored. The current nanomedicine was a boon to patients in cancer diagnosis and targeted drug delivery.[50,51] Based on the results of this study, we look forward to the synergistic effect of MCs and cytokines in TME on targeted therapy through nanotechnology in future work.

This study comprehensively analyzed the prognosis of MCs in CC patients through the TCGA dataset, which was verified in a clinical cohort. However, there continue to be some deficiencies in our study. First of all, this study was mainly based on bioinformatics analysis of the public database. Although it was validated in a clinical cohort, our study was small and retrospective research, and should be validated reliability in a prospective cohort with larger sample size. Second, since this study only verified the expression of MCs, the expression of more core genes needed to be further verified in experiments. Meanwhile, further experiments in vitro and in vivo will be carried out to reveal the potential mechanism of MCs in TME. Third, more attention should be paid to the therapeutic effect of MCs on CC in future studies. This study ignored the effect of high and low tumor-infiltrating MCs on therapy. So we cannot know anymore whether the number of MCs affected the treatment modality and the choice of therapeutic drugs to affect the patient's condition.

Conclusion

In summary, we performed a comprehensive analysis of MCs through clinical data and TCGA public data. Low tumor-infiltration MCs in CC tissues was beneficial for OS in patients. Furthermore, MCs were closely related to CSF2, CCL20, IL1A, IL1B, and CXCL8, suggesting that they collectively played a role in the immune response of cervical TME. Therefore, MCD may be a potential prognostic indicator and immunotherapy target for CC. We just hope the results of this study can provide new ideas for prognostic detection and treatment of CC in the future.

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Author Contributions

F.G., W-.N.K., and D.-W.L have contributed equally to this work. F.G., W-.N.K., and D.-W.L are responsible for conception of the research and preparation of the manuscript. G.Z., H-.L.W., and M.A. are responsible for interpretation and analysis of data. X.-Q.S. and Q.-N.S. are responsible for preparation of the manuscript. C.-L.M. and X-.M.M. are responsible for supervision this manuscript and revision for important intellectual content. All authors read and approved the submitted version.

Ethics Statement

This work has been approved by the Ethics Committee of the Tumor Hospital of Xinjiang Medical University (ethical approval number: K-2020013). All patients in the study obtained informed consent or alternative consent.

Data Availability

All data generated or analyzed during this study are included in this article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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