Editorial Commentary

The current status and reflections on 3D *in vitro* modeling of liver metastasis in colorectal cancer

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Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide and is identified as the second leading cause of cancer-related fatalities (1). The liver is the most common site for distant metastasis in CRC, with approximately 20% to 25% of newly diagnosed patients experiencing liver metastasis. As the disease progresses, this proportion can increase to as high as 50% (2), making it a primary cause of death in CRC patients.

Surgical resection and systemic chemotherapy are regarded as the primary therapeutic modalities for colorectal liver metastases (CRLM) (3-5). Although surgical resection is considered the "gold standard" for treating CRLM, approximately 80% of patients have lost this opportunity at the time of diagnosis (3). A considerable portion of patients, by the time of diagnosis, is no longer eligible for surgical resection. These patients may potentially benefit from liver transplantation (LT) (6,7). On the other hand, The effectiveness of chemotherapy in improving patient survival remains constrained (8). The biological complexity and clonal heterogeneity of CRLM result in diverse treatment responses among different patients. Consequently, the reliable application of preclinical models to predict drug treatment responses and assist in devising individualized chemotherapy regimens is a critical concern in CRLM treatment.

Conventional preclinical models of CRC liver metastasis, such as immortalized cell lines cultured in 2D environments, have provided valuable insights into cancer biology. However, these models fall short in faithfully preserving the original characteristics of the primary tumor due to tumor heterogeneity and the absence of a complex microenvironment, rendering them unsuitable for personalized therapies.

In recent years, 3D tumor models have garnered significant interest and gradually become a research hotspot. These 3D models far surpass the limitations of 2D monolayer cell cultures and costly, low-throughput animal models. They hold tremendous advantages and prospects in reproducing the biological and physical complexity of the tumor microenvironment. Various types of 3D tumor models have been developed, including randomly assembled 3D spheroids, patient-derived organoids (PDOs), cell-laden hydrogel platforms, microfluidic tumor chip platforms, and 3D bioprinting, among others.

Traditional patient-derived xenograft (PDX) models are considered superior preclinical models, as they can to some extent replicate the tumor microenvironment while retaining the characteristics of the original tumor. Wulf-Goldenberg *et al.* (9) described the establishment of metastatic PDX models in immune-compromised mice

and provided a stepwise guide on how to use particular PDX models as metastasis models and their proper characterization. Zhang *et al.* (10) established a xenograft mouse model using metastatic patient-derived samples, preserving the primary characteristics of the original patient tumors.

However, due to the extended experimental duration, high costs, limited scalability, and low success rates of PDX models, their ability to predict treatment responses is severely constrained, significantly limiting their application in relatively large-scale clinical studies (11,12).

On the other hand, *ex vivo* organoid cultures also serve as excellent *in vitro* 3D tumor models. Various tumor *ex vivo* models have been successfully established, demonstrating significant potential in predicting patient-specific drug responses (13).

PDO models are currently highly effective preclinical 3D models for CRLM. They show great potential in simulating the metastatic process of CRC and aiding in personalized treatment approaches for patients.

Li *et al.* (14) employed paired organoids derived from primary and liver metastatic tumors of CRC patients to simulate cancer metastasis. This research underscores the potential of patient-derived matched primary and metastatic cancer organoids as experimental models for investigating CRC progression.

Mo *et al.* (15) cultivated a living biobank comprising 50 CRLM organoids, meticulously sourced from primary tumors and their corresponding liver metastatic counterparts and undertook the value of CRLM PDOs in predicting chemotherapeutic drug responses and clinical prognoses. Their research unveiled that CRLM PDOs faithfully retained the histopathological and molecular characteristics of their parental tumors, skillfully capturing the intrapatient and interpatient heterogeneity. Remarkably, CRLM PDOs demonstrated promising potential in predicting the sensitivity to FOLFOX or FOLFIRI chemotherapy regimens and prognosticating clinical outcomes.

These findings underscore the potential of *in vitro* CRLM organoid models as a viable alternative to the time-consuming and costly PDX models, significantly contributing to the construction of robust *in vitro* models for CRLM and drug sensitivity testing. Nevertheless, it is worth noting that the success rate of organoid construction still greatly depends on factors such as tissue sample size, purity, the proliferative capacity of primary tissue cells, and the access to tumor tissue (biopsy or surgical procedure). Additionally, previous research has brought to light potential challenges associated with organoid tumor models in practical applications, including complexity, time and resource intensiveness, low success rates, uneven cell size distribution, and substantial heterogeneity. Consequently, these limitations impose significant constraints on the clinical translation of organoid tumor models.

3D bioprinting is a biomanufacturing technology that incorporates biological material elements. It involves digitizing and modeling through techniques such as computed tomography scans, magnetic resonance imaging, computer-aided design, computer-aided manufacturing tools, and mathematical modeling. This technology allows for the automated, precise layer-by-layer positioning and control of live cells, bio-inks, biochemical factors, and more, in a spatial arrangement that matches the natural structure of native tissues. This process leads to the construction of complex, functional 3D living human structures. Over the past 15 years, 3D bioprinting technology has provided various strategies for constructing biologically functional tissues. A variety of tissue cells have been used as source materials for 3D bioprinting, successfully reconstructing tissues such as the heart, blood vessels, and lungs *in vitro*. In cancer research, 3D bioprinting has also been successful in creating patient-derived tumor tissues, for instance, in cases of oral cancer and glioblastoma.

The advent of 3D bioprinting technology has opened up new avenues for the development of clinically relevant tumor models. On one hand, the utilization of bioinks in the printing process enables the adaptable customization of tissue model configurations and structural requirements, effectively overcoming the limitations associated with traditional organoid techniques. On the other hand, 3D bioprinting technology, guided by three-dimensional computer programming models, facilitates the automated and precise layer-by-layer positioning and control of live cells, bioinks, biochemical factors, and more. This confers advantages in terms of heightened precision, efficiency, and consistency.

It is this very capability to intricately define the positions of perfusable networks and various cell types that elevates 3D bioprinting technology above existing methods, enabling it to more accurately replicate the cancer microenvironment, providing a more faithful reflection of tumor formation, development, and responses to anticancer drugs. As such, it presents an innovative approach to the

more effective creation of highly intricate 3D structures utilizing live cells.

In summary, utilizing 3D bioprinting technology to develop personalized drug screening plans for CRLM is highly suitable. An ongoing clinical trial, currently registered as NCT04755907, is assessing the predictive capabilities of 3D bioprinted tumor models in response to chemotherapy for CRC and CRLM, with the published results indicating significant progress (16). This study demonstrates that patient-derived CRC and CRLM models, established using 3D bioprinting technology, exhibit robust *in vitro* growth while faithfully preserving specific biomarkers and characteristic mutation profiles of their parent tumors. Drug testing has unveiled notable tumor heterogeneity, encompassing inter-tumor variability among different patients and heterogeneity between primary tumors and their paired metastatic counterparts within the same patient. The correlation of drug response data from 3D bioprinted CRLM models with patients' clinical outcomes following neoadjuvant chemotherapy (NAC) further substantiates the role of 3D bioprinted cancer models as a reliable and efficient platform for personalized cancer treatment.

3D bioprinting technology provides a fresh perspective and method for developing novel human tumor models that closely resemble real pathological and physiological states. Our research team has previously successfully established 3D-printed models of liver cancer cell lines, as well as *ex vivo* microenvironment models of bile duct cancer cell lines, and 3D-printed models of patient-derived liver cancer cells, highlighting the reliable value of 3D printing in tumor drug screening (17-19). These results indicate that tumor models established through this method can maximally replicate the characteristics of the parent tumor and demonstrate favorable growth levels. Therefore, we affirm that 3D bioprinting technology holds significant clinical potential in preclinical tumor model research. However, the integration of patient-derived CRLM tumor cells into 3D bioprinting platforms for validating their personalized therapeutic potential is still in its early stages. Further optimization is needed in terms of standardizing the application protocol and improving modeling accuracy. This patient-derived 3D bioprinted tumor model holds excellent prospects for the clinical translation of tumor basic research and for personalized precision therapy for patients in the future. Further research is required to explore its full potential.

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