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1. Research Background

Colorectal cancer (CRC) is the third most common cancer worldwide. Approximately 2 million new CRC cases occur globally each year, with nearly 1 million deaths, ranking second among cancer-related deaths worldwide. Therefore, improving the prevention, screening, and treatment of colorectal cancer has become a priority in cancer prevention and control. Due to limitations in early diagnosis, many patients are already at an advanced stage when diagnosed. Among them, 15–25% develop liver metastases (colorectal liver metastases, CRLM), which is the most common metastatic pattern in CRC and a major cause of death in CRC patients. Therefore, most patients with advanced CRC and CRLM require systemic adjuvant therapy, mainly chemotherapy. Since the 2000s, combination chemotherapy based mainly on 5-fluorouracil (5-FU) and oxaliplatin, such as leucovorin + 5-FU + oxaliplatin (FOLFOX) and leucovorin + 5-FU plus irinotecan (FOLFIRI), has been the main chemotherapy regimen for patients with advanced CRC and has significantly improved the survival of CRC patients. In recent years, targeted drugs such as vascular endothelial growth factor (VEGF) inhibitors and epidermal growth factor receptor (EGFR) inhibitors combined with the above chemotherapy have further improved patients' median survival and quality of life. However, the 5-year survival rate of metastatic CRC, including CRLM, remains less than 15%. The reason is that both CRC and CRLM have high heterogeneity and a complex tumor microenvironment (TME). Different individuals show different treatment responses to the standard "one-size-fits-all" anticancer treatment approach, resulting in limited drug efficacy, which has become a bottleneck for further improving the efficacy of chemotherapy and targeted therapy. With the advent of the precision medicine era, achieving precision treatment has become a research direction in cancer treatment, an important direction of precision medicine. Cancer precision therapy, also called individualized medicine, refers to the development of more personalized treatment plans based on traditional standardized treatment combined with patient-specific molecular characteristics, striving to achieve the optimal therapeutic effect. The development of multi-omics big data, including genomics, transcriptomics, proteomics, and metabolomics, has laid a bioinformatics foundation for cancer precision medicine and has helped to more deeply understand the complex molecular landscape of CRC/CRLM in order to identify precise targets for existing drug treatments and novel drugs. However, to date, only a small proportion of targetable mutations identified through multi-omics technologies have been translated into clinical applications, resulting in only a minority of cancer patients benefiting. Another major limitation of clinical translational medicine is that less than 5% of drugs selected in preclinical research are truly effective and sufficiently safe in clinical trials. In addition, the low efficacy and drug resistance problems caused by the obvious

heterogeneity and complex TME of CRC/CRLM are increasingly prominent. The best way to address these limitations is to introduce advanced, reliable, and broadly applicable preclinical models combined with multi-omics bioinformatics to further improve preclinical precision validation and thereby expand patient benefit. Therefore, combining multi-omics technologies with the latest bioengineering technologies to develop reliable novel preclinical research platforms for clinical translation is an important breakthrough for advancing individualized treatment of CRC/CRLM. This study will use a novel bioengineering technology—3D multicellular bioprinting technology (3DMP)—to incorporate key cells in the TME to construct a novel 3DMP preclinical model that simulates the CRC/CRLM TME, and combine it with multi-omics technologies for individualized treatment and preclinical research in CRC/CRLM patients.

2. Research Purpose

This study intends to use 3D multicellular bioprinting technology to construct human-derived colorectal cancer primary lesion models, systematically evaluate the roles of CAFs and PBMC in tumor progression and immunosuppression, establish a research platform that accurately simulates tumor heterogeneity and the immune microenvironment, and promote the clinical translation of individualized therapy.

3. Research Methods

Non-interventional study, observational study.

This study will collect colorectal cancer (CRC) or colorectal cancer liver metastasis (CRLM) specimens, digest the tumor tissue into a single-cell suspension, and perform cell culture. The isolated colorectal tumor cell suspension will be mixed with hydrogel at an appropriate ratio, and a grid-like colorectal tumor tissue model will be constructed using 3D bioprinting equipment. At the same time, cancer-associated fibroblasts (CAFs), immune lymphocytes, and other components will be added to simulate the tumor microenvironment (TME).

After successful construction, all models will be cultured in vitro, and then various detection methods will be used to evaluate tumor cell growth, gene expression, drug tolerance, and other characteristics in the models. These models will provide an important platform for studying individualized treatment of colorectal cancer, drug sensitivity prediction, and the role of the tumor microenvironment, and will support clinical precision therapy.

4. Statistical Methods and Sample Size

All data will be expressed as mean ± standard deviation. In most statistical analyses, this study used the unpaired t-test. Survival analysis was performed using the Mantel-Cox log-rank test. Statistical analysis was performed using SPSS 23.0 software (IBM). A p value below 0.05 was considered statistically significant. For all tests, data from at least three independent experiments were used, and temporary samples or experiments were repeated three times.

A total of 30 colorectal cancer (CRC) or colorectal cancer liver metastasis (CRLM) specimens are expected to be obtained. Before tissue collection, patients must be communicated with and informed consent must be signed. This project is an observational study and does not involve intervention in patients; therefore, there is no risk.

5. Patient Inclusion and Exclusion Criteria and Sample Size

The study population consists of patients with colorectal cancer or colorectal cancer liver metastasis who require surgical treatment.

5.1 Inclusion Criteria

Adult patients (18–75 years) capable of independently signing informed consent; histologically confirmed advanced CRC and CRLM eligible for surgical resection; with or without neoadjuvant therapy; no contraindications to chemotherapy or targeted therapy; no history of other tumor diseases.

5.2 Exclusion Criteria

Patients who do not require surgical resection and postoperative adjuvant therapy; pregnant or lactating women and those with contraindications to the corresponding surgery, chemotherapy, or targeted therapy; patients who refuse surgery, chemotherapy, or targeted therapy.

6. Quality Control of the Study

A document-based management approach will be implemented. Research notebooks will be used to record research data in detail, accurately, and objectively, ensuring the authenticity, completeness, reliability, and comparability of the data. Strict laboratory quality control will be implemented in the clinical study, including recording adverse events and reporting serious adverse events. Statistical analysis of experimental data will be scientific, study reports will be written truthfully and rigorously, and research materials will be properly archived and stored at the end. Surplus specimens from the study will be destroyed.