

# **Correlation and Heterogeneity of the Immune Microenvironment and Histopathological Growth Patterns in Resectable Colorectal Cancer Liver Metastases**

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## **1. Background**

For patients with resectable colorectal cancer liver metastases (CRLM), a surgery-based treatment approach remains the primary therapeutic modality. However, the majority of patients experience recurrence within two years after hepatectomy, highlighting the critical importance of identifying effective biomarkers to predict postoperative recurrence and survival following resection of liver metastases. Histopathological growth patterns (HGP) of liver metastases have been proposed as potential prognostic biomarkers for patients with CRLM. Nevertheless, how HGPs influence patient prognosis and their relationship with the hepatic immune microenvironment remain unclear.

## **2. Aim and Content of the Study**

### **2.1 Aim of the study**

This study aims to retrospectively analyze the status and spatial heterogeneity of the tumor microenvironment (TME) in liver metastases from patients with CRLM, as well as the association between HGPs at the tumor-liver interface and postoperative recurrence following resection of liver metastases. Furthermore, this study seeks to explore the underlying mechanisms through which HGPs influence patient prognosis.

### **2.2 Content of the study**

- **Inclusion criteria:**

- a. Patients with colorectal adenocarcinoma who underwent liver metastasis resection at West China Hospital of Sichuan University;
- b. The liver resection specimens were confirmed to be liver metastases of colorectal cancer by histopathological examination;
- c. The liver metastasis surgical specimens could be obtained;
- d. It was confirmed postoperatively to be R0 resection;
- e. The clinical relevant data are complete.

- **Exclusion Criteria:**

- a. Only those who undergo liver ablation surgery are accepted;
- b. Patients who have not undergone R0 liver resection;
- c. Those who have undergone more than 3 times of liver metastasis resection;
- d. Those who die within one month after surgery or whose follow-up period is less than 6 months;
- e. Those whose clinical data are incomplete.

### **2.3 Materials and Methods**

This study is a retrospective investigation and was approved by the Biomedical Research Ethics Committee of West China Hospital, Sichuan University (Approval No. 2021,2024).

**Clinical Information and Analysis:** All data were derived from the advanced colorectal cancer cohort of the Colorectal Cancer Center. This study reports patient clinical outcomes, including baseline clinicopathological and molecular characteristics, treatment details, overall survival (OS), and recurrence-free survival (RFS).

**Histopathological Growth Pattern (HGP) Assessment:** According to the 2017 guidelines on histopathological growth patterns of liver metastases, HGPs are primarily classified into three types: the desmoplastic histopathological growth pattern (dHGP), the replacement histopathological growth pattern (rHGP), and the pushing histopathological growth pattern (pHGP). The tumor-liver interface was independently delineated by two pathologists using QuPath software, and distinct HGPs at the tumor-liver interface were identified. Formalin-fixed, paraffin-embedded (FFPE) sections of colorectal cancer liver metastases were stained with hematoxylin and eosin (H&E). The tumor-liver interface was independently delineated by two pathologists using QuPath software, and distinct histopathological growth patterns at the tumor-liver interface were identified.

**Immune Microenvironment Assessment:** Multiplex immunohistochemistry (mIHC) staining was performed on FFPE sections of liver metastases using two panels (Panel 1: CD4, CD8A, Foxp3, PD-L1, Panck; Panel 2: CD68, CD163, FAP,  $\alpha$ -SMA, Panck), encompassing a total of nine immune cell markers. Using QuPath pathology imaging software, tissue sections were divided into the tumor center (defined as regions  $>500\ \mu\text{m}$  from the liver-tumor interface) and the invasive tumor front (defined as a 1 mm region extending  $500\ \mu\text{m}$  on either side of the liver-tumor interface). Quantitative analysis of immune cell populations was performed in the tumor center, the invasive tumor front, and regions corresponding to different histopathological growth patterns.

**Statistical Methods and Study Endpoints:** Survival analysis was estimated using the Kaplan – Meier method and compared using the log-rank test. Quantitative data regarding the immune microenvironment were compared using the t-test or the Mann – Whitney U test, as appropriate. Changes between initial resection and secondary resection were analyzed using the nonparametric test for two paired samples. The endpoints of this study included patient clinical outcomes (OS and RFS), spatial distribution differences of immune cells within TME of liver metastases, distribution patterns of HGPs and their correlation with survival, and analysis of the immune microenvironment in relation to HGPs.

## 2.4 Outcome Measures

The primary endpoint of the study were OS, RFS, HGPs and TME. OS is defined as the time from the date of the first liver metastasis resection to death due to any cause or loss to follow-up. The definition of RFS is the time from the liver surgery to the first imaging evidence showing disease recurrence or death due to any cause, whichever occurs first. The histopathological growth pattern of the tumor-liver interface. Using QuPath pathology imaging software, tissue sections were divided into the tumor center (defined as regions  $>500\ \mu\text{m}$  from the liver-tumor interface) and the invasive tumor front (defined as a 1 mm region extending  $500\ \mu\text{m}$  on either side of the liver-tumor interface).

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### **3. Ethical principles and requirements of clinical research**

Clinical research will follow the Declaration of Helsinki of the World Medical Assembly and the Ethical Review Measures for Biomedical Research involving Human Subjects of the National Health and Family Planning Commission of the People's Republic of China and other relevant regulations, including the principles and requirements of informed consent, privacy protection, research free of charge and compensation, risk control, protection of special subjects and compensation for research-related damages. The clinical study was carried out after approval of the trial protocol by the ethical review board prior to the initiation of the study. Before each subject is enrolled in the study, the investigator is responsible for providing the subject or/and his/her legal representative with a complete and comprehensive introduction of the purpose, procedures and possible risks of the study, and signing a written informed consent form, so that the subject knows that their participation in the clinical study is completely voluntary. They may refuse to participate or withdraw from the study at any stage without discrimination or retaliation, and their medical treatment and rights and interests will not be affected. Informed consent should be retained as a clinical research document for future reference to effectively protect the personal privacy and data confidentiality of subjects.