

Prognostic Value of CD8+ TILs and MMR Status and Their Correlation with Clinical Outcomes in Rectal Cancer

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Research Article

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Abstract

Objective

To explore the relationship between CD8 + tumor-infiltrating lymphocytes (TILs) in rectal cancer, mismatch repair (MMR) status, and patient prognosis.

Methods

A total of 414 paraffin-embedded tissue samples from patients with pathologically confirmed stage I–III rectal cancer were included in this study. Tissue microarrays (TMAs) with a thickness of 4 µm were prepared, and immunohistochemical staining was performed using a CD8 antibody to detect the expression of CD8-positive cells. MMR status was assessed using immunohistochemical markers (MSH2, MSH6, MLH1, PMS2).

Results

CD8 + TILs were expressed on T lymphocytes. Density was significantly associated with tumor invasion depth, lymph node metastasis, and MMR status ($P < 0.05$), but not with age, sex, surgery type, or tumor size ($P > 0.05$). Patients with high CD8 + TIL density and stable MMR status had longer overall and progression-free survival. Univariate and multivariate Cox analyses indicated low CD8 + TIL density and unstable MMR status as independent risk factors for poor prognosis.

Conclusion

CD8 + TIL density and MMR status may serve as combined prognostic biomarkers in rectal cancer, with higher density and stable MMR status linked to better outcomes and potential guidance for immunotherapy strategies.

1 Introduction

Rectal cancer is a common malignant tumor originating from abnormal growth and division of malignant cells in the rectal lining. It is the third most common malignancy worldwide and ranks second in mortality^[1]. The high fatality rate of rectal cancer persists globally, with many patients diagnosed at advanced stages, leading to poor prognoses. Despite recent advancements in cancer treatment, rectal cancer management remains challenging, especially in advanced and recurrent cases^[2].

Recent studies have found that CD8 + tumor-infiltrating lymphocytes (TILs) in rectal cancer are closely related to tumor progression and prognosis^[3]. The level of CD8 + TIL infiltration is usually inversely correlated with clinical features and prognosis of rectal cancer. Some studies indicate that higher

infiltration levels of CD8 + TILs are associated with smaller tumor volumes, earlier stages, and fewer local lymph nodes. This suggests that the immune system serves as a defense mechanism capable of recognizing and eliminating abnormal cells, including tumor cells, thereby counteracting tumor metastasis^[4, 5]. The degree of CD8 + T cell infiltration reflects the activity of immune responses and the body's immune surveillance against tumors.

Research has shown that high-density CD8 + TILs are linked to stronger anti-tumor immune responses, which help inhibit tumor growth and spread, enhancing treatment efficacy and prognosis. This may be due to CD8 + TILs facilitating tumor immune clearance and resistance to recurrence^[6]. Additionally, CD8 + TILs have been widely studied in immunotherapy. Immunotherapy leverages the activation of the patient's own immune system to attack tumor cells, improving treatment outcomes by enhancing the anti-tumor effects of CD8 + TILs^[7]. For instance, inhibiting immune checkpoint molecules such as CTLA-4 and PD-1 can release the anti-tumor activity of CD8 + TILs, thereby increasing tumor sensitivity to immunotherapy. While the significance of CD8 + TILs in rectal cancer has been established, their mechanisms and interactions require further exploration.

Mismatch repair (MMR) deficiency is another important factor in rectal cancer, as it can influence the tumor microenvironment and immune cell density. MMR status, particularly instability (MSI), has been linked to altered immune cell infiltration, including CD8 + TILs. The combination of CD8 + TIL density and MMR status could potentially provide a more accurate prognostic marker for rectal cancer^[8].

2 Methods

2.1 General Information

We collected the data of 414 patients with rectal cancer who were treated at our hospital's rectal cancer diagnosis and treatment center. These patients were seen between January 2010 and December 2014 and all underwent radical surgery. Before surgery, none had received any treatment, and preoperative examinations did not reveal any abdominal or other organ metastases. Postoperative pathological results confirmed all cases were rectal adenocarcinoma. Patient information, including sex, age, type of surgery, depth of invasion, tumor size, and number of lymph nodes, was extracted from the hospital's medical records database. This study was approved by our hospital's Ethics Committee in 2020 (Ethics Approval No: 2020K-1418). Informed consent was obtained from all patients, who received standard postoperative chemotherapy and radiotherapy. Detailed follow-up instructions were provided, requiring patients to undergo tumor marker tests and chest-abdomen CT scans every 3 to 6 months for the first 2 years, and then every 6 to 12 months starting from the third year.

2.2 Materials

Immunohistochemistry: Rectal cancer tissue specimens were first fixed in 4% paraformaldehyde, then embedded in paraffin, and cut into 4 µm thick sections. These were subjected to routine

deparaffinization and hydration, followed by high-temperature antigen retrieval using citrate buffer. After blocking endogenous peroxidase activity, sections were incubated overnight at 4°C with primary antibodies for CD8 (1:50) and mismatch repair proteins (MSH2, MSH6, MLH1, PMS2). For MMR status, tumors were classified as stable (normal expression of all four proteins) or unstable (loss of expression of one or more MMR proteins). DAB staining was performed for visualization.

2.3 Results Analysis

CD8 + TILs Density Analysis: Tissue sections containing CD8 + tumor-infiltrating lymphocytes (TILs) from rectal cancer specimens were selected. These sections had been properly fixed and stained. High magnification microscopy was used to observe the tissue sections. CD8 + positive cells can be identified through visual inspection or using image analysis software. CD8 + positive cells are typically visualized through specific immunohistochemical staining or fluorescent staining. Subsequently, CD8 + TILs were counted. The number of CD8 + positive cells was counted in each selected field of view using high magnification microscopy, and the counting results were recorded. The counts from each field of view were summed to obtain the total count for the entire sample area. The total count was then divided by the tissue area to calculate the density of CD8 + TILs. A threshold of 10% was set for CD8 + TILs density; densities below 10% were categorized as low-density, and densities greater than or equal to 10% were categorized as high-density^[9].

2.4 Statistical Analysis

Statistical analysis was performed using SPSS 26.0 software. For the comparison of count data, the chi-square test was used, with numerical data presented as percentages. To assess the relationship between CD8 + TIL density, MMR status, and patient prognosis, we included MMR status as a categorical variable in our Cox regression models. We performed both univariate and multivariate Cox regression analyses to evaluate the independent prognostic value of CD8 + TIL density and MMR status, while adjusting for other clinicopathological factors.

3 Results

3.1 In rectal cancer tissues, CD8, as a type of immune cell, is located on the cell membrane (Figures 1).

3.2 The study found that the infiltration level of CD8+ TILs in rectal cancer tissues is closely related to patient prognosis. When comparing data between low-density and high-density groups in terms of age, gender, type of surgery, tumor staging, tumor size, and MMR status, we observed that CD8+ TIL density was significantly higher in tumors with stable MMR status ($P < 0.05$). The low-density CD8+ TIL group had a higher prevalence of MMR instability compared to the high-density group ($P < 0.05$). (Table 1).

3.3 In univariate Cox regression analysis, CD8+ TIL density and MMR status were found to be significantly associated with prognosis ($P < 0.05$), while age, gender, type of surgery, tumor invasion depth, tumor stage, and tumor size were not significantly related ($P > 0.05$). (Figure 2). Multivariate Cox

regression analysis revealed that low CD8+ TIL density and unstable MMR status were independent risk factors for poor prognosis in rectal cancer patients ($P < 0.05$). (Figure 3).

3.4 Patients in the high-density CD8+ TIL group exhibited longer overall survival (OS) and progression-free survival (PFS) compared to those with low-density CD8+ TILs ($P = 0.0346$). This suggests that a higher density of CD8+ TILs may be associated with a favorable prognosis. Additionally, tumors with stable MMR status showed better survival rates (Figure 4).

4 Discussion

Rectal cancer is a tumor with complex pathophysiological characteristics, and the immune system plays a pivotal role in its development and treatment. As an important type of immune cell, CD8 + tumor-infiltrating lymphocytes (TILs) have been extensively studied for their associations with the progression and prognosis of rectal cancer^[10–11]. In this study, we investigated the relationship between CD8 + TILs and tumor progression as well as prognosis in rectal cancer patients, and obtained several interesting findings. Through immunohistochemical analysis, we observed varying degrees of CD8 + TIL infiltration in rectal cancer patients. Higher CD8 + TIL density was more commonly detected in patients with advanced-stage disease, indicating that the activation of the immune system may exert an important role in controlling tumor progression. Statistical analyses revealed that CD8 + TIL density was significantly correlated with lymph node metastasis and mismatch repair (MMR) status, but showed no significant associations with age, gender, tumor size, and other clinical parameters. These results suggest that the infiltration of CD8 + TILs may be regulated not only by traditional clinicopathological features, but also by complex factors such as tumor genetic heterogeneity and immune escape mechanisms^[12].

Encouragingly, patients with high CD8 + TIL density exhibited better survival rates and lower recurrence rates, which is consistent with the findings in other malignancies (lung cancer, esophageal cancer, ovarian cancer, pancreatic cancer, and renal cancer) that high CD8 + TIL density serves as a favorable prognostic indicator^[13, 14, 15, 16]. In studies on primary rectal cancer, increased CD8 + TIL density was closely associated with tumor invasion, metastasis and favorable prognosis, which aligns with our results^[17]. This may be attributed to the fact that CD8 + TILs enhance immune clearance of tumors and resistance to recurrence. Therefore, CD8 + TILs may act as a potential prognostic marker for rectal cancer, facilitating the formulation of treatment decisions.

In the present study, we drew a positive conclusion that the density of CD8 + TILs in rectal cancer tissues was correlated with several clinical and pathological characteristics of patients. Specifically, there were no statistically significant differences between the low-density group and the high-density group in terms of patient age, gender, surgical type and tumor size ($P > 0.05$). However, the differences between the two groups in tumor invasion depth, lymph node metastasis and MMR status were statistically significant ($P < 0.05$). In particular, regarding tumor invasion depth, the proportion of T1-T2 stage cases in the low-density group was significantly higher than that in the high-density group ($P = 0.008$), while the high-

density group was predominantly composed of T3-T4 stage cases. This indicates that high CD8 + TIL density may be associated with shallower tumor invasion depth. In addition, the incidence of lymph node metastasis was higher in the low-density group ($P = 0.001$), suggesting that high CD8 + TIL density may be correlated with fewer lymph node metastases. With respect to MMR status, the number of patients with high microsatellite instability (MSI-H) in the low-density group was significantly lower than that in the high-density group ($P = 0.0001$). This implies that high CD8 + TIL density may be associated with a higher proportion of MSI-H cases, which sheds light on the tumor immune microenvironment and its sensitivity to immune checkpoint inhibitors.

In the present study, we performed univariate Cox regression analysis to investigate the impact of multiple clinical and pathological variables on survival outcomes in rectal cancer patients. The results of the analysis demonstrated that the density of CD8 + tumor-infiltrating lymphocytes (TILs) was a significant predictor of survival, P value of less than 0.00001, and a hazard ratio (HR) of 0.509 (95% confidence interval [CI]: 0.388, 0.667). These findings indicate that high CD8 + TIL density is significantly associated with a more favorable prognosis. Furthermore, mismatch repair (MMR) status also exhibited statistical significance, with an HR of 1.900 (95% CI: 1.220, 2.959), suggesting that patients with MMR deficiency have a markedly increased survival risk. Although other variables such as lymph node metastasis (HR = 1.124, $P = 0.040$) showed marginal significance, the remaining variables including age, gender, surgical type, tumor size, depth of invasion, and tumor stage did not reach statistical significance. These results indicate that CD8 + TIL density and MMR status have important value in the assessment of survival prognosis for rectal cancer and may provide an effective basis for the development of individualized clinical treatment strategies.

In this study, multivariate Cox regression analysis was performed to further explore the impact of multiple clinical and pathological variables on the survival outcomes of rectal cancer patients. The results showed that the density of CD8 + tumor-infiltrating lymphocytes (TILs) was a significant prognostic factor, P value of less than 0.001, and a hazard ratio (HR) of 0.548 (95% confidence interval [CI]: 0.409, 0.733), indicating that high CD8 + TIL density is significantly associated with better survival prognosis. Furthermore, mismatch repair (MMR) status also exhibited statistical significance, with an HR of 1.649 (95% CI: 1.120, 2.428), suggesting that patients with MMR deficiency face an increased survival risk. Although other variables, such as depth of invasion (HR = 0.798, $P = 0.085$) and surgical type (HR = 0.803, $P = 0.146$), approached significance, they failed to reach the statistical significance level, indicating that the impact of these factors on prognosis requires further investigation. In contrast, age, gender, tumor size, tumor stage, and lymph node metastasis did not show obvious correlations in this analysis. These results emphasize the critical role of CD8 + TIL density and MMR status in the prognostic assessment of rectal cancer, suggesting that these two factors should be used as important prognostic indicators in clinical practice to promote the development of individualized treatment and provide more optimal treatment regimens for patients.

However, there are still some unresolved issues regarding the relationship between CD8 + TILs and rectal cancer. First, the degree of CD8 + TIL infiltration may be influenced by other types of immune cells, such

as regulatory T cells (Tregs) and macrophages, which may affect the function and anti-tumor efficacy of CD8 + TILs^[18, 19]. Second, tumor genetic heterogeneity may lead to differences in lymphocyte density in different regions, thereby affecting their prognostic relevance. Future studies should further explore the specific mechanisms and functions of CD8 + TILs, including their roles in tumor antigen recognition and cytotoxic killing, as well as their interactions with other immune cell types. In addition, it is necessary to explore how to use knowledge about CD8 + TILs to develop more effective immunotherapeutic strategies, such as immune checkpoint inhibitors and CAR-T cell therapy^[20, 21]. Based on a large-scale cohort of rectal cancer patients and long-term follow-up data, this study systematically evaluated the relationship between CD8 + TIL infiltration, clinicopathological characteristics, and prognosis, providing new evidence for the clinical significance of the rectal cancer immune microenvironment, while verifying and expanding previous research findings.

Of course, our study is single-center; future validation through multi-center studies will provide more accurate results. The immune microenvironment involves not only CD8 + TILs but also other immune cells, which were not considered in this study, thus resulting in certain limitations.

Declarations

Ethics Approval Information

We adhere to the standard practice of the Fourth Hospital of Hebei Medical University, providing detailed information to each patient involved in the study and requesting their written consent to allow us to use their relevant information. In addition, this study has been approved by the Clinical Research Ethics Committee of the Fourth Hospital of Hebei Medical University (No. 2020KY418). These measures ensure the respect of patient rights and ensure that our research conforms to ethical standards and regulations.

Roles and Contributions of All Authors

In this study, Baokun Li was responsible for literature review and manuscript preparation. Zesong Meng and Feifei Wang contributed to data collection, organization, statistical analysis, with assistance from two pathologists in the Department of Pathology, the Fourth Hospital of Hebei Medical University. Chaoxi Zhou and Youqiang Liu participated in statistical analysis and manuscript writing. Xuhua Hu provided critical revision of the manuscript and material support. Fei Yang was responsible for manuscript revision and provided overall guidance on the core concept of the study. All authors have read and approved the final manuscript.

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Information Disclosure

The authors explicitly declare that they have no potential competing interests related to the research.

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There are no applicable acknowledgments.

Data Information

The datasets generated and/or analysed during the current study are not publicly available due, The data is currently under research and analysis and is not yet ready for public release. but are available from the corresponding author on reasonable request.

Consent to Participate

All individuals involved in this study have voluntarily provided written informed consent based on a clear understanding of the study's purpose, procedures, potential risks, and possible benefits. The informed consent documents are securely stored.

Consent to Publish

Not applicable

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Tables

Tables are available in the Supplementary Files section.

Figures

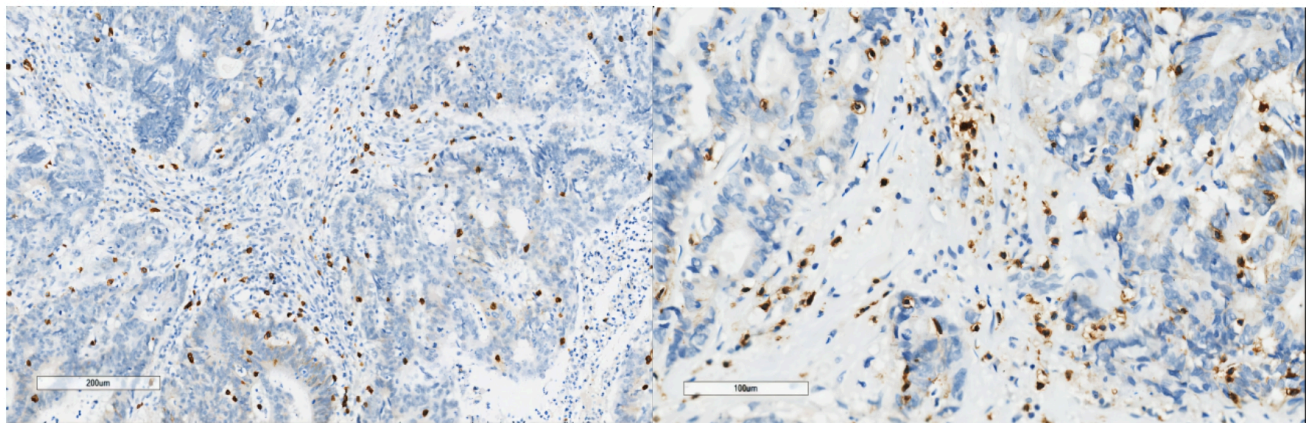


Figure 1

(A) This sample exhibits CD8-positive cells in moderately differentiated rectal adenocarcinoma, indicating the presence of active T cells. (B) This sample, at a higher magnification, reveals a more detailed cellular structure and quantifies the number of CD8-positive cells.

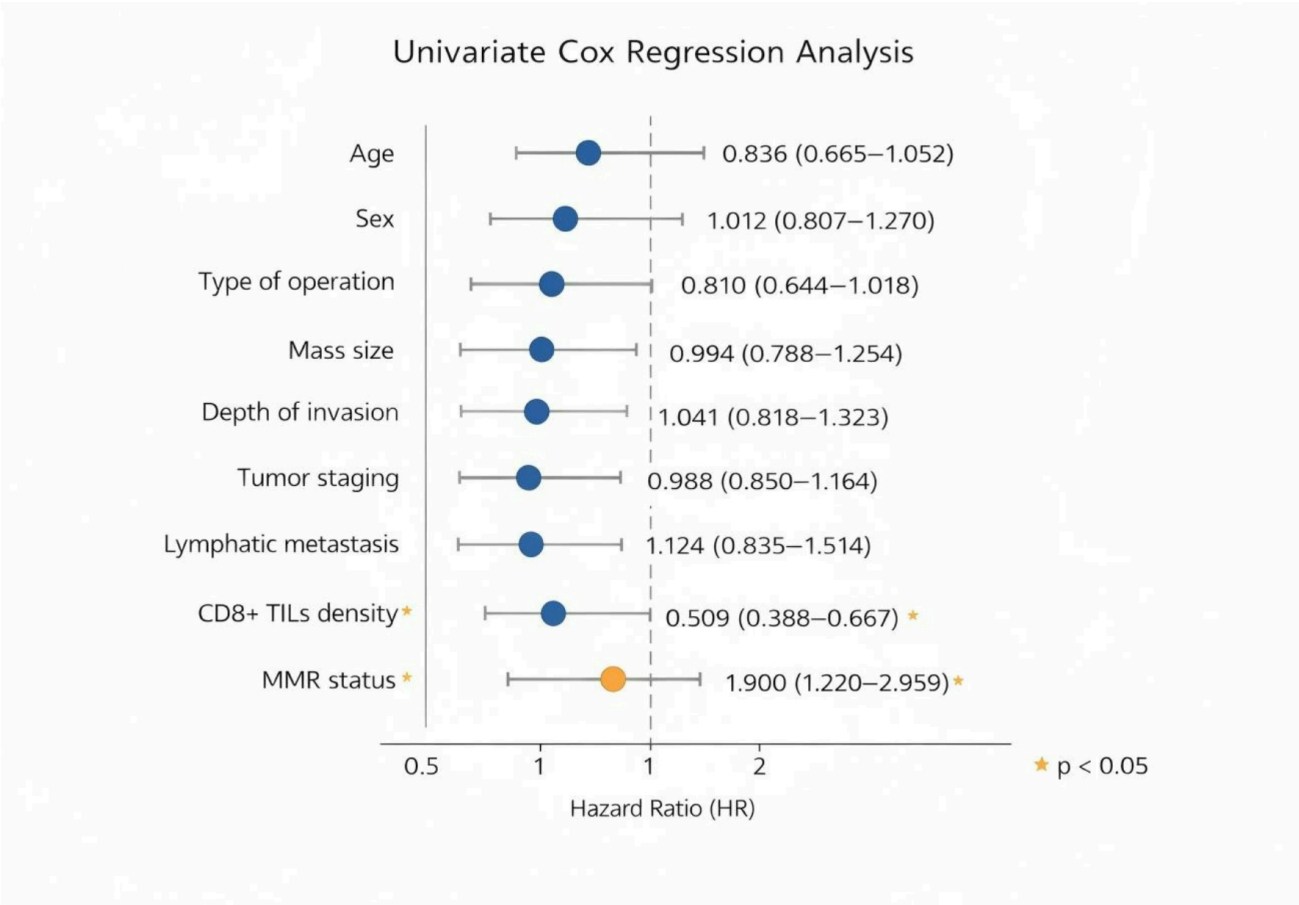


Figure 2

Univariate Analysis of Factors Affecting Prognosis in This Group of Rectal Cancer Patients.

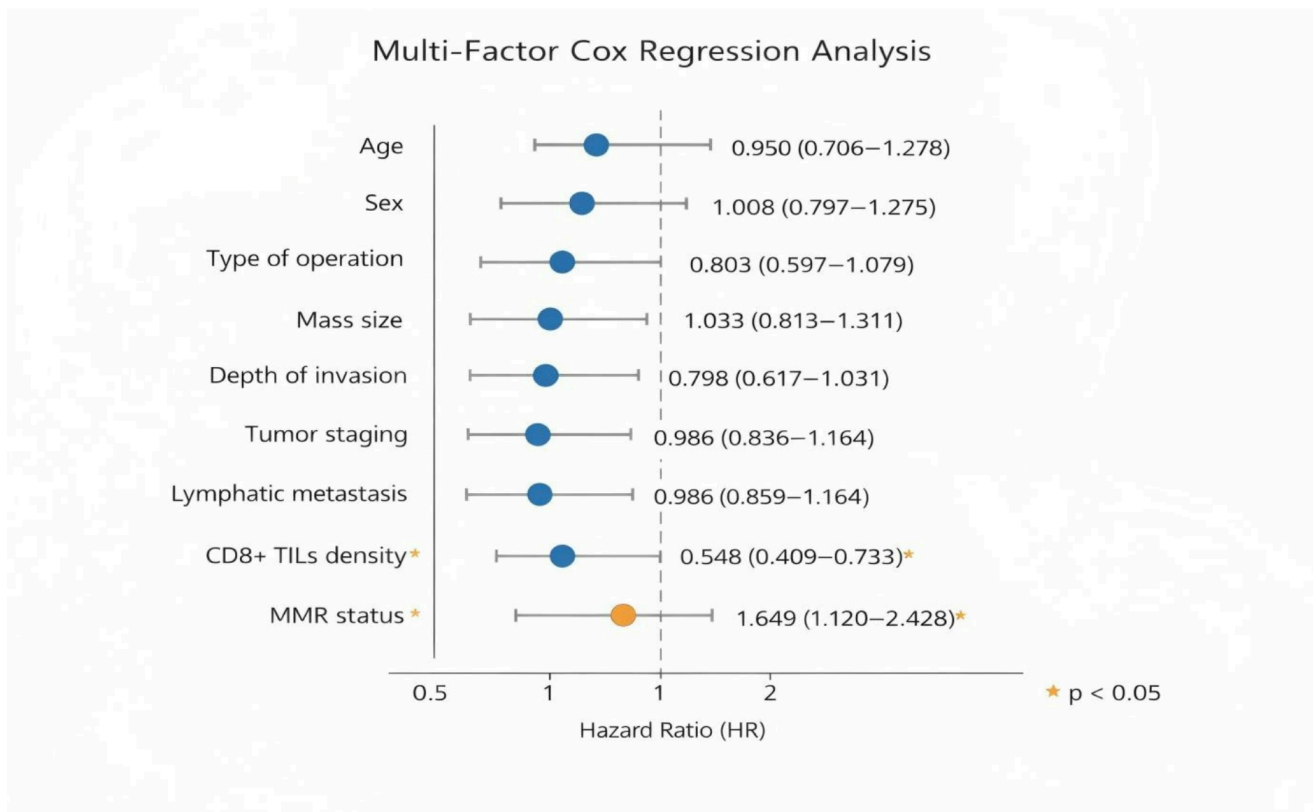


Figure 3

Multivariate analysis of factors influencing the prognosis of rectal cancer patients in this study group.

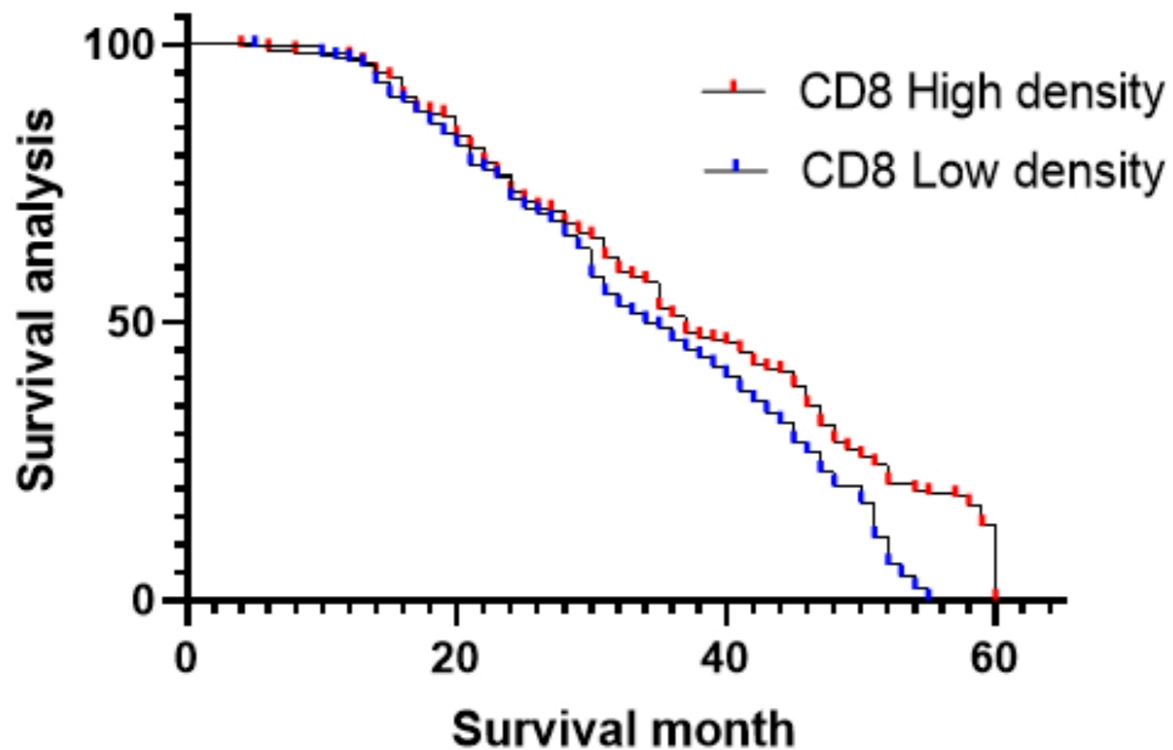


Figure 4

Survival curves of rectal cancer patients based on levels of tumor-infiltrating CD8+ T lymphocytes.
 $P=0.0346$

Supplementary Files

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