Chemosensitivity profiling of colorectal cancer patient-derived organoids reveals heterogeneous responses to 5-fluorouracil

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To establish patient-derived organoid (PDO) models from colorectal cancer (CRC) patients and evaluate their fidelity and utility in assessing 5-fluorouracil (5-FU) chemosensitivity. Tumour samples from 30 untreated CRC patients were cultured into PDOs. Successful models were validated by hematoxylin and eosin (HE) and immunohistochemical (IHC) staining for morphology and biomarker expression. RNA sequencing (RNA-seq) was performed to identify transcriptional changes. 5-FU sensitivity was tested using an ATP-based viability assay across early and late passages. Twenty-six PDO lines were successfully established (86.67% success rate) and retained the histopathological and molecular features of primary tumours. RNA-seq revealed significant dysregulation of extracellular matrix and immune-related pathways, consistent with the absence of a tumour microenvironment. 5-FU sensitivity varied widely among PDOs, with IC_{50} values ranging from 0.085 to 3.934 μg/ml in first-generation cultures and narrowing to 0.078–2.134 μg/ml by passage 4, implying enhanced drug efficacy under microenvironment-deficient conditions. CRC PDOs effectively recapitulate tumour characteristics and exhibit heterogeneous 5-FU responses, supporting their potential as personalised drug-testing platforms. However, the absence of tumour microenvironment components may influence sensitivity outcomes and should be considered in future applications.

Keywords: 5-fluorouracil, colorectal cancer, drug sensitivity, patient-derived organoid, RNA-seq

than half of the global CRC burden, with China alone contributing ~29% of all new cases[2]. Within China, CRC has become the second most frequently diagnosed cancer and the fourth leading cause of cancer death[3]. Although advancements in diagnosis and treatment techniques have improved the prognosis for patients with early-stage CRC, the majority of patients are diagnosed at intermediate or advanced stages, requiring chemotherapy or radiotherapy. However, the five-year survival rate for CRC patients has not demonstrated significant improvement, largely due to drug resistance[4,5]. Currently, only 5% of anticancer drugs that show efficacy in preclinical studies are approved following phase III clinical trials, with the attrition rate for anticancer drugs being significantly higher compared to other therapeutic areas[6]. Therefore, the development of an innovative and effective *in vitro* tumour research model that accurately replicates the key physiological characteristics of human tissues has long been a primary objective for researchers and clinicians engaged in tumour drug research and development.

Recently, patient-derived organoid (PDO) models have emerged as a new generation of three-dimensional *in vitro* culture systems[7,8]. These models, cultivated and expanded from a patient's own tumour tissue, have demonstrated the capacity to effectively mimic the physiological and histological characteristics of the original tumour. As a result, PDOs are considered a promising tool for evaluating patient-specific drug sensitivity[9–11]. In the present study, we established PDO models for CRC, which were assessed at both morphological and histological levels. This model was subsequently employed in clinical drug sensitivity testing for CRC patients, with the objective of providing a foundation for guiding CRC treatment and facilitating the realisation of precise therapeutic interventions.