Research article

Construction and validation of a chemokine family-based signature for the prediction of prognosis and therapeutic response in colon cancer

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ABSTRACT

The role of chemokines in predicting the prognosis of colon cancer has not been mentioned. Chemokines have been shown to be associated with immune cell chemotaxis and activation, so the expression of chemokine genes in tumor tissue may be related to prognosis. We used a least absolute shrinkage and selection operator (LASSO) model based on chemokine gene families to construct a model that can predict the prognosis of colon cancer patients. We divided patients into high-risk groups and low-risk groups to study the prognosis. Then, we evaluated the relationship between the different risk groups in infiltration of immune cells. It was found that there was less immune cell infiltration in the high-risk group. We conducted a functional enrichment analysis based on model stratification, and explored the biological signal pathways enriched in the high and low-risk groups, which provided ideas for studying the mechanism behind its impact on prognosis. In addition, the chemokine-related gene signature could predict the response of patients to immunotherapy and chemotherapy.

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1. Introduction

Colon cancer is one of the most common tumors that seriously threaten life and health. The incidence and mortality of colon cancer ranks third among all cancer [1,2]. With the increasing aging of the global population, colon cancer patients are increasing year by year [3]. The early diagnosis of colon cancer is mainly based on endoscopy, but the popularity of colonoscopy is still limited due to the difference in people's awareness and economic level [4]. Therefore, only a small number of people have the opportunity to undergo surgical treatment, and most of the patients are already at an advanced stage when they are diagnosed, and they have lost the chance of a radical cure. The treatment of advanced colon cancer is very limited, and most patients lack specific drugs [5].

Immunotherapy is a promising treatment method that has emerged in recent years [6]. At present, it has achieved good results in many types of cancer, including melanoma [7], lung cancer [8], and urothelial cancer [9]. Immunotherapy has also initially achieved good results in colon cancer [10]. At present, the commonly used clinical immune checkpoint inhibitor treatment marker is the expression of PD-L1. According to the results of clinical trials, patients with PD-L1 expression exceeding 10% are more likely to benefit from immune checkpoint inhibitor treatment [11]. However, no matter it is for traditional therapy or immunotherapy, there is currently a lack of very effective prognostic indicators.

In cancer, chemokines are widely present in the tumor microenvironment and can coordinate the transportation of immune cells [12]. They play a key role in the migration of immune cells to tumors. They can not only shape the immune characteristics of the tumor microenvironment, but also tend to promote tumorigenesis [13]. In addition, chemokines can directly target non-immune cells in the tumor microenvironment, including cancer, stroma, and vascular endothelial cells. Therefore, chemokines are involved in a variety of cancer development processes, such as angiogenesis, metastasis, cancer cell proliferation, stemness, and invasiveness [14]. Therefore, they are key determinants of disease progression and have a great impact on patient prognosis and treatment response. Because of their multiple roles in tumor immune microenvironment and tumor biology, chemokine networks have become potential targets for immunotherapy [15,16].

In this article, we constructed a prognostic model for predicting the prognosis of colon cancer patients based on chemokine family genes. This prognostic model based on the transcriptome data of colon cancer patients would help clinicians to judge the prognosis of patients. Moreover, the model also had a certain predictive effect on predicting the efficacy of immunotherapy and chemotherapy, and could assist other indicators in clinical drug evaluation.

2. Material and methods

2.1. Dataset collection

The RNA sequencing (RNA-seq) data generated from the Illumina HiSeq RNA-Seq platform and corresponding clinical information of colon cancer patients were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) and used as the training set. The chemokine gene set was obtained from the ImmPort website (https://www.immport.org/home).

2.2. Construction and evaluation of prediction signature

The least absolute shrinkage and selection operator (LASSO) Cox regression by the "glmnet" R package (version: 4.0–2; https://cran.r-project.org/web/packages/glmnet/index.html) was used to filter variables, and finally Seven genes were used to construct a prognostic model.

Seven chemokine genes were identified to have nonzero coefficients in the model, which was derived from the surv cutpoint function of the "survminer" R package (Version: 0.4.3; https://cran.r-project.org/web/packages/survminer/index.html). Risk score = sum of coefficients normalized mRNA expression of chemokine genes: CCL22, CX3CL1, SEMA4C, SEMA4D, SEMA5B, SEMA6C, XCL1 (risk = "CCL22"*-0.18075990649556" +"CX3CL1"*"0.039409758585357" +"SEMA4C"*"0.154811228091522"+"SEMA4D"*"0.150021227472605" +"SEMA5B"*"0.0628259972143246"+"SEMA6C"*"0.141817652549588"+"XCL1"*"0.168943499705618").

Colon cancer patients were divided into high-risk groups and low-risk groups according to the median value of the risk score. The Kaplan–Meier survival curve was used to compare the overall survival rate of patients in the high and low-risk groups. Time-dependent ROC curve analysis was used to evaluate the predictive power of gene signature and various clinicopathological features.

2.3. Establishment and validation of nomogram

The nomogram was constructed from clinicopathological characteristics (age, gender, grade, stage, TMN stage) and risk scores based on prognostic signature, and predicts OS of COAD patients at 1, 3 and 5 years. C-index and the calibration curve were used to evaluate the predictive ability of the nomogram.

2.4. Differential gene analysis

We screened out differently expressed genes (DEGs) between tumor and normal tissues using the R package of "edgeR" by the standard of adjusted p value < 0.05 and log2 (fold change) > 1).

2.5. Functional enrichment analysis

The biological functions of differentially expressed DEGs were comprehensively detected by gene ontology (GO) enrichment and Kyoto genome Encyclopedia (KEGG) analysis methods to clarify the biological functions and pathways related to risk score by R software version 4.0.2. Gene Set Enrichment Analysis (GSEA) analysis was conducted to clarify the underlying mechanism of the different group of COAD patients with different risk score by R software version 4.0.2.
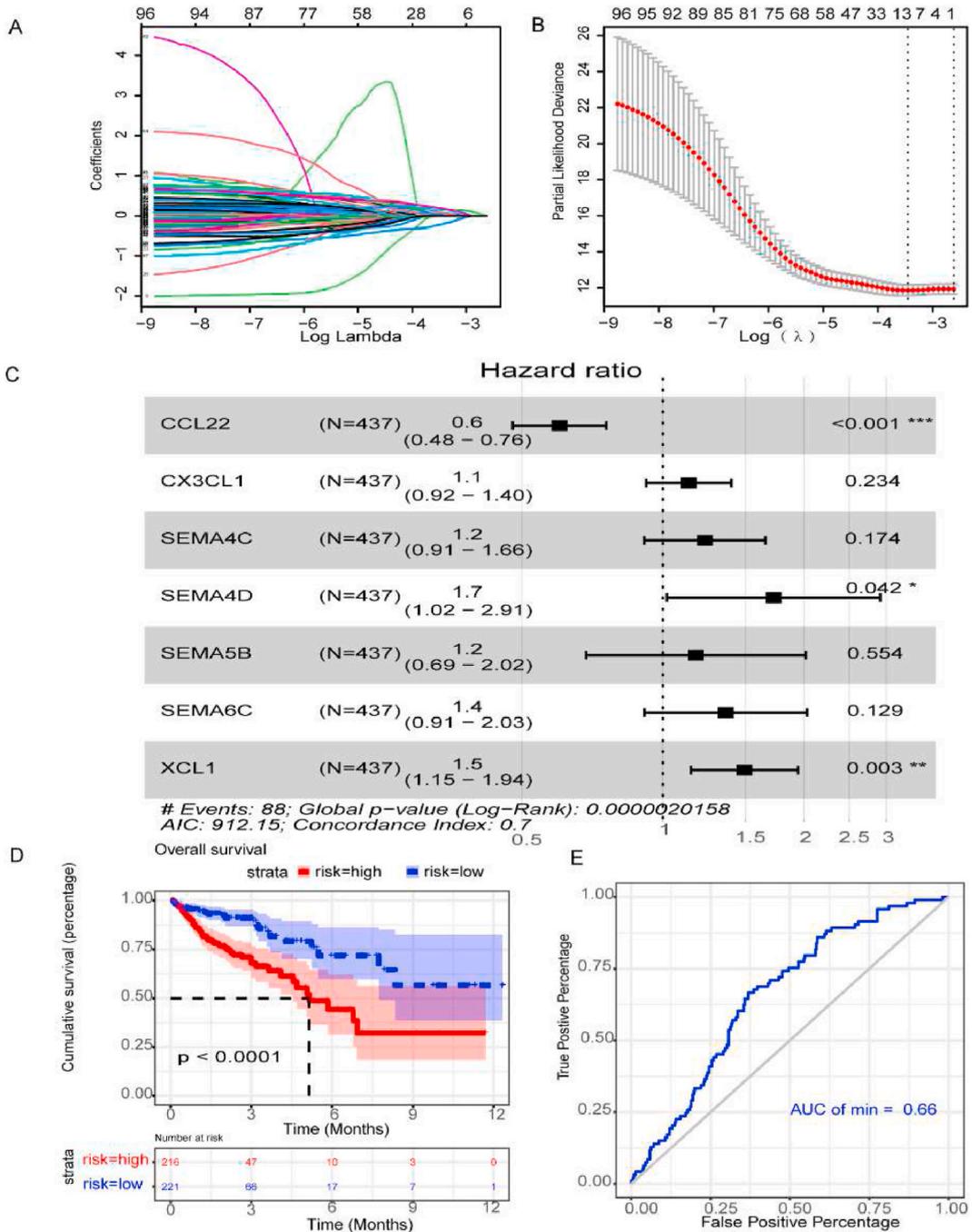
Fig. 1. Prognostic model construction and survival analysis based on chemokine genes. A. One-hundred-fold cross-validation for tuning parameter selection in the LASSO model. B. LASSO coefficient profiles of the most useful prognostic genes. C. Results of the multivariate Cox regression analyses of genes in the model regarding OS in the TCGA COAD cohort. D. Kaplan–Meier curves of OS of TCGA COAD cohort based on risk score. E. ROC analysis of chemokine genes signature for prediction of OS.

2.6. Gene Set Variation Analysis

Gene Set Variation Analysis (GSVA) was used to study the association of prognostic gene signature and cancer hallmarks by R software version 4.0.2. The hallmark gene set was used to obtain the GSVA score of each gene set of each sample of COAD.

2.7. Immune cell infiltration analysis

We used a variety of methods including xcell (https://xcell.ucsf.edu), ssGSEA, MCPcounter, and ConsensusTME [17] (www.consensustme.org) methods for immune cell infiltration analysis by R software version 4.0.2.

2.8. Estimation of immunotherapy and chemotherapy responses

We used the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do) to analyze the correlation between the prognostic model's risk score and chemotherapeutic drugs. The database is mainly established based on the 60 types of cancer cells listed by the National Cancer Institute Cancer Research Center (NCI) [18].
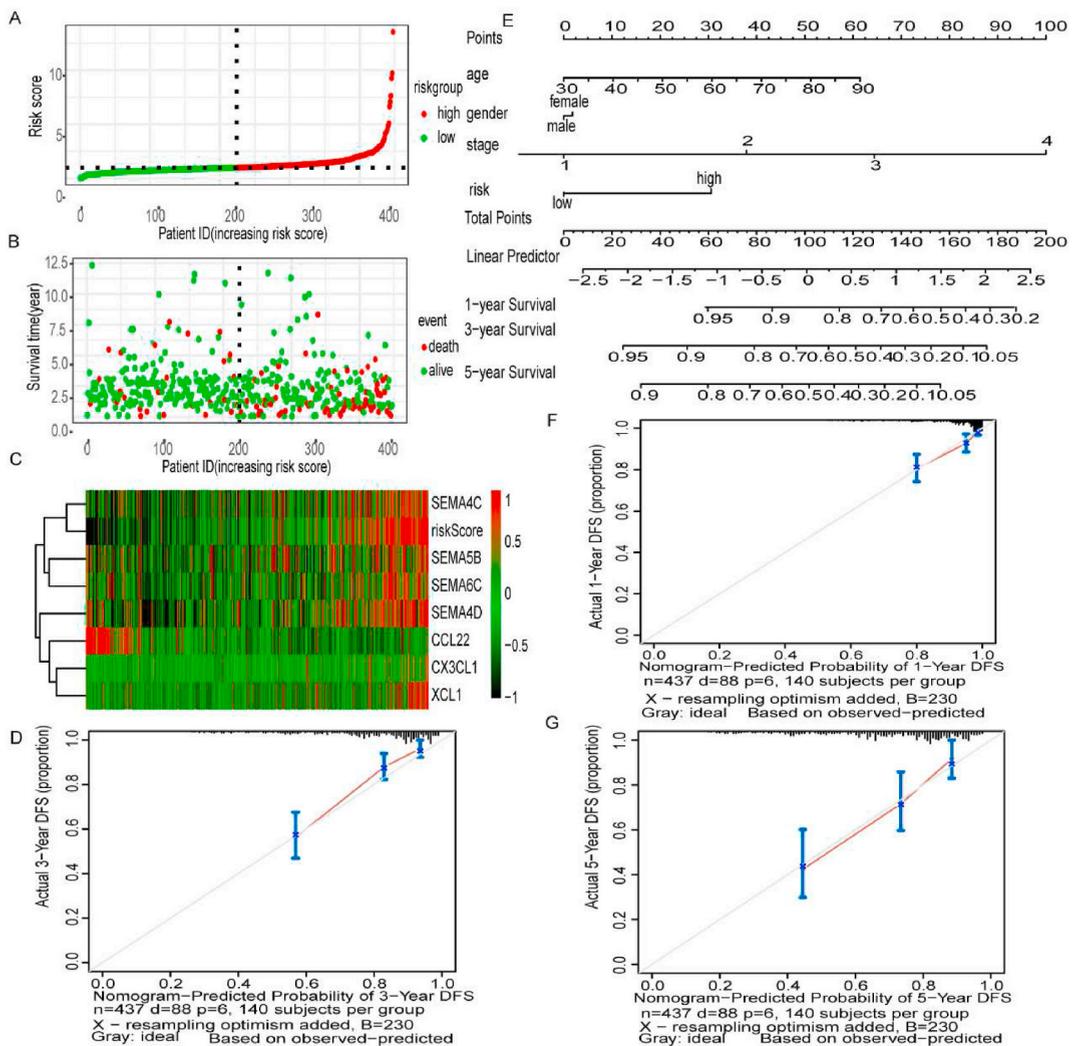
Fig. 2. The 7-gene prognostic model and nomogram chart based on chemokine genes. A, The risk scores for patients of the TCGA COAD cohort. B. The survival of each patient in the TCGA COAD cohort. C. Expression distribution of the seven genes in the TCGA COAD cohort, with red indicating higher expression and green indicating lower expression. D. Nomogram predicting overall survival for TCGA COAD cohort. E, F, G. The calibration plots of the nomogram. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.9. Statistical analyses

All statistical analyses in this article used R software version 4.0.2, and the difference was considered statistically significant when $p < 0.05$. The Kaplan–Meier survival curve was used to compare the overall survival rate of patients in the high and low-risk groups. Time-dependent ROC curve analysis was used to evaluate the predictive power of gene signature and various clinicopathological features.

3. Results

3.1. Construction and validation of a chemokine genes prognostic signature in COAD patients

First, we constructed a prognostic model based on chemokine genes using the TCGA colon cancer dataset as a training set. The LASSO Cox regression model was used to identify the genes with the most robust prognostic value. Tenfold cross-validation was applied to overcome overfitting. Finally, 7 candidate genes (CCL22, CX3CL1, SEMA4C, SEMA4D, SEMA5B, SEMA6C and XCL1) were identified to have nonzero LASSO coefficients and were included in the gene signature model (Fig. 1A, B, C).

Then, we performed prognostic analysis of the chemokine genes in the TCGA COAD cohort. The TCGA samples of cancer patients were separated into high- and low-risk groups based on the median of the model's risk score. Kaplan-Meier curves showed that patients in the high-risk group exhibited worse OS in the TCGA COAD cohort (P < 0.001, Fig. 1D). To illustrate the predictive performance of the chemokine genes-based model, we calculated the C-index and time-dependent area under the ROC curve. Results demonstrated that the area under the curve (AUC) was 0.66 (Fig. 1E).
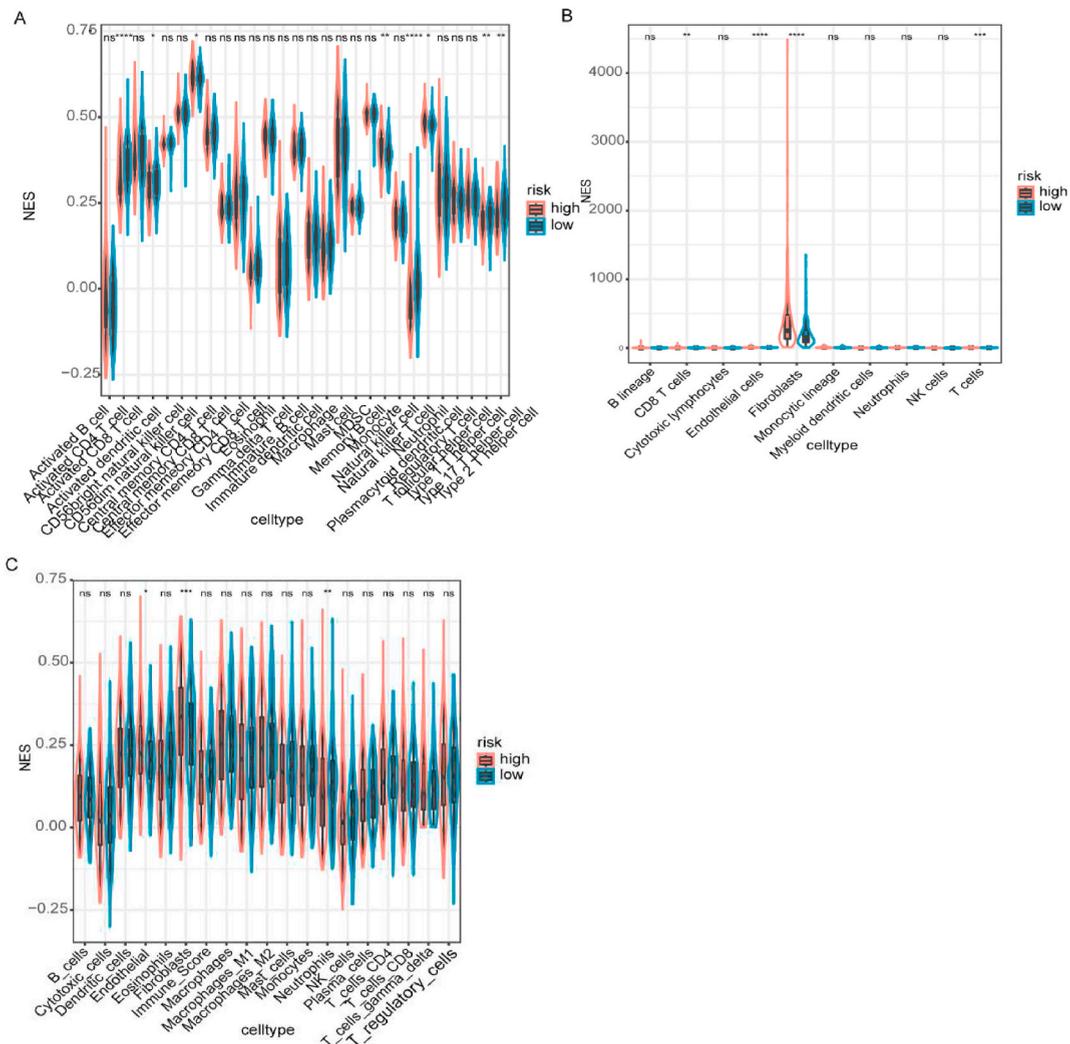
Fig. 3. Immune cell infiltration analysis from four independent databases. A. ssGSEA. B. ConsensusTME. C. MCPcounter.

The risk scores for patients of the TCGA COAD cohort were shown in Fig. 2 (Fig. 2A–C). We built a Nomogram model to predict the prognosis of patients. The Nomogram prediction model not only incorporated the risk score of the chemokine gene model, but also covered clinical characteristics including age, gender, and pathological stage (Fig. 2D). Calibration was performed for the nomogram. The calibration curve showed that the probability predicted by the nomogram was consistent with the ideal reference line for 1-year, 3-year and 5-year survival rates (Fig. 2E, F, G). We also evaluated the predicted discrimination of the nomogram by the C index, which quantifies the level of agreement between the probability derived from the nomogram and the actual death observation. This model would help clinicians to judge the prognosis of patients.

3.2. The relationship between chemokine gene-based signature and the proportion of immune cell infiltration

We performed Immune cell infiltration analysis by ssGSEA, xcell, MCPcounter and ConsensusTME.

According to the analysis of the ssGSEA method, in the high-risk group, the proportion of central memory CD4 T cell, natural killer cell and Plasmacytoid dendritic cell infiltration was higher, while in the low-risk group, Activated CD4 T cell, Activated dendritic cell, Neutrophil, Type 17 T helper cell and Type 2 T helper cell was higher (Fig. 3A). The analysis of the xcell database showed that strocytes, B-cells, CD4$^+$ memory T-cells, Cdc, DC, Macrophages, Neutrophils, Plasma cells and Th2 cells had higher tumor microenvironment infiltration in the low-risk group (Fig. 3B). The analysis results of the MCPcounter and ConsensusTME database showed that the samples in the high-risk group had more Endothelial and Fibroblasts, and the samples in the low-risk group had a higher proportion of Neutrophils infiltration (Fig. 3B and C).
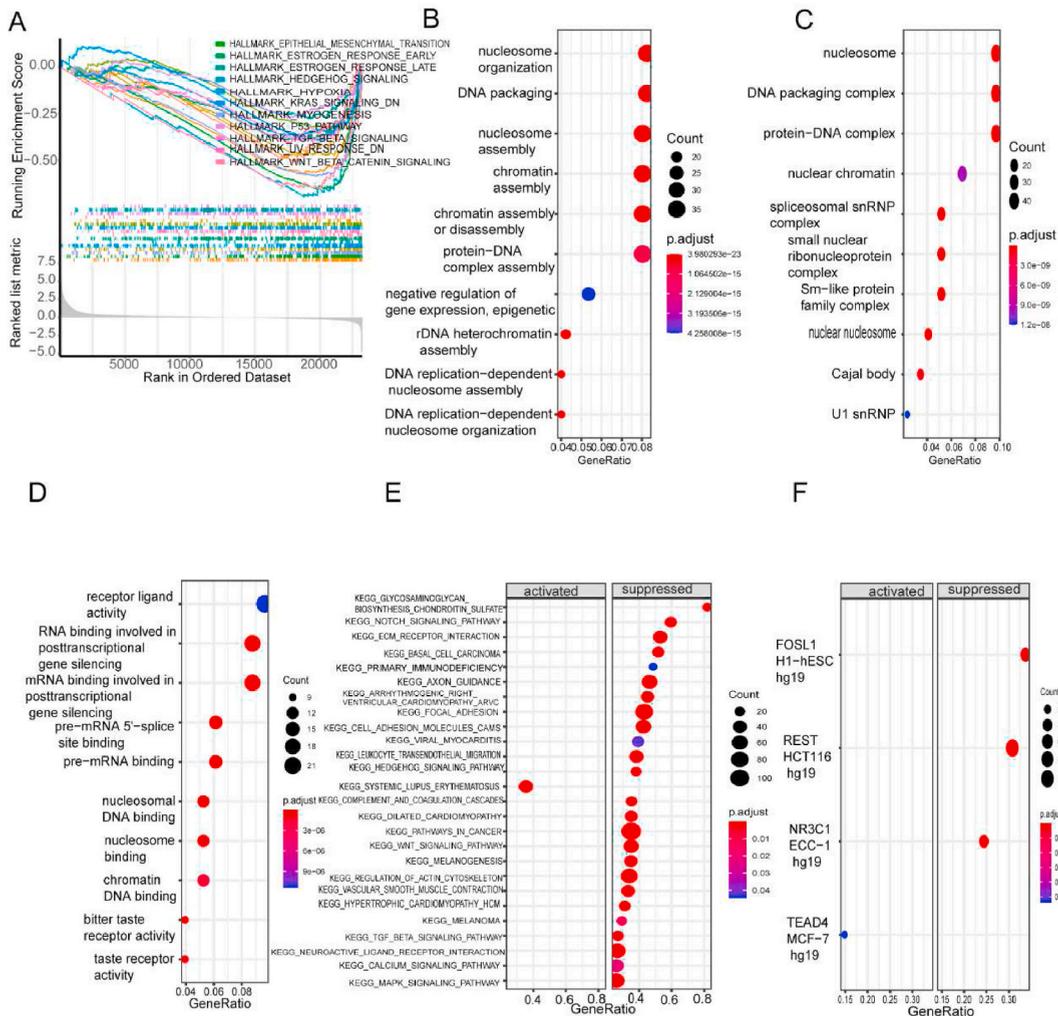
Fig. 4. Functional enrichment analysis of expression profile data of patients in high-risk group and low-risk group. A. Gene Set Enrichment Analysis of different model groups. B, C, D. Gene Ontology annotation. E. The Kyoto Encyclopedia of Genes and Genomes pathway analysis. F. Analysis of transcription factors in different model groups.

3.3. Functional enrichment analysis of high and low risk groups

Subsequently, we conducted signal pathway enrichment analysis on the high-risk group and the low-risk group. We found that the signal pathways enriched in the high-risk group and the low-risk group were different.

Compared with the high-risk group, many signal pathways were inhibited in the low-risk group. These signaling pathways included those related to the occurrence and development of tumors, such as hedgehog pathway, notch pathway, leukocyte transendothelial pathway, viral myocarditis, cell adhesion molecules cams, focal adhesion, basal cell carcinoma, MAPK pathway, TGFβ pathway, melanogenesis, wnt pathway, pathways in cancer. In addition, some signaling pathways that affected immunity and cell function were also inhibited in the low-risk group. These signaling pathways included primary immunodeficiency, ECM receptor interaction, calcium pathway, neuroactive ligand receptor interaction, regulation of actin cytoskeleton, complement and coagulation cascades (Fig. 4B–E).

Transcription factor analysis showed that in the high-risk group, the functions of FOSL1, REST and NR3C1 transcription factors were inhibited, while the function of transcription factor TEAD4 was activated (Fig. 4F).

GSEA analysis results showed that epithelial mesenchymal transition, estrogen response, hedgehog, hypoxia, KRAS, myogenesis, P53, TGFβ, UV response, wnt β catenin signaling pathways were inhibited in the low-risk group (Fig. 4A).

3.4. The effect of the chemokine gene-based signature on immunotherapy prediction

Next, we analyzed the expression of genes reported in the literature that may have a predictive effect on the efficacy of immune checkpoint inhibitors in different risk groups (Fig. 5A–E). The expression of CD274 in the low-risk group was higher. Clinical studies have shown that patients with high expression of CD274 were more likely to benefit from treatment with the PD1/PD-L1 immune checkpoint inhibitors [19]. Immune checkpoint B7–H3 protein expression was associated with poor outcome in prostate cancer [20].
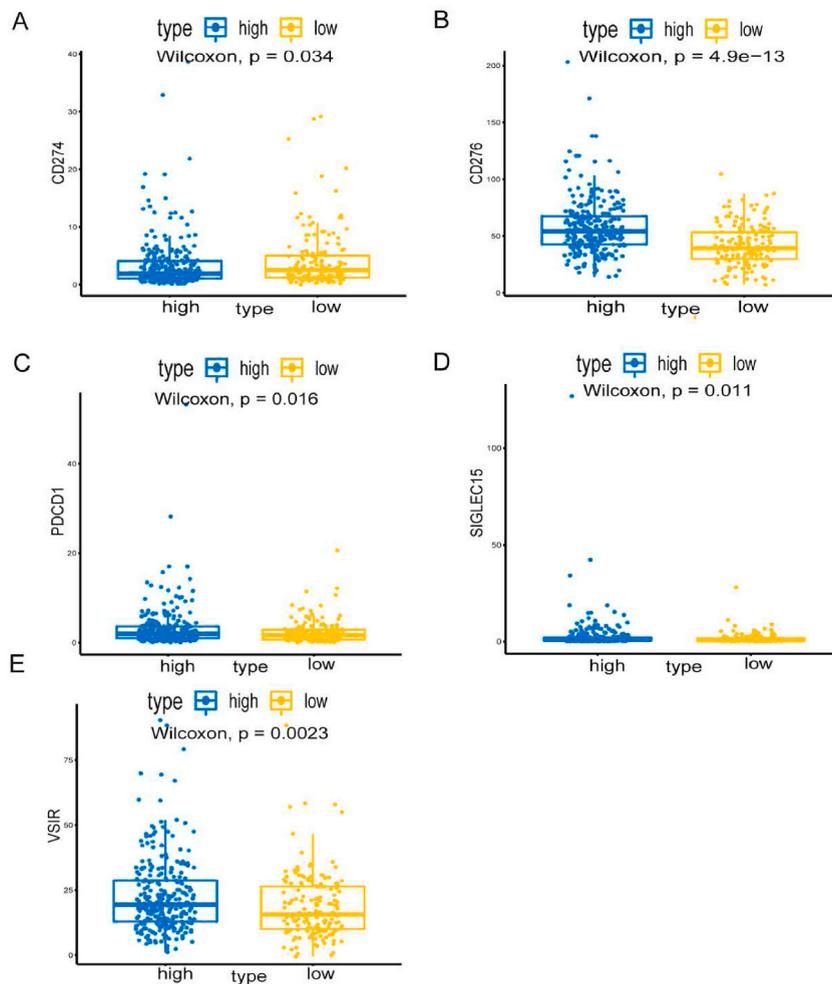
Fig. 5. The expression of genes associated with the efficacy of immune checkpoint inhibitors. A-E. CD247, CD276, PDCD1, Siglec15, and VSIR, respectively.

3.5. The predictive ability of the chemokine gene-based signature on chemotherapy response

Chemotherapy plays an important role in the treatment of colon cancer. However, the effects of different choices of chemotherapeutic drugs were different. Finally, we predicted the relationship between the risk score and the efficacy of chemotherapy drugs with the dataset downloaded from the CellMiner database [18]. The risk score of the chemokine gene-based model was positively correlated with the IC50 of the Methylprednisolone (Cor = 0.415, p < 0.001), Nelarabine (Cor = 0.401, p = 0.001), ZM−336372(Cor = 0.344, p = 0.007), Y−27632(Cor = 0.342, p = 0.007) and Fludarabine (Cor = 0.335, p = 0.009) (Fig. 6A–E).

4. Discussion

Regarding colon cancer prognosis indicators, there is currently a lack of very effective indicators [21]. Our prognostic model based on chemokine gene families can provide clinicians with a new solution based on transcriptome sequencing data to determine the prognosis of patients. It can be combined with other clinical indicators to assist clinicians in making more accurate prognostic judgments for patients.

This article emphasizes the important role of chemokines in the prognosis of colon cancer for the first time. Chemokines play an important role in the occurrence and development of tumors and the regulation of tumor microenvironment. Our prognostic model based on chemokine family genes can predict the stratification of colon cancer patients and predict the prognosis of patients in different groups. According to our model, the prognosis of patients in the high-risk group is worse than that in the low-risk group.

Seven chemokine genes are included in our model, namely CCL22, CX3CL1, SEMA4C, SEMA4D, SEMA5B, SEMA6C and XCL1.
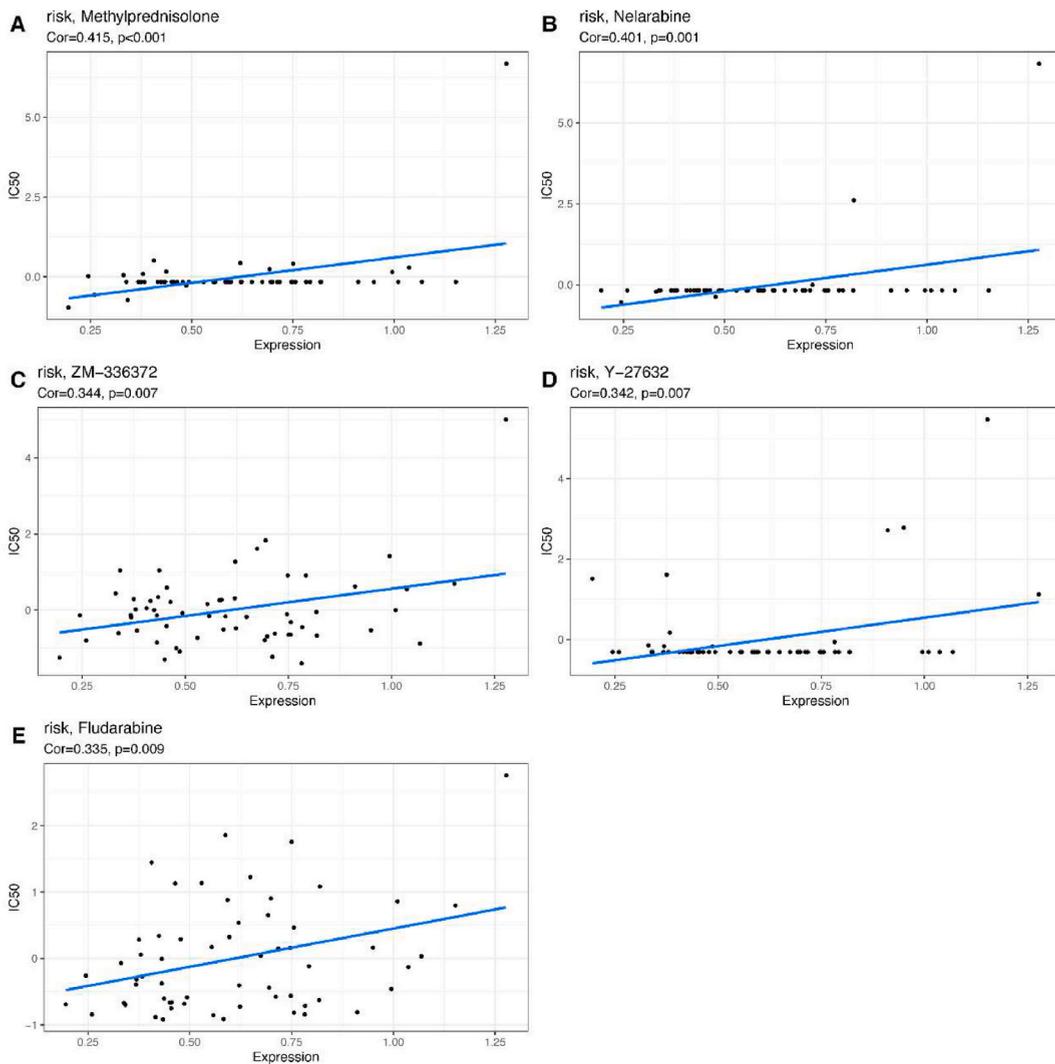
Fig. 6. The relationship between risk score and the efficacy of chemotherapy drugs. A-E. Methylprednisolone, Nelarabine, ZM-336372, Y-27632, and Fludarabine, respectively.

CCL22 plays an important role in the occurrence and development of tumors. CCL22 can bind to CCR4 on Treg cells, thereby promoting the migration of Treg cells to the tumor microenvironment [22]. As a multifunctional chemokine, CX3CL1 can participate in a variety of biological processes, such as immune cell attraction and enhancement of tumor immune cell interaction, as well as enhancement of tumor cell proliferation and metastasis. In human tumors and animal experiments, it has been shown that overexpression of CX3CL1 leads to a slight increase in tumor growth [23]. Transcriptome data and immunohistochemical staining of tissue microarrays from CRC patients showed that the expression of SEMA4C mRNA and protein is related to the pathological stage and metastasis of CRC patients [24]. SEMA4D is overexpressed in colon cancer and can regulate the function of the immune system [25]. SEMA5B is a HIF target gene highly expressed in RCC, which can promote tumor growth in vivo [26]. SEMA6C were expressed in fetal brain [27]. XCL1+ T-cell clusters are related to the tumor mutational burden high status of cancer patients [28]. However, the more precise role of these genes and the interaction between genes need further experimental studies to confirm.

Moreover, according to the prognosis model we constructed, we can further explore the reasons for the difference in prognosis of patients in different groups. Immune cell infiltration analysis shows that patients in the low-risk group have more killer immune cell infiltration. The immune microenvironment of patients in the high-risk group contains fewer killer immune cells, and more suppressive immune cells, which can promote tumor occurrence, development and even migration.

Prognostic models based on chemokine gene families can also predict the response of patients to immunotherapy and chemotherapy. Different groups of patients have different enriched signaling pathways. Inhibition or activation of these signaling pathways may cause patients to have different therapeutic responses to drug treatments.

However, our study also has some limitations. First, this study lacks the verification of basic experimental data and prospective clinical research results. Secondly, the biological functions of the genes included in the model need more experimental studies to clarify in order to better explain their clinical significance. Furthermore, the clinical information we have obtained is limited, and the results of this model need to be verified in more prospective clinical trials in order to better serve the clinic.

In short, we have constructed a prognostic model based on the gene family of chemokines. This model can assist clinicians in the prognosis of colon cancer patients and make certain judgments on whether the patients are effective in some immunotherapy and chemotherapeutic drugs. The model also explained the heterogeneity of the tumor to a certain extent. The different levels of immune cell infiltration in different patients may be the reason for the different prognosis and therapeutic response.

Author contributions

H.-M.Z. and X.-X.W. designed the study and contributed to study materials and consumables. H.-M.Z., X.-X.W., and L.A. conducted the study. X.-X.W., L.-H.W., L.A., W.P., J.-Y.R., and Q. Z. collected data. H.-M.Z., X.-X.W., and L.-H.W. performed the statistical analyses and interpreted the data. X.-X.W. and L.-H.W. wrote the manuscript. All authors approved the final manuscript.

Data availability

Publicly available datasets analyzed in this study are available in the Cancer Genome Atlas (Repository (cancer.gov)).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16478.

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