RESEARCH HIGHLIGHT

LICOB: a powerful organoid platform for drug discovery

Haojie Jin[1,2,3]✉, Zhen Sun[1,3] and René Bernards[1,2]✉

Cell Research (2024) 34:11–12; https://doi.org/10.1038/s41422-023-00878-0

A comprehensive patient-derived organoid biobank was established, which recapitulates the histological and molecular features of primary liver cancer, enabling drug discovery.

Treatment of primary liver cancer (PLC) remains problematic as most PLC patients are diagnosed at an advanced stage.[1] The paucity of recurrent and targetable mutations in PLC limits therapeutic options to buckshot approaches in which drugs with limited selectivity are used to target multiple cancer-relevant protein kinases. Hence, there is an urgent need to develop new drugs for PLC treatment, but a lack of suitable preclinical models that mimic key tumor characteristics is a major impediment. To address this, Ji et al. established a large-scale biobank, Liver Cancer Organoid Biobank (LICOB), containing 65 patient-derived organoids (PDOs).[2]

An organoid is a three-dimensional structure with the ability to self-renew that recapitulates the original tissue structure and function.[3] PDOs represent flexible ex vivo models in evaluating personalized therapeutic options for cancer patients. Unlike animal models based on patient-derived xenografts (PDXs), PDOs can support high-throughput drug screening. Ji et al. established organoid cultures from surgically resected liver cancer tissue, which included the three main types of PLC, hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular-cholangiocarcinoma (CHC), as well as a rare type, hepatoblastoma (HB). Intra- and inter-tumor heterogeneities greatly influence drug resistance and are considered as one of the major factors contributing to treatment failure.[4] In the PDO biobank established by Ji et al., multi-omics analyses including genomic (somatic mutation and copy number variations), epigenomic, transcriptomic, and proteomic analyses consistently showed that LICOB preserved the histological and molecular characteristics and inter-tumor heterogeneity of different types of PLCs. Importantly, the multi-omics profiles of LICOB organoids were highly similar to those of the original tumors, with the exception of a few somatic mutations. This indicates that LICOB can maintain the original pathology and genetic features of the primary cancer (Fig. 1).

Based on the LICOB-derived multi-omics data, PLCs could be classified into four subtypes, namely L-ICC (ICC-dominated), L-PL (proliferation-dominated), L-LM (lipid metabolism pathway-dominated), and L-DM (drug metabolism pathway-dominated), and the latter three categories were characterized similarly to the HCC tissue-based clustering results reported previously.[5] In contrast, the cell line models showed mainly proliferative features, indicating their inability to recapitulate the molecular subtype diversity of PLC.

To investigate whether use of the LICOB can reveal drug response heterogeneity, Ji et al. conducted a high-throughput screening of 76 anticancer drugs using the PDO models in LICOB. Their results confirmed that the drug response patterns of PLCs are heterogeneous. Specifically, the four LICOB clustering subtypes, intra-L-ICC, and different HBV infection status exhibited distinct drug response patterns. Consistently, a previous study showed the ability of 27 liver cancer PDOs, which were established by using multi-regional sampling in three ICC and two HCC patients, to reveal intra- and inter-tumor drug response heterogeneities.[6] These results demonstrate that liver cancer PDOs can be utilized as a powerful drug testing platform.

The authors then developed a model for predicting drug response using the multi-omics data. Their predicted resistance or sensitivity signatures were closely related to the drug mechanism of action. Interestingly, the authors found that the efficacy of tivantinib, a selective MET inhibitor, was associated with upregulation of genes required for MET activation rather than expression of MET itself, which is consistent with previous clinical studies showing no significant efficacy of tivantinib among HCC patients with high MET expression. In addition, the LICOB data could also predict drug resistance mechanisms. For example, the authors investigated lenvatinib response and EGFR-related pathways which confer resistance to lenvatinib treatment in HCC.[7] They found that lenvatinib resistance was positively correlated with high expression levels of proteins involved in EGFR tyrosine kinase inhibitor resistance pathway and negatively correlated with DNA methylation of associated genes, further supporting LICOB as a powerful model to study the drug resistance mechanism.

To explore whether LICOB can serve as a platform for discovering new therapeutic targets for PLC, the authors performed differential protein expression analysis of four LICOB clustering subtypes. As one example, they found that glucose-6-phosphate dehydrogenase (G6PD), a rate-limiting enzyme in the pentose phosphate pathway that provides substrate for nucleotide synthesis and maintains redox homeostasis, was significantly elevated in the L-DM subtype. Further knockdown of G6PD significantly inhibited the proliferation of PDOs of the L-DM subtype. Thus, the authors conclude that the G6PD inhibitor is a potential therapeutic agent for the L-DM subtype.

Rational drug combinations can delay drug resistance.[8] Ji et al. developed a network-based approach for predicting appropriate drug combinations using multi-omics datasets from LICOB. It is based on the rationale that if the pathway targeted by one drug is activated in a sample that is resistant to another drug, the two

[1]State Key Laboratory of Systems Medicine for Cancer, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [2]Division of Molecular Carcinogenesis, Oncode Institute, The Netherlands Cancer Institute, Amsterdam, The Netherlands. [3]These authors contributed equally: Haojie Jin, Zhen Sun.
✉email: hjjin1986@126.com; r.bernards@nki.nl

Published online: 20 September 2023

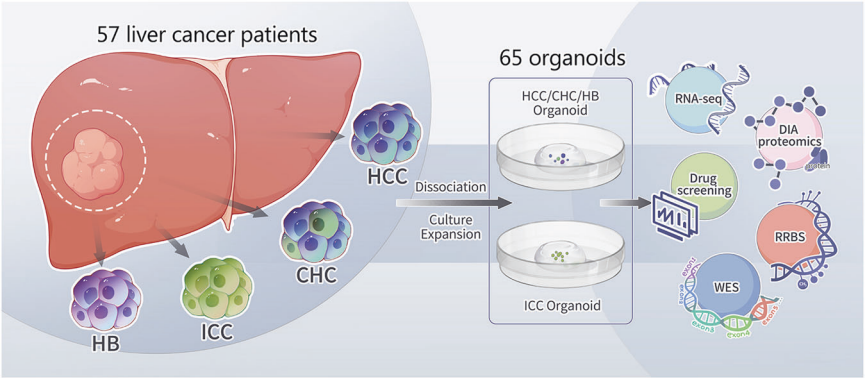

Fig. 1 LICOB establishment and multi-omics analyses of liver cancer organoids. PLC tissues were collected from patients to generate LICOB. In total, 65 organoids were established from 57 patients, including HCC ($n = 44$), ICC ($n = 12$), CHC ($n = 4$) and HB organoids ($n = 5$). Multi-omics analyses of these organoids were performed by using reduced representation bisulfite sequencing (RRBS), whole-exome sequencing (WES), RNA-seq, and label-free proteomics. The sensitivity of the organoids to 76 approved or investigational drugs was tested.

drugs are likely to have a synergistic effect. Based on this approach, the authors tested the synergistic therapeutic effects of lenvatinib, a first-line therapy for unresectable HCC, in combination with the mTOR inhibitor temsirolimus. Among the four PDOs with high predictive scores, lenvatinib alone had a limited effect on cell proliferation, while the addition of temsirolimus resulted in a synergistic inhibitory effect. This synergistic effect was validated by in vivo experiments.

To make the pharmaco-proteogenomic data generated from LICOB widely available to the community, the authors have developed an interactive web portal (http://cancerdiversity.asia/LICOB/). Specifically, six modules including mutations, copy number variations, methylation, mRNA, protein, and pathway analyses are provided, enabling users to explore correlations with drug responses. Researchers can also search the drug response module for predicted combination rankings among the 76 tested drugs. Of interest, users can upload the omics data of pre-treatment samples to predict drug response profiles based on the models trained in LICOB. However, whether the predicted drug ranking based on the current model can be used as a basis for clinical decision-making still needs to be validated. The authors will continue to add the number of PDOs and drug response data to make this tool an even more informative platform.

Several studies exploring the feasibility of liver cancer organoids as a research model have been published and consistently demonstrated that it could well preserve parental tissue features and reveal tumor and drug response heterogeneity.[6,9] However, since the success rate of establishing liver cancer organoids is lower than that of other cancer types, the large-scale liver cancer organoid biobank with pharmaco-proteogenomic characteristics of the Chinese population is a major step forward. Interestingly, an increasing number of PDO-based clinical trials in recent years suggest a trend towards an increasing reliance on PDOs for clinical decision-making in personalized medicine. Therefore, the new therapeutic targets and potential drug combinations for liver cancer predicted by the multi-omics data of LICOB deserve to be advanced to preclinical and clinical studies for validation as it may bring new therapeutic opportunities for liver cancer.

While the unique strengths and benefits of using PDO models are clear, there are some challenges with current PDO technology. First, the current PDO models in LICOB lack a tumor microenvironment (TME). This is relevant as many established drugs inhibit angiogenesis in the TME. Moreover, the lack of immune components hinders predictive modeling of immunotherapeutic responses. Several approaches have been proposed, including co-culture with immune cells and non-tumor stromal cells, organoid-on-a-chip models combined with microfluidics, and 3D bioprinting technologies to try to establish organoid systems capable of mimicking the complexity of TME, and these might be adopted into LICOB as new subsystems.[10] Finally, it is relevant to point out that PDOs have unique requirements for in vitro proliferation, including the use of ROCK and TGF-β inhibitors. How the presence of these drugs in the culture media affects drug responses remains to be studied.

REFERENCES

1. Sung, H. et al. *CA Cancer J. Clin.* **71**, 209–249 (2021).
2. Ji, S. et al. *Sci. Transl. Med.* **15**, eadg3358 (2023).
3. Lancaster, M. A. et al. *Science* **345**, 1247125 (2014).
4. Crucitta, S. et al. *Cancer Treat. Rev.* **104**, 102340 (2022).
5. Gao, Q. et al. *Cell* **179**, 561–577 (2019).
6. Li, L. et al. *JCI Insight* **4**, e121490 (2019).
7. Jin, H. et al. *Nature* **595**, 730–734 (2021).
8. Jin, H. et al. *Nat. Rev. Drug Discov.* **22**, 213–234 (2022).
9. Broutier, L. et al. *Nat. Med.* **23**, 1424–1435 (2017).
10. van Renterghem, A. W. J. et al. *Nat. Rev. Clin. Oncol.* **20**, 305–317 (2023).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Haojie Jin or René. Bernards.