The density of CD8+ tumour-infiltrating T lymphocytes in rectal cancer cells and its relationship with the clinicopathological characteristics and prognosis of patients

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Research Article

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Abstract

Objective: To explore the relationship between CD8+ tumor infiltrating T lymphocyte density and clinicopathological features and prognosis of patients with rectal cancer.

Methods: For immunohistochemistry, paraffin-embedded TMA samples were cut into 4-μm sections. CD8 (ZA-0508, ZSGBBIO) Specific procedure: Tissue sections were baked, dewaxed and rehydrated. Endogenous peroxides were closed by incubating at 37°C for 15 minutes with a methanolic solution of 3% hydrogen peroxide. Antigen repair is then carried out by autoclaving in EDTA antigen repair solution (pH=8) for approximately 10 minutes. Sections were incubated with primary antibody for approximately 1.5 hours at 37°C. After washing, tissue sections were treated with HRPRABBIT/MOUSE secondary antibody (K5007, 20029103, Dako) for 30 minutes at room temperature. Sections were immunostained with diaminobenzidine tetrahydrochloride (DAB, K5007, 20019193, Dako) and restained with hematoxylin.

Results:CD8+ TILS were expressed in rectal cancer tissues in T lymphocytes, which were localised to the cell membrane (Figure 1).

The relationship between the density of CD8+ TILs in rectal cancer tissues and the clinicopathological characteristics of patients, low density group and high density group, there was no statistically significant difference ($P>0.05$) in terms of age, gender, type of surgery and size of the mass, and there was a statistically significant difference ($P<0.05$) when comparing the depth of infiltration and lymph node metastasis, as shown in Table 1.

For survival analysis, Kaplan-Meier analysis showed that patients with high density CD8+ TILS had a better prognosis than those with low density CD8+ TILS. Figure 2.

Multi-factor Cox regression analysis with prognosis as the dependent variable (Table 2 for variable assignment) showed that low density CD8+ TILs was an independent risk factor for poor prognosis in patients with rectal cancer ($p<0.05$), see Table 3.

Conclusion: This study illustrates that CD8 expression can be used as an indicator of prognosis in rectal cancer patients, and also provides more effective guidance for immunotherapy in rectal cancer patients at a later stage.

1 Introduction

Colorectal cancer (CRC) has the third highest incidence and the second highest mortality rate in the world [1] , and in a study of survival rates for rectal cancer, it was noted that its 5-year survival rate was about 53% [2] . We need to find a more effective prognostic indicator to provide new evidence for clinical treatment.

With the proposed tumour immune microenvironment (TIME), which plays an important role in tumourigenesis and development, especially against cancer cells [3], tumour infiltrating T cells (TILs), are more effective against chemotherapy and have a better prognosis in patients with a higher density of tumour microenvironment that is involved in or inhibits the proliferation and migration of malignant tumour cells (TILs) [4–5], studies have demonstrated that tumourinfiltrating Tlymphocytes (TILs) can alter the tumour microenvironment and participate in or inhibit the proliferation and migration of malignant cells Among the many types of TILs, only some are prognostic, and studies have confirmed that CD8 + TILs are cytotoxic immune cells that can directly kill or kill cancer cells [6], therefore, if we understand the immune status of tumour cells in rectal cancer patients, then it will be valuable as a guide for both patient prognosis and biomarker screening for immunotherapy. The aim of this study was to analyse the density of CD8 + TILs in rectal cancer tissue cells and its relationship with the clinicopathological characteristics and prognosis of patients.

2 Methods

2.1 General data We collected 404 rectal cancer patients who all underwent D2 radical surgery from January 2010 to December 2014 who attended the Colorectal Cancer Treatment Center of the Fourth Hospital of Hebei Medical University, all without any preoperative treatment, with preoperative examination excluding distant organ metastases, and all with postoperative pathology of rectal adenocarcinoma. The study was conducted according to the Declaration of Helsinki (revised in 2013) and was reviewed and approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (Ethics: 2020K-1418), and all patients signed an informed consent notification form. Tumour markers and abdominal computed tomography scans were performed 3–6 months in a year, after which the review was changed to 6–12 months.

2.2 Materials

2.2.1 Immunohistochemistry

Paraffin-embedded TMA samples were cut into 4-μm sections.CD8 (ZA-0508, ZSGBBIO) Specific procedure: tissue sections were baked, dewaxed and rehydrated. Endogenous peroxides were closed by incubating at 37°C for 15 minutes with a methanolic solution of 3% hydrogen peroxide. Antigen repair is then carried out by autoclaving in EDTA antigen repair solution (pH = 8) for approximately 10 minutes. Sections were incubated with primary antibody for approximately 1.5 hours at 37°C. After washing, tissue sections were treated with HRPRABBIT/MOUSE secondary antibody (K5007, 20029103, Dako) for 30 minutes at room temperature. Sections were immunostained with diaminobenzidine tetrahydrochloride (DAB, K5007, 20019193, Dako) and restained with hematoxylin.
2.3 Analysis of results

2.3.1 CD8 + TILS density analysis: CD8 + TILS was visible in the tumour cell membrane or cytoplasm after being positively stained as yellowish to brownish granules. The percentage of positive cells was obtained

under high magnification microscopy (400x), with less than 10% representing low CD8 + TILS expression, 10%-39% being moderate expression and 40%-90% being high expression. The median number of CD8 + TILS was used as the basis for dividing the number of CD8 + TILS-positive cells in patients into low-density and high-density groups [7] .

2.4 Statistical analysis. SPSS 26.0 statistical software was used for statistical analysis of the data. All count data were expressed as numbers and rates (%). For comparisons that existed between comparison groups X2 tests were used and correlations between variables were summed using Spearman correlations. The Kaplan-Meier method was used to estimate survival rates. Factors influencing the prognosis of patients with rectal cancer were analysed using one-way and multi-way Cox regression analysis. $p < 0.05$ was statistically significant.

3 Results

3.1 CD8+ TILS were expressed in rectal cancer tissues in T lymphocytes, which were localised to the cell membrane (Figure 1).

3.2 The relationship between the density of CD8+ TILs in rectal cancer tissues and the clinicopathological characteristics of patients, the low density group and high density group, there was no statistically significant difference ($P>0.05$) in terms of age, gender, type of surgery and size of the mass, and there was a statistically significant difference ($P<0.05$) when comparing the depth of infiltration and lymph node metastasis, as shown in Table 1.

3.3 Survival analysis, Kaplan-Meier analysis showed that patients with high density CD8+ TILS had a better prognosis than those with low density CD8+ TILS. Figure 2.

3.4 Multi-factor Cox regression analysis with prognosis as the dependent variable (Table 2 for variable assignment) showed that low density CD8+ TILs was an independent risk factor for poor prognosis in patients with rectal cancer ($p<0.05$), see Table 3.

4 Discussion

We know that the immune microenvironment plays a crucial role in the development of tumours, which includes tumour-associated macrophages, T cells, B cells, dendritic cells (DCs) and neutrophils [8–10] . This further illustrates that the immune microenvironment as a whole works against tumour cells. In our study, we found that among these immune cells, CD8 + TILs are even a very important immune cell, which mainly mediates the body's cellular immune response and plays an important role in the body's anti-tumour process [11] , and it can be found that the increased density of CD8 + T cells in the tumour plays a crucial role in suppressing the tumour plays a crucial role, especially in cancers such as lung, oesophageal, ovarian, pancreatic and renal cancers, which can be used as good prognostic indicators [12–14] . In a study of primary colorectal cancer, elevated CD8 + TILS density was found to be closely

associated with tumour infiltration, metastasis and better prognosis [15–16], which is largely consistent with these reports in our results.

In the present study, we first analysed the relationship between CD8 + TILs and clinicopathology. We selected 404 colorectal cancer patients without any preoperative adjuvant therapy, and immunohistochemistry was used to detect the expression of CD8 + TILS density. In the pathological tissues of rectal cancer patients we saw that CD8 + TILS density correlated with patient age, gender, type of surgery and acute tumour size There was no correlation with patient age, gender, and surgical type of acute tumor size, while there was a strong and statistically significant correlation in the depth of tumor infiltration and lymph node metastasis. There was a statistical difference between the KaplanMeier curves of patients in the low-density group and the high-density group. In the multi-factor Cox regression analysis of factors influencing prognosis of rectal cancer patients, we found that CD8 + TILS density was closely related to the prognosis of patients, and the survival of patients with high-density rectal cancer with CD8 + TILs was longer than that of patients with low-density rectal cancer, and CD8 + TILS density was an independent risk factor for poor prognosis in patients with rectal cancer. Therefore, the study in this trial illustrates that CD8 expression can be used as an indicator of prognosis for rectal cancer patients and a more effective guide for rectal cancer patients to undergo immunotherapy at a later stage.

Of course, our study is a single-centre study, then the results will be more accurate if we can post-combine with multi-centre studies for validation. For autoimmunity, our immune environment involves not only PD-L1 and CD8 + TILS, but also more immune cells [17–18], and obviously we did not take these factors into account, so our study also has shortcomings place.

Declarations

Ethical Approval and Informed Consent

In our study, written informed consent for the use of patient information was obtained from all as is routine practice at the Fourth Hospital of Hebei Medical University, and the study was approved by the Clinical Research Ethics Committee of the Fourth Hospital of Hebei Medical University.

Author Contributions

Baokun Li and Guiying Wang were responsible for the literature search, design of the study and writing of the article; Feifei Wang and Fei Yang performed data collection, acquisition and statistical analysis, Jiaxu Han and Zesong Meng and were responsible for the production of the figures; Guanglin Wang and Chaoxi Zhou critically reviewed the intellectual content of the article and provided material support, Bin Yu performed quality control and proofreading of the article . All authors confirmed the final draft of the paper.Funding

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Disclosure

The authors declare no potential competing interests

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Patient consent for publication

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Tables 1-3 is available in the Supplementary Files section.

Figures

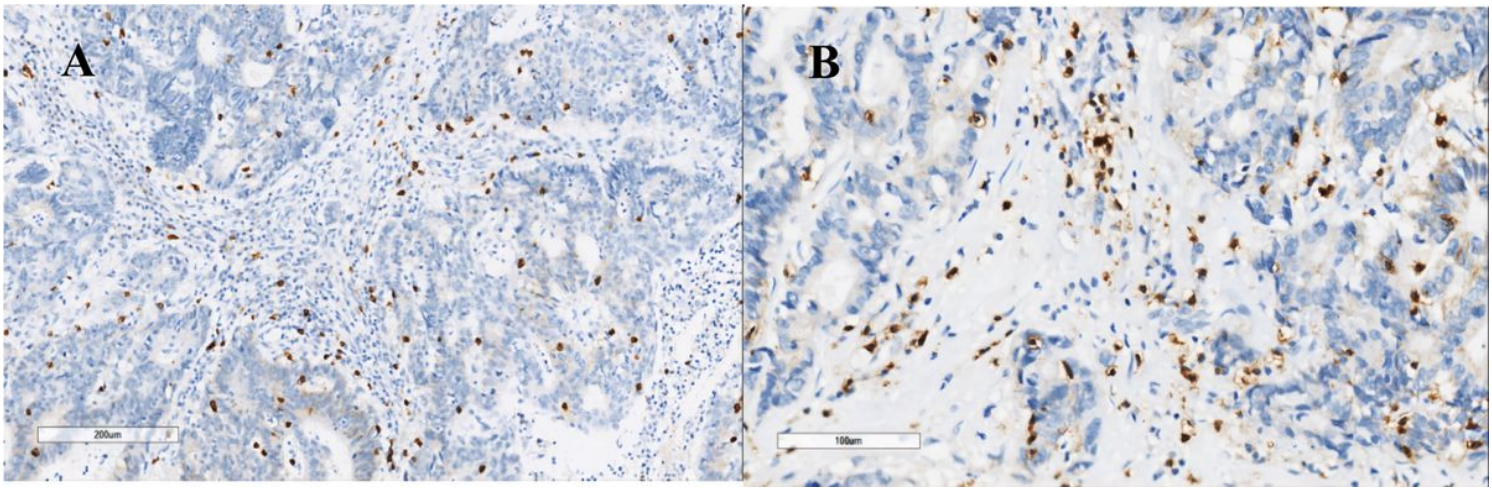

Figure 1

(A) Microscopic image of CD8 protein expression (EnVision assay) in a moderately differentiated rectal adenocarcinoma (×10). (B) Microscopic image of CD8 protein expression (EnVision assay) in a highly differentiated rectal adenocarcinoma (×20).

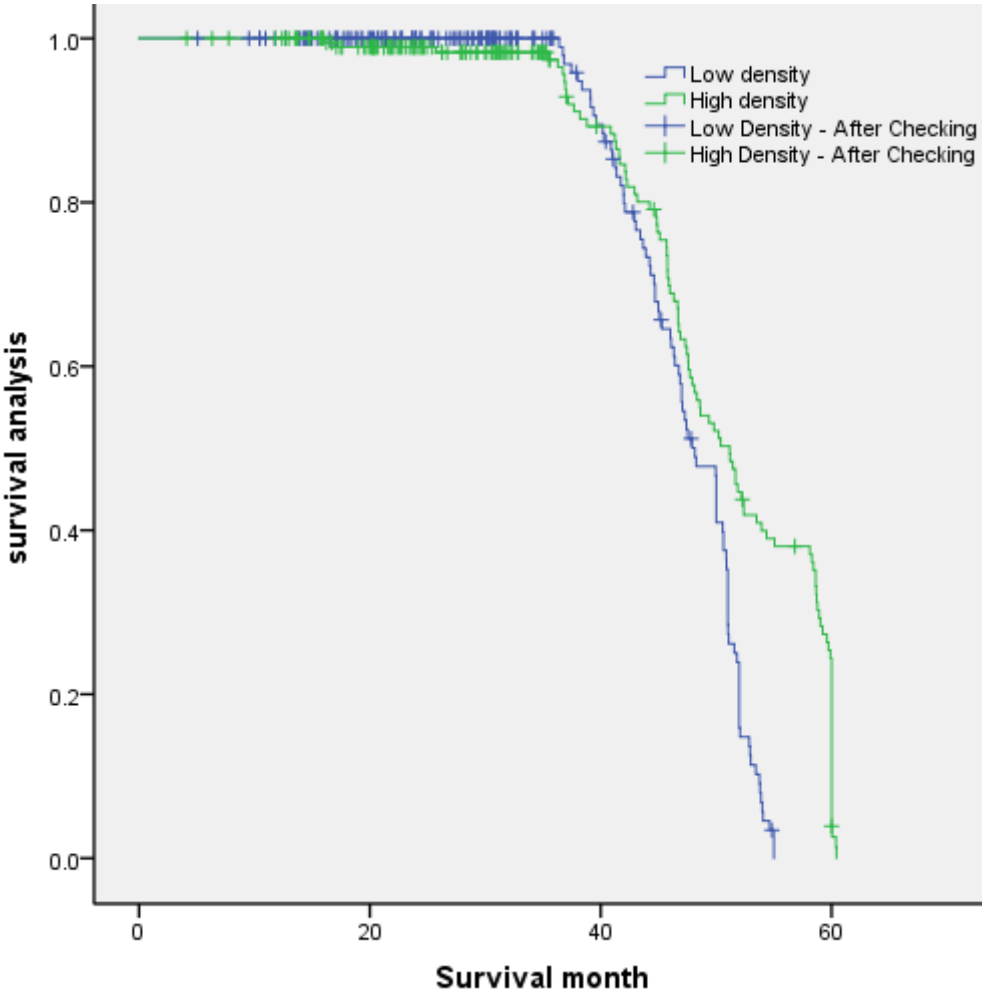

Figure 2

Kaplan-Meier curves in rectal cancer patients with different densities of CD8+ TILs

Supplementary Files

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- Table13.docx