Tumor infiltrated macrophage-the fighter defends against recurrence of stage Ⅲ colorectal cancer

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Abstract

Background

Biomarkers which can robustly predict the recurrence of stage II colorectal cancer (CRC) have been urgent needed. Immune cells highly related to the recurrence and prognosis of CRC. However, whether the immune cells take part in the recurrence of stage II CRC has not known.

Methods

Explored the roles and possible mechanism of immune cells in the recurrence of stage II CRC by public databases.

Results

Tumor Immune Estimation Resource (TIMER) analysis indicated the macrophage infiltration level in stage II CRC: Significantly higher in the nonrecurrence group than that in the recurrence group; Significantly negative correlated with the recurrence-related hub gene transferrin (TF); Significantly positive correlated with the improved disease-free survival (DFS) and overall survival (OS). And macrophage infiltration related-gene recurrent model (GRM) (area under the curve (AUC) = 0.906) provide a better performance than the GRM established previously (AUC = 0.882) to predict the recurrence of stage II CRC. Gene Set Enrichment Analysis (GSEA) analysis indicated macrophage (M1) infiltrated in the tumor microenvironment (TME) defending against recurrence as a dynamic process: M1 was activated by receiving the stimulus signals (LPS/IFN- γ) which released by CRC cells, then infiltrating and recognizing the tumor to secrete IL-6 to directly or indirectly act on TF or create anti-tumor TME to kill tumor cells.

Conclusions

This study maybe provides a novel immunological perspective to explore the mechanism of recurrence and improve clinical practice performance in stage II CRC.

Background

Radical resection is recognized as the foundation treatment for stage II CRC. However, postoperative adjuvant therapy remains a topic of interest and debate. American Society of Clinical Oncology (ASCO) recommend adjuvant chemotherapy not routine use but for patients with high risks which characterized by the clinic pathological factors of stage II CRC.¹ However, according to this recommendation, multiple unsatisfied and inconsistent clinical results indicated its not robust,^{2,3} so the efficient and reliable biomarkers to select patients with high risks for recurrence,as well as patients who should receive targeted surveillance and additional adjuvant therapy are urgent needed.

Nowadays, with the popularization of big data analysis and the progress of bioinformatics technology, a series of biomarkers was identified for predicting the recurrence, metastasis, chemo sensitivity, and

prognosis of CRC. Previously, based on TCGA database, we established the GRM which consisted of ZNF561, WFS1, SLC2A1, and PTGR1 to predict the recurrence risk of stage II CRC by random forest.⁴ And the excellent predictive performance was validated by ROC analysis with the AUC of 0.882. However, validating the GRM in prospective, multicenter, randomized controlled clinical trials, improving the diagnostic efficacy of the GRM, and multi-dimensional exploration of the recurrence mechanisms of stage II CRC have been our goal.

TME which consists of tumor cells and other surrounding non-tumor cells, such as immune cells, endothelial cells, and fibroblasts,⁵ has been recognized as an important hallmark and plays a critical role in tumor progression and metastasis.^{6,7,8} The immune cells infiltration levels highly related to the recurrence, metastasis, treatment response, and prognosis of CRC have been widely reported.^{9, 10, 11, 12, 13, 14, 15} And the immune cell infiltration has shown superior prognostic power than the clinic pathological factors as TNM staging.¹⁶ Recently, a large international validation effort demonstrated the reliable performance of the immune cell infiltration for estimating CRC patients' risk of recurrence.¹⁷ Given the time to recurrence strongly correlate with the strength of the in-situ adaptive immune reaction in CRC.¹⁷ We hypothesis whether the immune cells in the TME have a superior power for predicting recurrence than the high risks defined by clinicopathological factors of stage II CRC?¹

In this study, we explored 6 types of immune infiltrating cells in stage II CRC between recurrence and nonrecurrence group using TIMER, and found the macrophage infiltration level in the nonrecurrence group was significantly higher than that in the recurrence group, the macrophage infiltration level was significantly negative correlated with the recurrence-related hub gene TF and was significantly positive correlated with the improved DFS and OS. In addition, macrophage infiltration related-GRM (0.906) provide a better performance than GRM (0.882) to predict the recurrence of stage II CRC. Based on the results of GSEA analysis, we speculate the macrophage infiltrated in the TME was M1 phenotype and defended against recurrence maybe a dynamic spatiotemporal process as follows: 1. Tumor cells release LPS or IFN- γ antigens. 2. Macrophages (M1) receive the stimulus signals (LPS or IFN- γ) and activation. 3. Trafficking of macrophages (M1) to tumor. 4. Macrophages (M1) infiltrate into tumor. 5. Macrophages (M1) recognize tumor then secrete IL-6 to directly or indirectly act on TF or create anti-tumor TME to kill tumor cells. Finally, inhibit the recurrence of stage II CRC. Notably, the regulation of IL-6 was high quality. In conclusion, this study maybe provides a novel immunological perspective to explore the mechanism of recurrence and improve clinical therapy practice in stage II CRC.

Methods

Data collection and preprocessing

The specific inclusion and exclusion criteria, methodology application, and parameters setting were as previous description: The RNA-Seq dataset of CRC, which included human whole transcriptome sequencing dataset and corresponding survival profiles, was downloaded from TCGA database. All the

data in the dataset used were pathological stage II (T3-4N0M0), without postoperative adjuvant therapy and followed up for at least 2 years. According to recurrence or not, they were divided into recurrence group and non-recurrence group.⁴

Estimation of the immune cell infiltration levels between nonrecurrence and recurrence groups

TIMER (<https://cistrome.shinyapps.io/timer/>) applies a deconvolution statistical method¹⁸ to systematic analysis of immune infiltration levels of B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells from gene expression profiles.¹⁹ In order to explore whether there is a statistical difference in the level of tumor immune infiltration between the nonrecurrence and the recurrence group, the gene expression profiles data were used to estimate infiltration levels by TIMER. All analyses were performed with GraphPad Prism 7.0 and $P < 0.05$ was considered statistically significant.

Evaluation of the diagnostic efficiency of the macrophage infiltration level by ROC analysis

Previously, based on TCGA database, we established the GRM to predict the recurrence risk of stage II CRC by random forest. And the excellent predictive performance was validated by ROC analysis with the AUC of 0.882. The immune cells infiltration levels in predicting the recurrence, metastasis, treatment response, and prognosis of CRC have been widely reported.^{9, 10, 11, 12, 13, 14, 15} And immune-related prognostic model was constructed and validated in CRC too.²⁰ In order to explore whether the macrophage infiltration level play an important role in predicting the recurrence in stage II CRC or not, we assessed the diagnostic efficiency of the macrophage infiltration level combined GRM (macrophage infiltration related-GRM) by ROC. And the AUC were compared between macrophage infiltration related-GRM and GRM.

Exploring the clinical values of the macrophage infiltration level

As macrophage infiltration level was negative correlated with recurrence in stage II CRC, and recurrence is recognized as a poor prognostic factor. Given the immune cells infiltration levels are highly relevant to the progression and prognosis of CRC,^{9, 10, 11, 12, 13, 14, 15, 20} we aim to explore whether the macrophage infiltration level could predict the prognosis of stage II CRC.

To compare the differences of DFS and OS in the macrophage infiltration level between nonrecurrence and recurrence groups of stage II CRC, the Kaplan-Meier method with log-rank test were used. All analyses were performed with GraphPad Prism 7.0 and SPSS version 19.0 (IBM), and $P < 0.05$ was considered statistically significant.

Exploring the correlation between hub genes and macrophage infiltration level

Previously, we identified 4 hub genes ABCG2, CACNA1F, CYP19A1, and TF, which associated with the recurrence risk of stage II CRC.⁴ And the high expression of the ABCG2, CACNA1F, CYP19A1, and TF was associated with poor OS, with P values of <0.05 , <0.05 , <0.01 , and <0.05 , respectively. As we validated in

this article the macrophage infiltration level was significant positive correlated with the improved DFS and OS. We aimed to explore whether there are correlations between the hub genes and the macrophage infiltration level in the recurrence of stage II CRC. The correlation was analyzed using Pearson Correlation, and $P < 0.05$ was considered statistically significant.

Gene Set Enrichment Analysis

In order to better understand the immune status and the underlying mechanism for the recurrence of stage II CRC, we further studied the immunologic signatures gene set (C7) by GSEA between the nonrecurrence and the recurrence groups (<http://software.broadinstitute.org/gsea/index.jsp>).²¹ And FDR $< 25\%$, $P < 0.01$ was considered statistically significant.

Results

Identification of the data set

Information on the 124 patients who met our research criteria was obtained from the TCGA database. After a 2-year follow-up, 24 patients experienced recurrence, and 100 patients did not experience recurrence.

The macrophage infiltration level was significantly higher in the nonrecurrence group than that in the recurrence group of stage II CRC

We assessed the correlations of immune infiltration levels with recurrence in stage II CRC by TIMER. The results showed that the only significant difference between nonrecurrence group and recurrence group was the macrophage infiltration level ($P = 0.0444$), and the macrophage infiltration level in the nonrecurrence group was significantly higher than that in the recurrence group of stage II CRC [Figure 1].

The high macrophage infiltration level was associated with improved survival

The results of survival analysis showed that the patients with high macrophage infiltration level has a significant higher DFS and OS than the patients with the low macrophage infiltration level, and the P values were 0.024 and 0.019 respectively [Figure 2].

The macrophage infiltration related-GRM provide a better predictive recurrence performance than GRM

The ROC curve defined an optimal threshold to predict the recurrence risk of stage II CRC, and the AUC values of the ROC for GRM and macrophage infiltration related-GRM were 0.882, 0.906 respectively [Figure 3]. Which means the combined analysis of the macrophage infiltration level plus GRM could provide a better predictive recurrence performance than GRM in stage II CRC, and showed remarkable sensitivity and specificity when the cut off value was 0.840.

There was a negative correlation between TF and the macrophage infiltration level

The correlation analysis between hub genes and macrophage infiltration level indicated there was only TF has a negative correlation with the macrophage infiltration level ($r = -0.31$, $p = 0.0008$) [Figure 4].

The high macrophage infiltration level was associated with the antitumor-related gene set

GSEA was conducted to validate the association of the macrophage infiltration level and immunologic signatures between the recurrence and nonrecurrence groups of stage II CRC. Total 3549 gene sets were upregulated in the nonrecurrence group, 72 gene sets were significant at $FDR < 25\%$, 119 gene sets were significantly enriched at nominal P value < 0.01 . However, although there were 1323 gene sets were upregulated in the recurrence group, but there was no gene set were significant at $FDR < 25\%$. Which suggesting that nonrecurrence positively associated with intra-tumoral immune response.

As $FDR < 25\%$, P value < 0.01 considered significant, we identified 72 gene sets to further study. According to the above result that the macrophage infiltration level in the nonrecurrence group was significantly higher than that in the recurrence group of stage II CRC. Based on the mechanism that infiltrated immune cells can induce the host immune response by releasing cytokines and other factors, which directly or indirectly inhibit or promote the progression of cancers.¹¹ We focused our interest on the gene sets which about macrophage and related cytokines. Interestingly, the 5 significantly enriched gene sets of nonrecurrence group were all with an anti-tumor effect [Figure 5].

Discussion

The efforts on the researches about postoperative adjuvant therapy of stage II CRC have been decades. However, the guideline remains unclear, the controversies remain exist. “Who”, “how”, and “duration time” are always the interest topics, and Intergroup 0089,²¹ GENCORE trial,^{22, 23} QUASAR,²⁴ IDEA Collaboration,²⁵ and the researches from Chau et al,²⁶ Kumar et al,²⁷ Tsai et al²⁸ are the representative of large samples. Undoubtedly, “who” is the foundation of “how” and “duration time”, so we have been committed to research at the source.

It is important to note that this study does not contradict - but rather extends our previous study. The 5-year survival rate for stage II CRC is about 80%, and the improvement in survival following adjuvant chemotherapy is less than 5%.²⁹ Facing such a relatively narrow window of benefit for adjuvant therapy and combined its well-recognized heterogeneous disease. The more accurate of the model for the prediction of the recurrence is, the better guide the clinical practice is. In this study, macrophage infiltration related-GRM (AUC = 0.906) provide a better performance for the prediction of the recurrence than previous GRM (AUC = 0.882). Which put the goal of further improving the diagnostic efficacy of the GRM of stage II CRC into practice. In addition, high risk patients with recurrence should receive targeted surveillance and more active adjuvant therapy.

As we all known, iron is necessary to maintain cell growth and proliferation. The faster the cells grow and proliferate, such as tumor cells, the greater the demand for iron. transferrin is an important chelator with a primary function of iron transportation. TF, transferrin receptor (TFR), and Fe^{3+} form the TF-TRF- Fe^{3+}

complex on the cell surface, then Fe³⁺ is transported into the cell by endocytosis, subsequently, Fe³⁺ is reduced to Fe²⁺ to perform biological functions. TF-TRF complex return to the cell surface through exocytosis. Continue to form the TF-TRF-Fe³⁺ complex. The cycle then repeats itself. Higher transferrin saturation was associated with increased cancer risk (HR 1.68 (95% CI: 1.18, 2.38; P < 0.01)) and increased risk of cancer death (HR: 0.65; 95% CI: 0.42, 0.99; P < 0.05).³⁰ Based on the biological properties of intracellular transportation and tumor selectivity, TF, TFR and TF-TRF-Fe³⁺ complex has been widely developed as active-target drug delivery platforms for cancer therapy.^{31, 32} Simultaneous assay of feces hemoglobin (Hb) and TF showed high accuracy and effectiveness for colorectal cancer screening, which with a sensitivity and specificity of 68.2% and 92.6%, respectively.³³ Previously, we reported TF is a hub gene which play an important role in promoting the recurrence of stage II CRC. In this study, we further validated the macrophage infiltration level was significant negative correlated with the recurrence-related hub gene TF. Which indicated macrophage may defend against the recurrence of stage II CRC by targeting recurrence-related hub gene TF.

As macrophage is one of the most abundant cells in the TME.³⁴ And the infiltrated macrophage was associated with poor clinical outcome in numerous solid tumors, including bladder,³⁵ breast,³⁶ renal,³⁷ prostate,³⁸ gastric,³⁹ glioma, endometrial and cervical cancers.⁴⁰ Hence, significant attention has been drawn towards development of cancer immunotherapies targeting macrophage. However, macrophage reported plays inconsistent even contradictory roles in CRC.^{41, 42} In this study, still focus on stage II CRC, we found the macrophage infiltration level in the nonrecurrence group was significantly higher than that in the recurrence group, the macrophage infiltration level was significantly negative correlated with the recurrence-related hub gene TF and was significantly positive correlated with the improved DFS and OS. Which means macrophage infiltrated in the TME could defends against recurrence and then improves the prognosis of stage II CRC.

Macrophage is one of the earlier and more amount infiltrated immune cells in the TME of CRC,¹¹ and exhibit different phenotypes and functions in response to various microenvironmental signals which generated from tumor and stromal cells.⁴³ According to their activation, macrophages are divided into three subtypes (M0, M1 and M2). M0 is the unactivated subtype and without inflammatory or tumor-related function. Depending on the different activation pathways, M0 can differentiate into two activated subtypes, M1 and M2, which exhibit pro-tumor or anti-tumor roles respectively. As the evidence indicated that the infiltrated macrophage could recruited by tumor cell through several different mechanisms.^{44, 45} During inflammation, cancer and other pathological processes, macrophage is among the first responders, recognizing pathogen-associated patterns (PAMPs) like LPS or IFN- γ .⁴⁶ LPS engages the Toll-like receptor 4 (TLR-4) on the surface of macrophage to activate transcription factors e.g. interferon regulatory factors (IRFs) and nuclear factor kappaB (NF- κ B), then activates the inflammatory response and also participates in the host innate immunity.^{45, 46, 47} Releasing a variety of cytokines, including interleukin (IL)-1 β , IL-6, IL-12, IL-16, tumor necrosis factor- α (TNF- α),⁴⁸ nitric oxide,⁴⁹ interleukin-1 h,^{50, 51}

and reactive oxygen intermediates,^{52, 53} facilitating the local recruitment of more macrophage and leukocyte infiltrate to fight against pathogenic insult.⁵⁴ Finally, killing the tumor in the TME.⁵⁵

According to the analysis results from GSEA, we speculate the macrophage defends against recurrence maybe a dynamic spatiotemporal process as Chen and Mellman⁵⁶ proposed cancer-immunity cycle (CIC) [Figure 6]: 1. Tumor cells release (LPS (GSE14000, GSE14769) or IFN- γ (GSE1925)) antigens. 2. Macrophages (M1) receive the stimulus signals (LPS or IFN- γ) and activation. 3. Trafficking of macrophages (M1) to tumor. 4. Macrophages (M1) infiltrate into tumor. 5. Macrophages (M1) recognize tumor then secrete IL-6 (GSE411) to directly or indirectly act on TF or create anti-tumor TME to kill tumor cells. Finally, inhibit the recurrence of stage II CRC. In addition, according to the result of GSE411 (PMID: 12754506),⁵⁷ suppressor of cytokine signaling (SOCS) protein is feedback inhibitor of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signaling pathway.⁵⁸ When Socs3-deficient, IL-6 induces a wider transcriptional response in Socs3-deficient macrophage than in wild-type cell. Thus, SOCS3 functions to control the quality of the response to IL-6. In our study, the nonrecurrence group was significantly enriched in this up-regulated gene set, which means regulation of IL-6 during the suppression of the recurrence of the stage II CRC was of high quality.

However, in fact, TME is so complex which with multiple cells interacting and numerous mechanisms intersecting, that it is difficult to explain any biological process such as the recurrence of stage II CRC from a single perspective. On the one hand, the macrophages(M1) shapes the anti-tumor microenvironment by providing immunosuppressive effect through a myriad of mechanisms, However, on the other hand, to gain fitness advantages, cancer cells can actively engage in constructing ecological niches by escaping the killing of macrophages (M1) through circumvent these inhibitory signals. These macrophage (M1)-tumor-microenvironment interactions can have significant implications for the recurrence of stage II CRC. Unfortunately, there is no significant immunologic signatures gene set enriched in the recurrence group, to some extent, its limited our comprehensive exploration of the possible mechanisms for the recurrence of stage II CRC.

However, our current study has the following limitations: 1. Our study is the retrospective analysis from TCGA database, therefore, the evidence level is imperfect. 2. Our study was based on mRNA evaluations from the TCGA database, therefore, it is not as persuasive as the level of protein expression. Due to the restrictions on the type of data run by TIMER, this immune risk score model could not be verified in GEO database. And we have not use clinical samples to further verify its authenticity. 3. As the clinical information of TCGA database was partial absence. It was not possible to implement a comparison of predictive effectiveness between our macrophage infiltration related-GRM and high-risk factors defined by the NCCN guidelines. While intriguing and hypothesis generating, our study requires further validation with a prospective, multicenter, randomized controlled clinical trial. As not only the quantity, but also the high ratio of macrophages to tumor cells and the position especially the tumor front positively influences the prognosis in CRC.⁵⁹ These are all what we need to pay attention to in order to ensure the accuracy of the research in the future. In addition, at present, immunotherapy is mostly used in patients with

advanced CRC. However, the clinical value of immune cell infiltration in the recurrence and prognosis of early stage CRC has been increasingly discovered.^{9, 17} Which providing a novel immunological perspective to improve clinical therapy performance and broadening the treatment thoughts of the early stage CRC, as well as assist clinicians in selecting targets for immunotherapies and individualize treatment strategies for these early patients. We wish this study will provide a novel immunological perspective to explore the mechanism of recurrence and improve clinical therapy practice in stage II CRC.

Conclusions

Previously, based on TCGA database, we established the GRM by random forest to predict the recurrence risk of stage II CRC with the AUC of 0.882. In this study, from the perspective of immune cell infiltration in the TME, we found the macrophage infiltration level in the nonrecurrence group was significantly higher than that in the recurrence group, the macrophage infiltration level was significantly negative correlated with the recurrence-related hub gene TF and was significantly positive correlated with the improved DFS and OS. In addition, macrophage infiltration related-GRM (0.906) provide a better performance than GRM (0.882) to predict the recurrence of stage II CRC. Based on the results of GSEA analysis, we speculate the macrophage infiltrated in the TME was M1 phenotype and defended against recurrence maybe a dynamic spatiotemporal process as follows: 1. Tumor cells release LPS or IFN- γ antigens. 2. Macrophages (M1) receive the stimulus signals (LPS or IFN- γ) and activation. 3. Trafficking of macrophages (M1) to tumor. 4. Macrophages (M1) infiltrate into tumor. 5. Macrophages (M1) recognize tumor then secrete IL-6 to directly or indirectly act on TF or create anti-tumor TME to kill tumor cells. Finally, inhibit the recurrence of stage II CRC. Notably, the regulation of IL-6 was high quality. In conclusion, this study maybe provides a novel immunological perspective to explore the mechanism of recurrence and improve clinical therapy practice in stage II CRC.

Abbreviations

ASCO

American Society of Clinical Oncology

AUC

area under the curve

CRC

colorectal cancer

CIC

cancer-immunity cycle

DFS

disease-free survival

IRFs

interferon regulatory factors

GRM

gene recurrent model
GSEA
Gene Set Enrichment Analysis
NF- κ B
nuclear factor kappaB
TNF- α
tumor necrosis factor- α
OS
overall survival
PAMPs
pathogen-associated patterns
SOCS
suppressor of cytokine signaling
TF
transferrin
TFR
transferrin receptor
TIMER
Tumor Immune Estimation Resource
TLR-4
Toll-like receptor 4
TME
The tumor microenvironment

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1

The multiple linear regression model was constructed with the TBP and PIP to predict lung metastasis.

	Model^a (p value)	Model^b
ANOVA(F)	15.682 (≤ 0.001)	8.413 (0.008)
Constant	3.303 (0.017)	145.197 (≤ 0.001)
PIP coefficients	0.14 (≤ 0.001)	5.222 (0.008)
TBP coefficients	0.147 (0.10)	
Adjusted R Square	0.561	0.244
Durbin-Watson	1.494	1.926
Std. Residual	-1.594 ~ 2.759	-1.028 ~ 3.312
a Predictors: (Constant), PIP, TBP; Dependent Variable: $\log_{10}^{(\text{Flux})}$		
b Predictors: (Constant), PIP; Dependent Variable: lung weight		

Table 2

Physical characteristics (concentration and size) of supernatants. (1) Within a certain period of culture time (30 min-24 h), extension of the culture time promoted release. (2) Coculture promoted release. (3)

There were no significant differences in EV size among the supernatants.

Sample	Size(nm)	Concentration
4T1 (30 min)	61.14 ± 16.45	5.45 × 10 ⁹
4T1 (24 h)	57.63 ± 16.91	9.47 × 10 ⁹
PLT (30 min)	64.26 ± 27.31	2.39 × 10 ¹⁰
PLT (24 h)	57.35 ± 19.73	3.28 × 10 ¹⁰
4T1 + PLT (30 min)	61.79 ± 24.34	2.97 × 10 ¹⁰
4T1 + PLT (24 h)	57.37 ± 19.55	5.73 × 10 ¹⁰
4T1: murine breast cancer cell line.		
PLT: platelets obtained from a BALB/c mouse.		
4T1 + PLT: coculture of the murine breast cancer cell line and the PLTs obtained from the BALB/c mouse.		

Table 3

The procoagulant activity of the 24 h coculture supernatant was significantly higher than that of the 24 h platelets supernatant.

Sample	Size(nm)	Concentration
PLT (24 h)	82.36 ± 28.29	5.84 × 10 ⁹
4T1 + PLT (24 h)	76.91 ± 29.26	9.86 × 10 ⁹
PLT: platelets obtained from a BALB/c mouse.		
4T1 + PLT: coculture of a murine breast cancer cell line and the PLTs obtained from the BALB/c mouse.		

Figures

Complete separation the main nutrient vessel

proximal ligation of the main nutrient vessel

Figure 1

Estimation of immune cells infiltration levels between nonrecurrence and recurrence groups of stage II CRC. $P < 0.05$ was considered statistically significant.

Figure 2

Comparing the differences of DFS and OS in the macrophage infiltration level between nonrecurrence and recurrence groups of stage $\geq$ CRC. $P < 0.05$ was considered statistically significant.

Figure 3

Assessing the diagnostic efficiency of GRM and macrophage infiltration related-GRM by ROC.

Figure 4

Exploring the correlation between the recurrence related hub gene TF and the macrophage infiltration level of stage $\geq$ CRC.

Figure 5

Analysis the gene set which about macrophage or macrophage-related cytokines between the nonrecurrence and the recurrence groups of stage $\geq$ CRC by GSEA.

Figure 6

Macrophage defends against recurrence maybe a dynamic spatiotemporal process.