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Development and advanced applications of hepatobiliary tumor organoid models in drug response prediction

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Abstract

Hepatobiliary tumors, including hepatocellular carcinoma, intrahepatic cholangiocarcinoma, and gallbladder cancer, represent major causes of cancer-related mortality worldwide. Due to the lack of early symptoms, many patients are diagnosed at advanced stages, missing the optimal treatment window. Additionally, the elevated heterogeneity of hepatobiliary tumors and limited responsiveness to traditional chemotherapy posit formidable treatment challenges. The organoid model represents an advanced in vitro approach that simulates the tumor microenvironment and biological characteristics, providing a platform more accurately mirrors physiological states for tumor research. Compared to traditional two-dimensional cell culture and animal models, organoid models more accurately reflect the biological characteristics of tumor cells, offering a high degree of individualization, and are well suited for large-scale screening and drug testing. This review outlines the development of hepatobiliary tumor organoid models and their advanced applications in drug response prediction. It explores the extensive applications and challenges of organoid models in simulating liver pathophysiological mechanisms, studying tumor development mechanisms, drug screening, and developing individualized treatment strategies. The goal is to offer novel insights and methods for precision medicine in hepatobiliary tumors.

Key words hepatobiliary tumors, organoid models, drug response prediction, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, gallbladder cancer, three-dimensional culture technology, individualized medicine, drug screening, precision medicine

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Introduction

Hepatobiliary tumors, including hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and gallbladder cancer (GBC), rank among the leading causes of cancer-related deaths worldwide [1-3]. Due to its asymptomatic nature, most patients are diagnosed at an advanced stage, missing the optimal treatment window. Additionally, the high heterogeneity of hepatobiliary tumors, poor response to traditional chemotherapy drugs, and frequent development of resistance posit substantial treatment challenges [4, 5]. Therefore, developing new therapeutic strategies and drugs to improve patient survival and quality of life is urgently needed in medical research.

In tumor research, organoid models have garnered significant attention as emerging in vitro models due to their ability to simulate the tumor microenvironment and biological characteristics [6-8]. Organoids are miniature tissues formed from adult stem cells or pluripotent stem cells through three-dimensional culture techniques in vitro, possessing self-renewal and multi-directional differentiation capabilities [9-11]. These miniature tissues can maintain the histological, molecular, and genetic characteristics of the original tumors and partially simulate the tumor microenvironment and biological behavior, providing a platform more accurately mirrors physiological states for tumor research [12].

Compared to traditional two-dimensional cell culture and animal models, organoid models offer several advantages [13-16]. Organoid models more accurately reflect the biological characteristics of tumor cells, including cell-to-cell interactions, the influence of the extracellular matrix, and the dynamics of the tumor microenvironment. Additionally, the high degree of individualization of organoid models enables the establishment of personalized organoids using the patient's tumor cells or tissue samples, thereby facilitating personalized medicine. Furthermore, organoid models are faster to establish, cost-effective, and well-suited for large-scale screening and drug testing.

Organoid models are rapidly gaining traction in hepatobiliary tumor research. These models are employed to study the mechanisms of tumor development, drug screening, and the development of personalized treatment strategies [17, 18]. Organoid models enable researchers to rapidly evaluate drug cytotoxic effects on tumor cells and predict drug sensitivity and resistance, thereby providing more precise treatment plans for patients [19]. Furthermore, organoid models can assess the impact of the tumor microenvironment on drug response, explore mechanisms of tumor immune evasion, and contribute to the development of new immunotherapy strategies [20].

In summary, the clinical treatment of hepatobiliary tumors presents numerous challenges. Organoid models, as an innovative research tool, offer new perspectives and methods for the mechanistic study and drug development of hepatobiliary tumors. Given the ongoing advancements and applications of organoid technology, it is reasonable to believe that these models will play an important role in the precision medicine of hepatobiliary tumors.

Fundamental principles and techniques of organoid models

Organoid models represent cutting-edge technologies in current biomedical research and are defined by their ability to mimic the structure and function of native organs [21-23]. In vitro, organoids exhibit remarkable biological characteristics, self-organize into three-dimensional tissue structures, and simulate the physiological functions and pathological processes of their original organs (**Figure 1**) [24, 25].

Three-dimensional (3D) culture techniques are crucial for organoid models [26]. Unlike traditional two-dimensional (2D) cell cultures, 3D culture techniques provide cells with an environment resembling physiological conditions. In this environment, cells can interact with the extracellular matrix, forming tissues with specific spatial structures [27-29]. Furthermore, 3D cultures promote intercellular communication and signal transmission, which is essential for maintaining cellular phenotype and function [30]. Another significant aspect is the self-renewal ability of organoid models. Under specific culture conditions and signaling pathway regulations, stem cells within organoid models can self-renew and continuously produce new cells, thus maintaining the long-term stability of the model [31, 32] This self-renewal capability provides important insights into stem cell biology and offers potential applications in regenerative medicine and cell therapy.

Organoid models have unique advantages in simulating liver pathophysiological mechanisms [33-36]. The liver is a highly complex organ with functions involving metabolism, detoxification, and immunity, among others. Traditional 2D cell cultures fail to adequately simulate these complex physiological functions, while organoid models offer partial recreation of liver functions. For instance, liver organoids can express liver-specific enzymes and transport proteins, conduct drug metabolism, and secrete bile [37]. Furthermore, liver organoids provide valuable insights into the development and progression of liver diseases, serving as significant models for exploring the molecular mechanisms underlying these conditions [38]. Despite their considerable potential in simulating liver pathophysiology, liver organoid models face several challenges. For instance, accurately simulating the liver microenvironment, including blood flow, oxygen supply, and interactions with immune cells, remains a critical issue in current research [39-41]. Additionally, improving the stability and reproducibility of organoid models and their application in large-scale drug screening and toxicity testing are crucial issues that require further attention [42].

In summary, as an emerging in vitro model, organoid models have broad application prospects in simulating liver pathophysiological mechanisms. Through continuous technological innovation and optimization, these models are expected to provide more accurate and effective tools for liver disease research and treatment. Further research and application of organoid models are expected to enhance their role in hepatobiliary tumor research and treatment, bringing new hope to patients.

Research progress of hepatobiliary tumor organoid models

The research progress of hepatobiliary tumor organoid models marks a significant milestone in tumor biology and personalized medicine. These models provide new perspectives in understanding the complexity of tumors and offer powerful tools for developing new therapeutic strategies and drug screening [43-46].

Constructing hepatobiliary tumor organoid models involves extracting cells from patient-derived tumor tissues and culturing these cells in vitro using specialized media and 3D culture techniques to develop organoids that exhibit tumor-like characteristics. These models accurately simulate the histological structure, cellular heterogeneity, and microenvironmental traits of primary tumors, providing a highly mimetic system for studying the biological behavior of tumors.

In the realm of disease models, hepatobiliary tumor organoid models are extensively utilized to study tumor initiation, development, metastasis, and drug resistance. Researchers can observe tumor cell growth, division, and interactions in a simulated vivo environment. Additionally, organoid models facilitate the study of intercellular within the tumor microenvironment and how tumors evade immune surveillance [47].

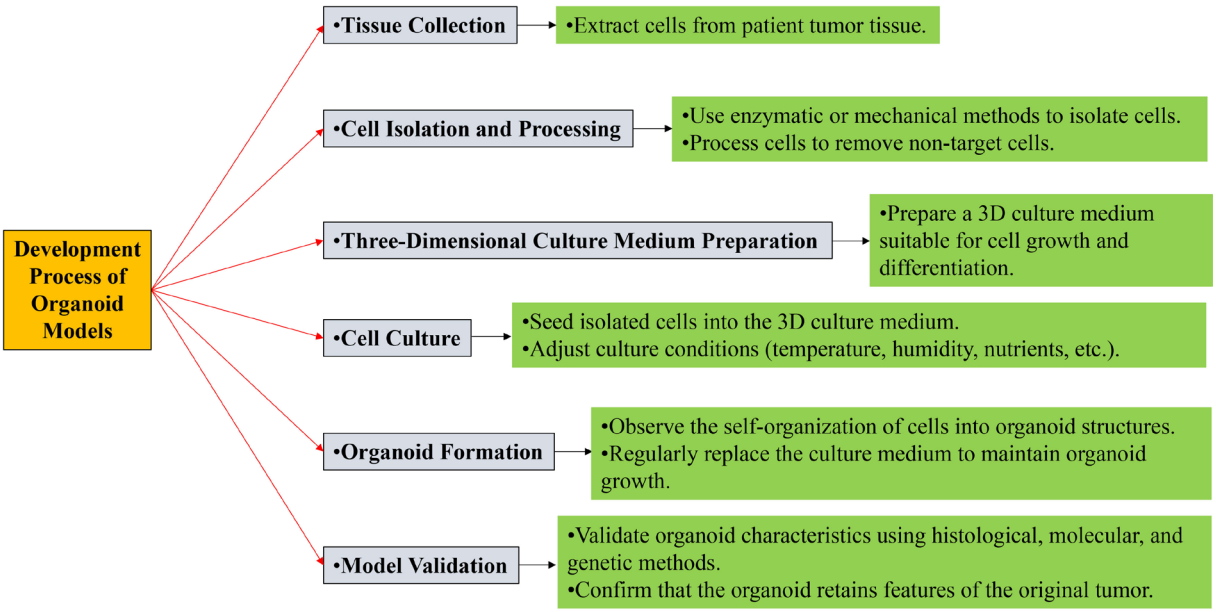

Figure 1. Development process of organoid models.

The advantage of organoid models lies in their ability to faithfully preserve the phenotypic characteristics of primary tumors. Compared to two-dimensional cell cultures and animal models, organoid models better simulate tumours' 3D structure and intercellular interactions [48, 49]. This highly mimetic feature makes organoid models ideal tools for studying tumor heterogeneity and the influence of the microenvironment. Furthermore, the individualized nature of organoid models also makes personalized medicine possible. By using a patient's tumor cells to construct organoids, personalized treatment strategies can be developed for each patient.

Of particular interest are Patient-Derived Hepatobiliary Organoids (PDHO). PDHO models hold significant potential in predicting drug sensitivity for hepatobiliary tumors [50-52]. By applying different drugs to tumor cells within PDHO models, researchers can assess the drug efficacy and toxicity, providing more precise treatment options for patients. Additionally, PDHO models are valuable for high-throughput screening in drug development [53-55]. Researchers can quickly identify compounds with potential antitumor activity by testing numerous compounds on these models, accelerating the discovery and development of new drugs. However, the establishment and application of PDHO models also face challenges. For example, the culture conditions of organoids need to be optimized for different types of tumors to ensure the model's stability and reproducibility. Additionally, due to tumor heterogeneity, a single organoid model may not fully represent the behavior of all tumor cells, necessitating multiple models to comprehensively understand tumor complexity. Despite these challenges, the application prospects of PDHO models in hepatobiliary tumor research and treatment remain very promising.

With technological advancements and a deeper understanding of tumor biology, hepatobiliary tumor organoid models will increasingly become vital in tumor research and treatment. These models provide critical insights into tumor complexity and develop more effective and personalized treatment strategies, ultimately improving patient outcomes and quality of life.

Application of multi-omics technologies in organoid models

The application of multi-omics technologies plays a crucial role in organoid models, providing unprecedented perspectives into understanding the molecular mechanisms and drug responses of hepatobiliary tumors. These technologies include whole-genome sequencing (WGS), RNA sequencing (RNA-seq), mass spectrometry (MS), and single-cell RNA sequencing (scRNA-seq), each revealing the complexity of tumor cells in unique ways [56-60].

Whole-genome sequencing (WGS) offers a comprehensive view of the tumor cell genome, including mutations, copy number variations, and structural variations. WGS enables researchers to identify driver genes and potential biomarkers in tumor cells, which is crucial for understanding tumor development and guiding targeted therapies. In organoid models, WGS can help researchers evaluate whether the models retain the genetic characteristics of the original tumors, ensuring their representativeness and reliability.

RNA sequencing (RNA-seq) is essential for exploring the transcriptome of tumor cells, revealing gene expression patterns and regulatory networks. RNA-seq can detect changes in gene expression levels, reflecting tumor cell responses to environmental changes and drug treatments. In organoid models, RNA-seq aids in studying intercellular communication within the tumor microenvironment and the adaptive strategies tumor cells use to evade treatment by altering gene expression.

Mass spectrometry (MS) is a powerful proteomics tool that quantitatively analyses protein expression and modification states within tumor cells. MS technology enables researchers to understand the functional state of tumor cells at the protein level, identifying key signaling pathways and biological processes. In organoid models, MS technology helps reveal the metabolic characteristics of tumor cells and the molecular mechanisms of drug action.

Single-cell RNA sequencing (scRNA-seq) has emerged as a novel technology in recent years, enabling the resolution of tumor

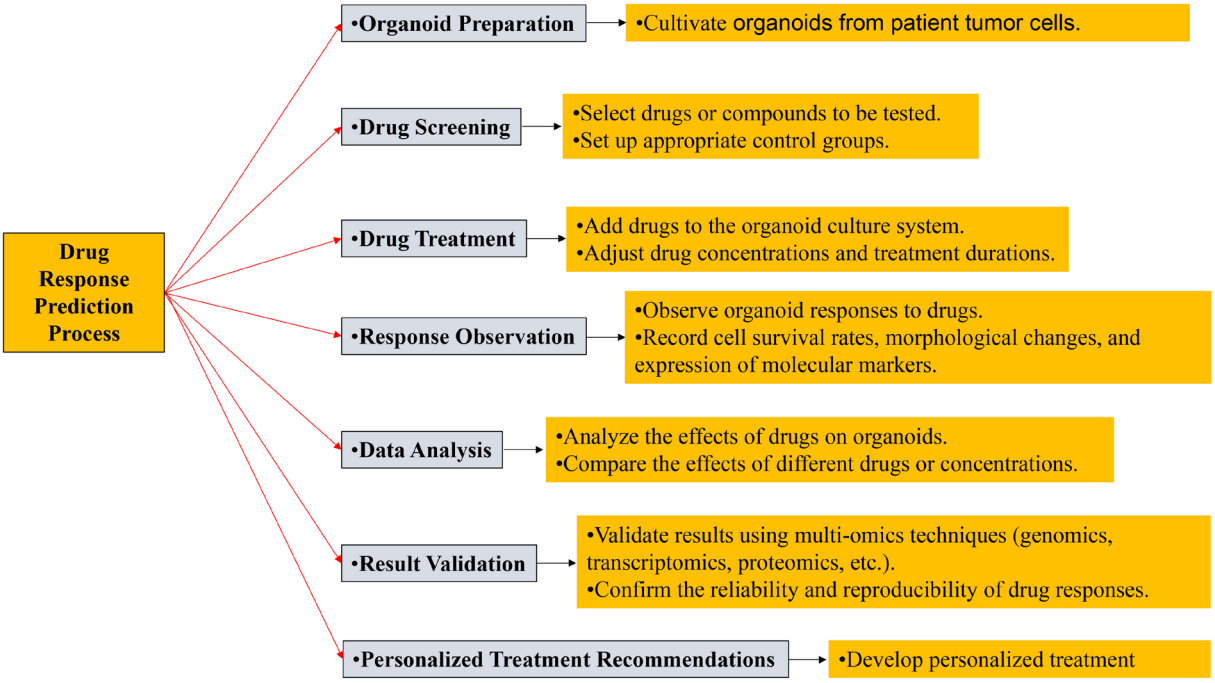

Figure 2. Drug response prediction process.

cell heterogeneity at the single-cell level. The application of scRNA-seq reveals unique gene expression patterns of different cell subpopulations within tumors, providing new insights into tumor complexity and treatment resistance. In organoid models, scRNA-seq helps identify different cell types and states within the tumor microenvironment, elucidating their collective influence tumor development and treatment response.

By integrating these multi-omics technologies, researchers can comprehensively understand the molecular mechanisms of hepatobiliary tumors at multiple levels. For instance, combined analysis of WGS and RNA-seq can identify mutations in tumor cells and study how these mutations affect gene expression and tumor behavior [61]. The combination of MS and scRNA-seq exposes the tumor cell heterogeneity at the protein and single-cell levels, providing clues for developing new therapeutic strategies. Furthermore, the application of multi-omics technologies in organoid models facilitates the prediction of drug responses and the development of personalized treatments [62]. By analyzing the molecular responses of tumor cells to different drugs, researchers can identify biomarkers that predict drug sensitivity or resistance. Combining patients' clinical information with multi-omics data from organoid models can help design personalized treatment plans for each patient, improving treatment outcomes.

In summary, the integration of multi-omics technologies with organoid models has greatly advanced our understanding of the molecular mechanisms of hepatobiliary tumors and provided new strategies and tools for precision oncology. The continuous development and refinement of these technologies reasonably suggest their pivotal role in future tumor research and therapeutic approaches.

Advanced applications in drug response prediction

Predicting drug response is crucial in cancer treatment as it directly affects treatment efficacy and patient survival rates. Organoid models, as an emerging in vitro model, are playing an increasingly important role in drug screening and efficacy evaluation. These models simulate the tumor microenvironment and biological characteristics and provide more precise and personalized data for drug response prediction (**Figure 2**) [63, 64]. The role of organoid models in drug screening and efficacy evaluation is evident. By testing different drugs on tumor cells within organoid models, researchers can quickly assess the efficacy and toxicity of the drugs. This assessment includes the direct cytotoxic effects on tumor cells and the impact on the tumor microenvironment, such as tumor angiogenesis and immune evasion [65]. More importantly, organoid models can simulate tumor heterogeneity, reflecting the responses of different tumor cell subpopulations to drugs, thus providing more comprehensive data for drug screening.

In the context of personalized immunotherapy, in vitro screening platforms based on organoids for neoantigen peptide activity have shown great potential. Neoantigen peptides, produced by tumor-specific nonsynonymous mutations, can stimulate the immune system to attack tumor cells [66, 67]. Researchers can identify candidate peptides that activate the immune system by screening neoantigen peptides with immunogenicity in organoid models. This method can enhance the specificity and efficacy of immunotherapy while reducing side effects. Drug-target interaction prediction methods, such as the wSDTNBI method, also play an important role in drug discovery. The wSDTNBI method is a network-based drug-target interaction prediction method that predicts the activity of drugs against specific targets by analyzing the network relationships between drugs and targets. This method can predict interactions between drugs and targets and evaluate the strength of these interactions, providing more precise guidance for drug discovery.

In practice, the wSDTNBI method has been used to predict the activity of various drugs against different targets [68]. Researchers can quickly identify compounds with potential activity through this method, accelerating drug discovery and development. Moreover, the wSDTNBI method does not rely on the three-

dimensional structure of targets or negative samples, making it widely applicable in drug discovery.

Despite their significant potential in predicting drug responses, organoid models and drug-target interaction prediction methods face several challenges. Constructing and maintaining organoid models demands complex techniques and conditions. Furthermore, the significant heterogeneity in tumor cells from different patients can impact the representativeness and reproducibility of the models [69, 70]. Additionally, while drug-target interaction prediction methods can provide rapid predictions, their accuracy and reliability need further validation and optimization.

In summary, organoid models and drug-target interaction prediction methods hold significant potential for improving drug response prediction. With continuous technological advancements and optimization, these methods will provide more precise and personalized strategies for cancer treatment, thereby improving treatment outcomes and patient survival rates. In future, interdisciplinary collaboration and technological innovation are expected to achieve greater breakthroughs in cancer treatment.

Clinical applications and challenges

In clinical treatment, organoid models hold broad application prospects as they open new avenues for personalized therapy and drug response prediction. These models can be cultured from a patient's tumor cells, preserving tumor heterogeneity and microenvironmental characteristics, offering unparalleled advantages in simulating tumor behavior and predicting drug responses [71]. Screening drugs on organoid models can help clinicians tailor personalized treatment plans, improving therapeutic outcomes and reducing unnecessary side effects.

However, organoid models face several challenges in clinical applications. The primary challenge lies in the intrinsic complexity of these models. The heterogeneity of tumor cells and the diversity of the microenvironment require organoid models to accurately simulate these features, necessitating complex culture techniques and conditions. Moreover, individual variability poses an additional challenge. Significant intratumoral heterogeneity exists among patients with the same type of cancer, requiring organoid models to accurately reflect the tumor characteristics of each patient.

To overcome these challenges, researchers are exploring methods to optimize culture techniques and integrate multi-omics data [72]. By improving cultural media composition and cultural conditions, the stability and representativeness of organoid models can be enhanced. Simultaneously, integrating multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, offer a deeper insight into the molecular mechanisms of tumors, thereby improving the predictive accuracy of the models.

The development direction of organoid models and drug response prediction technologies in hepatobiliary tumor research will become clearer in the future. Recent technological advances coupled with a deeper understanding of tumor biology enable these models to more accurately simulate the tumor microenvironment and biological behavior, providing stronger support for drug screening and personalized therapy. Additionally, interdisciplinary collaboration and technological innovation will drive progress in this field. Combining knowledge and techniques from bioinformatics, systems biology, and clinical medicine can develop more efficient and accurate models and predictive methods.

Conclusion

The importance of organoid models in hepatobiliary tumor research and drug response prediction is undeniable. These models provide new tools for dissecting tumor complexity and crafting personalized therapy and drug development strategies. However, further research and technological innovations are necessary to fully elucidate organoid models' potential. Key areas for improvement include optimizing culture methods, integrating multi-omics data, developing new predictive algorithms, and enhancing model reproducibility and stability. Continued advancements in these areas are expected to yield significant breakthroughs in cancer treatment, providing patients with more effective and safer therapeutic options.

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Availability of data and materials

Data and materials are available on request from the authors.

Ethical policy

Not applicable.

Author contributions

CHZ and HTL conceptualized, designed, conducted research, and wrote the first draft; YXL contributed to the revision and figure production; SC provided supervision and revision of the draft.

Competing interests

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References

1. Argemi J, Ponz-Sarvise M, Sangro B: Immunotherapies for hepatocellular carcinoma and intrahepatic cholangiocarcinoma: current and developing strategies. Adv Cancer Res 2022, 156: 367-413.
2. Hormati A, Hajrezaei Z, Jazi K, Aslani Kolur Z, Rezvan S, Ahmadpour S: Gastrointestinal and pancratohepatobiliary cancers: a comprehensive review on epidemiology and risk factors worldwide. Middle East J Dig Dis 2022, 14(1): 5-23.
3. Liao X, Zhang D: The 8th edition American Joint Committee on cancer staging for hepato-pancreato-biliary cancer: a review and update. Arch Pathol Lab Med 2021, 145(5): 543-553.
4. Marin JJG, Prete MG, Lamarca A, Tavolari S, Landa-Magdalena A, Brandi G, Segatto O, Vogel A, Macias RIR, Rodrigues PM, et al: Current and novel therapeutic opportunities for systemic therapy in biliary cancer. Br J Cancer 2020, 123(7): 1047-1059.
5. Lohitesh K, Chowdhury R, Mukherjee S: Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight. Cancer Cell Int 2018, 18: 1-15.
6. Mao S, Pang Y, Liu T, Shao Y, He J, Yang H, Mao Y, Sun W: Bioprinting of in vitro tumor models for personalized cancer treatment: a review. Biofabrication 2020, 12(4): 042001.
7. Clevers H, Tuveson DA: Organoid models for cancer research. Annu Rev Cancer Biol 2019, 3(1): 223-234.
8. Manduca N, Maccafeo E, De Maria R, Sistigu A, Musella M: 3D cancer models: One step closer to in vitro human studies. Front Immunol 2023, 14: 1175503.

9. Jin H, Xue Z, Liu J, Ma B, Yang J, Lei L: Advancing Organoid Engineering for Tissue Regeneration and Biofunctional Reconstruction. Biomater Res 2024, 28: 0016.
10. Long HY, Qian ZP, Lan Q, Xu YJ, Da JJ, Yu FX, Zha Y: Human pluripotent stem cell-derived kidney organoids: Current progress and challenges. World J Stem Cells 2024, 16(2): 114-125.
11. Wu H, Wang G, Shang Y, Liu N, Zhen Y, Chen L, Li H, An Y: Organoids and their research progress in plastic and reconstructive surgery. Aesthet Plast Surg 2023, 47(2): 880-891.
12. Tan ML, Ling L, Fischbach C: Engineering strategies to capture the biological and biophysical tumor microenvironment in vitro. Adv Drug Deliv Rev 2021, 176: 113852.
13. Kaushik G, Ponnusamy MP, Batra SK: Concise review: current status of three-dimensional organoids as preclinical models. Stem Cells 2018, 36(9): 1329-1340.
14. Wu X, Su J, Wei J, Jiang N, Ge X: Recent advances in three-dimensional stem cell culture systems and applications. Stem Cells Int 2021, 2021: 9477332.
15. Lee SY, Koo IS, Hwang HJ, Lee DW: In Vitro three-dimensional (3D) cell culture tools for spheroid and organoid models. SLAS Discov 2023, 28(4): 119-137.
16. Gunti S, Hoke ATK, Vu KP, London NR Jr: Organoid and spheroid tumor models: techniques and applications. Cancers 2021, 13(4): 874.
17. Dienstmann R, De Dosso S, Felip E, Tabernero J: Drug development to overcome resistance to EGFR inhibitors in lung and colorectal cancer. Mol Oncol 2012, 6(1): 15-26.
18. Dermawan JK, Rubin BP: Molecular pathogenesis of gastrointestinal stromal tumor: a paradigm for personalized medicine. Annu Rev Pathol 2022, 17(1): 323-344.
19. Verduin M, Hoeben A, De Ruysscher D, Vooijs M: Patient-derived cancer organoids as predictors of treatment response. Front Oncol 2021, 11: 641980.
20. Rahman MM, Wells G, Rantala JK, Helleday T, Muthana M, Danson SJ: In-vitro assays for immuno-oncology drug efficacy assessment and screening for personalized cancer therapy: scopes and challenges. Expert Rev Clin Immunol 2024, 20(8): 821-838.
21. Prasad M, Kumar R, Buragohain L, Kumari A, Ghosh M: Organoid technology: A reliable developmental biology tool for organ-specific nanotoxicity evaluation. Front Cell Dev Biol 2021, 9: 696668.
22. Sorour Sadeghzade, Mohammad Hosseini Hooshiar, Hossein Akbari, Mahzad Haji Mahdi Tajer, Kiarash Kaki Sahneh , Saba Yousefpour Ziaei, Fatemeh Jalali, Elaheh Akouchakian: Recent advances in Organ-on-a-Chip models: How precision engineering integrates cutting edge technologies in fabrication and characterization. Appl Mater Today 2024, 38: 102231.
23. Wang Y, Qin J: Advances in human organoids-on-chips in biomedical research. Life Med 2023, 2(1): lnad007.
24. Jackson EL, Lu H: Three-dimensional models for studying development and disease: moving on from organisms to organs-on-a-chip and organoids. Integr Biol 2016, 8(6): 672-683.
25. Luo Z, Zhou X, Mandal K, He N, Wennerberg W, Qu M, Jiang X, Sun W, Khademhosseini A: Reconstructing the tumor architecture into organoids. Adv Drug Deliv Rev 2021, 176: 113839.
26. Ren X, Chen W, Yang Q, Li X, Xu L: Patient-derived cancer organoids for drug screening: basic technology and clinical application. J Gastroenterol Hepatol 2022, 37(8): 1446-1454.
27. Knight E, Przyborski S: Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. J Anat 2015, 227(6): 746-756.
28. Habanjar O, Diab-Assaf M, Caldefie-Chezet F, Delort L: 3D cell culture systems: tumor application, advantages, and disadvantages. Int J Mol Sci 2021, 22(22): 12200.
29. Marziye M, Vahid A, Bethany RH, Stephen DW, Xavier B, Dae KH: Advanced cell culture platforms: a growing quest for emulating natural tissues. Mater Horiz 2019, 6(1): 45-71.
30. Ravi M, Ramesh A, Pattabhi A: Contributions of 3D cell cultures for cancer research. J Cell Physiol 2017, 232(10): 2679-2697.
31. Wu X, Su J, Wei J, Jiang N, Ge X: Recent advances in three-dimensional stem cell culture systems and applications. Stem Cells Int 2021, 2021: 9477332.
32. Tang XY, Wu S, Wang D, Chu C, Hong Y, Tao M, Hu H, Xu M, Guo X, Liu Y: Human organoids in basic research and clinical applications. Signal Transduct Target Ther 2022, 7(1): 168.
33. Lee SW, Jung DJ, Jeong GS: Gaining new biological and therapeutic applications into the liver with 3D in vitro liver models. Tissue Eng Regen Med 2020, 17(6): 731-745.
34. Akbari S, Arslan N, Senturk S, Erdal E: Next-generation liver medicine using organoid models. Front Cell Dev Biol 2019, 7: 345.
35. Fiorotto R, Amenduni M, Mariotti V, Fabris L, Spirli C, Strazzabosco M: Liver diseases in the dish: iPSC and organoids as a new approach to modeling liver diseases. Biochim Biophys Acta Mol Basis Dis 2019, 1865(5): 920-928.
36. Bao YL, Wang L, Pan HT, Zhang TR, Chen YH, Xu SJ, Mao XL, Li SW: Animal and organoid models of liver fibrosis. Front Physiol 2021, 12: 666138.
37. Mitaka T, Ooe H: Characterization of hepatic-organoid cultures. Drug Metab Rev 2010, 42(3): 472-481.
38. Yuan B, Zhao X, Wang X, Liu E, Liu C, Zong Y, Jiang Y, Hou M, Chen Y, Chen L, et al: Patient-derived organoids for personalized gallbladder cancer modelling and drug screening. Clin Transl Med 2022, 12(1): e678.
39. Clark AM, Ma B, Taylor DL, Griffith L, Wells A: Liver metastases: Microenvironments and ex-vivo models. Exp Biol Med 2016, 241(15): 1639-1652.
40. Bao YL, Wang L, Pan HT, Zhang TR, Chen YH, Xu SJ, Mao XL, Li SW: Engineering liver microtissues for disease modeling and regenerative medicine. Adv Funct Mater 2020, 30(44): 1909553.
41. Huang D, Gibeley SB, Xu C, Xiao Y, Celik O, Ginsberg HN, Leong KW: 3D liver models on a microplatform: well-defined culture, engineering of liver tissue and liver-on-a-chip. Lab Chip 2015, 15(19): 3822-3837.
42. Fang H, Xu H, Yu J, Cao H, Li L: Human Hepatobiliary Organoids: Recent Advances in Drug Toxicity Verification and Drug Screening. Biomolecules 2024, 14(7): 794.
43. Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST: Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv Drug Deliv Rev 2017, 108: 25-38.
44. Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M: Opportunities and challenges in phenotypic drug discovery: an industry perspective. Nat Rev Drug Discov 2017, 16(8): 531-543.
45. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB: Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov 2009, 8(10): 806-823.
46. Castro F, Leite Pereira C, Helena Macedo M, Almeida A, José Silveira M, Dias S, Patrícia Cardoso A, José Oliveira M, Sarmento B: Advances on colorectal cancer 3D models: The needed translational technology for nanomedicine screening. Adv Drug Deliv Rev 2021, 175: 113824.
47. Zhang J, Tavakoli H, Ma L, Li X, Han L, Li X: Immunotherapy discovery on tumor organoid-on-a-chip platforms that recapitulate the tumor microenvironment. Adv Drug Deliv Rev 2022, 187: 114365.
48. Imparato G, Urciuolo F, Netti PA: In vitro three-dimensional models in cancer research: a review. Int Mater Rev 2015, 60(6): 297-311.
49. Van Zundert I, Fortuni B, Rocha S: From 2D to 3D cancer cell models—the enigmas of drug delivery research. Nanomaterials 2020, 10(11): 2236.
50. Zhu Y, Tang S, Yuan Q, Fu J, He J, Liu Z, Zhao X, Li Y, Zhao Y, Zhang Y, et al: Integrated characterization of hepatobiliary tumor organoids provides a potential landscape of pharmacogenomic interactions. Cell Rep Med 2024, 5(2): 101375.

51. Wang W, Ma L, Xing Z, Yuan T, Bao J, Zhu Y, Zhao X, Zhao Y, Zong Y, Zhang Y, et al: Tumor-Specific CircRNA-Derived Antigen Peptide Identification for Hepatobiliary Tumors. Eng 2023, 22: 159-170.
52. Zhu Y, Tang S, Yuan Q, Fu J, He J, Liu Z, Zhao X, Li Y, Zhao Y, Zhang Y, et al: Integrated characterization of hepatobiliary tumor organoids provides a potential landscape of pharmacogenomic interactions. Cell Rep Med 2024, 5(2): 101375.
53. Saito Y: Establishment of an organoid bank of biliary tract and pancreatic cancers and its application for personalized therapy and future treatment. J Gastroenterol Hepatol 2019, 34(11): 1906-1910.
54. Liu XM, Chapman GB, Wang H, Durante W: Adenovirus-mediated heme oxygenase-1 gene expression stimulates apoptosis in vascular smooth muscle cells. Circulation 2002, 105(1): 79-84.
55. Nageswara Rao AA, Warad DM, Weaver AL, Schleck CD, Rodriguez V: Cross-cultural medical care training and education: a national survey of pediatric hematology/oncology fellows-in-training and fellowship program directors. J Cancer Educ 2019, 34(3): 478-487.
56. Rossi E, Zamarchi R: Single-cell analysis of circulating tumor cells: how far have we come in the-omics era? Front Genet 2019, 10: 958.
57. Caligola S, De Sanctis F, Canè S, Ugel S: Breaking the immune complexity of the tumor microenvironment using single-cell technologies. Front Genet 2022, 13: 867880.
58. Liu Z, Li H, Dang Q, Weng S, Duo M, Lv J, Han X: Integrative insights and clinical applications of single-cell sequencing in cancer immunotherapy. Cell Mol Life Sci 2022, 79(11): 577.
59. Finotello F, Rieder D, Hackl H, Trajanoski Z: Next-generation computational tools for interrogating cancer immunity. Nat Rev Genet 2019, 20(12): 724-746.
60. Dutta AK, Alberge JB, Sklavenitis-Pistofidis R, Lightbody ED, Getz G, Ghobrial IM: Single-cell profiling of tumour evolution in multiple myeloma—opportunities for precision medicine. Nat Rev Clin Oncol 2022, 19(4): 223-236.
61. de Joode K, van de Geer WS, van Leenders GJLH, Hamberg P, Westgeest HM, Beeker A, Oosting SF, van Rooijen JM, Beerepoot LV, Labots M, et al: The genomic and transcriptomic landscape of advanced renal cell cancer for individualized treatment strategies. Sci Rep 2023, 13(1): 10720.
62. Feng F, Shen B, Mou X, Li Y, Li H: Large-scale pharmacogenomic studies and drug response prediction for personalized cancer medicine. J Genet Genomics 2021, 48(7): 540-551.
63. Lee RY, Wu Y, Goh D, Tan V, Ng CW, Lim JCT, Lau MC, Yeong JPS: Application of artificial intelligence to in vitro tumor modeling and characterization of the tumor microenvironment. Adv Healthc Mater 2023, 12(14): 2202457.
64. Kuenzi BM, Park J, Fong SH, Sanchez KS, Lee J, Kreisberg JF, Ma J, Ideker T: Predicting drug response and synergy using a deep learning model of human cancer cells. Cancer Cell 2020, 38(5): 672-684.e6.
65. Jiang X, Wang J, Deng X, Xiong F, Zhang S, Gong Z, Li X, Cao K, Deng H, He Y, et al: The role of microenvironment in tumor angiogenesis. J Exp Clin Cancer Res 2020, 39(1): 204.
66. Fang X, Guo Z, Liang J, Wen J, Liu Y, Guan X, Li H: Neoantigens and their potential applications in tumor immunotherapy. Oncol Lett 2022, 23(3): 1-9.
67. Benvenuto M, Focaccetti C, Izzi V, Masuelli L, Modesti A, Bei R: Tumor antigens heterogeneity and immune response-targeting neoantigens in breast cancer. Semin Cancer Biol 2021, 72: 65-75.
68. Amador CA, Barrientos V, Peña J, Herrada AA, González M, Valdés S, Carrasco L, Alzamora R, Figueroa F, Kalergis AM, et al: Spironolactone decreases DOCA–salt–induced organ damage by blocking the activation of T helper 17 and the downregulation of regulatory T lymphocytes. Hypertension 2014, 63(4): 797-803.
69. LeSavage BL, Suhar RA, Broguiere N, Lutolf MP, Heilshorn SC: Next-generation cancer organoids. Nat Mater 2022, 21(2): 143-159.
70. Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K, Lin L, Fong ELS, Balachander GM, Chen Z, Soragni A, et al: Organoids. Nat Rev Methods Primers 2022, 2(1): 94.
71. Salemme V, Centonze G, Avalle L, Natalini D, Piccolantonio A, Arina P, Morellato A, Ala U, Taverna D, Turco E, et al: The role of tumor microenvironment in drug resistance: emerging technologies to unravel breast cancer heterogeneity. Front Oncol 2023, 13: 1170264.
72. Graw S, Chappell K, Washam CL, Gies A, Bird J, Robeson MS 2nd, Byrum SD: Multi-omics data integration considerations and study design for biological systems and disease. Mol Omics 2021, 17(2): 170-185.