REVIEW

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Current Applications of Colorectal Cancer Organoids: A Review

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ABSTRACT

Colorectal cancer is a prevalent malignancy, with advanced and metastatic forms exhibiting poor treatment outcomes and high relapse rates. To enhance patient outcomes, a comprehensive understanding of the pathophysiological processes and the development of targeted therapies are imperative. The high heterogeneity of colorectal cancer demands precise and personalized treatment strategies. Colorectal cancer organoids, a three-dimensional in vitro model, have emerged as a valuable tool for replicating tumor biology and exhibit promise in scientific research, disease modeling, drug screening, and personalized medicine. In this review, we present an overview of colorectal cancer organoids and explore their applications in research and personalized medicine, while also discussing potential future developments in this field.

Key words: organoid – colorectal cancer – drug screening – personalized medicine – tumor microenvironment.

Abbreviations: CRC: colorectal cancer; ECM: extracellular matrix; EOCRC: early onset CRC; GEMM: genetically engineered mouse models; GETO: genetically engineered tumor organoids; PDTO: patient-derived tumor organoids; PDTX: patient-derived tumor xenograft; TILs: tumor-infiltrating lymphocytes; 5-FU: 5-Fluorouracil.

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INTRODUCTION

Colorectal cancer (CRC) ranks as the third most common malignant tumor and the second leading cause of cancer-related deaths worldwide [1]. Approximately 20% of patients are diagnosed with metastatic CRC, and the five-year survival rate is less than 20%. Advanced CRC often yields poor curative effects, high recurrence rates following surgical resection, and unfavorable prognoses [2]. Clinical trials have demonstrated that detecting somatic gene mutations and customizing treatment regimens based on distinct molecular characteristics enhance the overall survival of patients with metastatic CRC. For instance, in 50% of patients with the KRAS/NRAS/BRAF wild-type genotype, combined cetuximab and panitumumab chemotherapy is more effective than chemotherapy alone, resulting in a median survival time extension of 2-4 months [3]. Therefore, it is crucial to explore the pathophysiological processes of CRC for developing targeted drugs with improved therapeutic efficacy. Moreover, the high heterogeneity of CRC necessitates individualized treatment to optimize therapeutic outcomes, minimize drug toxicity, and enhance the overall survival and quality of life of patients. CRC organoids, a novel three-dimensional (3D) *in vitro* model, offer an effective means of replicating the biological characteristics of in vivo tumors. They have been employed in CRC scientific research and exhibit promising potential in disease modeling, drug screening, and personalized medical treatment.

COLORECTAL CANCER ORGANOIDS

Advantages of Tumor Organoids

Organoids, often referred to as „mini-organs," are three-dimensional (3D) structures derived from adult or pluripotent stem cells and cultured *in vitro* [4-6]. Organoids enable amplification, cloning, and gene editing at a stable genetic level. There are two main types of tumor organoids: patient-derived tumor organoids (PDTO) obtained from tumor tissues during surgery and tumor organoids created through gene editing with CRISPR-Cas9 technology [4, 7]. With CRISPR-

Cas9, normal tissue-derived organoids can be genetically engineered to model malignant cancer. For example, successive introduction of mutations in KRAS, APC, SMAD4, and TP53 in wild-type intestinal organoids generated an *in vitro* model of colorectal cancer [4]. Genetically engineered tumor organoids (GETO) allow the study of the effects of single or multiple gene mutations on tumor initiation and progression, providing valuable insights into the pathophysiological processes of cancer. However, a limitation of GETO is their inability to accurately model patient tumors, which typically result from multiple known or unknown gene mutations. On the other hand, PDTO are more suitable for drug screening and personalized medicine, as they can more accurately replicate patient tumors. Nonetheless, they may not be ideal for studying the comprehensive pathophysiological processes of colorectal cancer.

Conventional tumor research models include human tumor-derived cell lines, spheroid cultures, organoid cultures, patient-derived tumor xenograft (PDTX) mouse models, and genetically engineered mouse models (GEMM). However, the validity of these models in simulating the biological characteristics of human tumors and evaluating drug efficacy remains controversial, and each model has its own limitations (Table I). Cell lines lack tumor microenvironment components, which can distort the biological characteristics of tumors. Spheroids, self-assembled 3D cellular aggregates, closely resemble the cellular microenvironment in vivo due to cell-matrix interactions. However, their biological characteristics change as they undergo passages [8, 9]. Xenotransplantation models lose the heterogeneity of tumors in vivo, and genetic variations may easily occur during the passage of genetically engineered mice [10]. In contrast, organoid cultures effectively preserve the genetic, proteomic, morphological, and pharmacokinetic characteristics of the parent tumor [11]. Compared with traditional models, tumor organoids retain the molecular characteristics of the original tumor cells and reduce the heterogeneity of tumors. Through multiple passages, they maintain stable genes and demonstrate great potential in personalized cancer treatment, particularly in preclinical drug screening and predicting patients‘ responses to selected treatment regimens [4, 12].

Culture of CRC Organoids

In the organoid culture system, intestinal stem cells undergo self-renewal and form crypt-like organoid structures in an extracellular matrix [13]. The key to successful culture lies in simulating the microenvironment that supports the growth of intestinal stem cells [14, 15]. Human colorectal organoids are cultured using a laminin-rich extracellular matrix (Matrigel) and specific factors that create a microenvironment suitable for intestinal stem cells. These factors include epithelial growth factor (EGF), a bone morphogenetic protein (BMP) signaling pathway inhibitor (Noggin), an agonist of the Wnt signaling pathway (R-spondin 1), an exogenous ligand of the Wnt signaling pathway (Wnt 3A), an inhibitor of transforming growth factor-β (A83-01), and an inhibitor of p38 (SB202190) [16]. Since 2011, this culture system has been successfully applied to derive patient-derived colorectal cancer organoids (Fig. 1) [17]. Interestingly, it has been observed that the requirement for niche factors in organoids decreases gradually during the progression from normal tissue to adenoma and then adenocarcinoma. The specific genetic mutations present in tumors determine the need for different niche factors, and matching colorectal cancers with similar genetic characteristics to corresponding niche factors can optimize colorectal cancer organoid culture [18]. The successful cultivation of organoids encompasses various critical aspects, including morphology, survival time, cell types, and histopathological characterization. Taking colorectal cancer organoids derived from patient samples as a case in point, the process involves the isolation and seeding of human colon cancer tissue in an extracellular matrix and culture medium. On the initial day, observable is a circular healing phenomenon among cell clusters, signifying the survival of organoid cells. Progressing to the third day, the organoids undergo expansion, some exhibiting folds with the increasing volume. By the sixth day, a substantial increase in diameter occurs, reaching 100-200 μm or more, concurrently forming a discernible hollow lumen. Fluorescent staining

Table I. Comparison of tumor cell lines, spheroid culture, PDTX, GETO, and PDTO models

	Tumor cell lines	Spheroid culture	PDTX	GETO	PDTO
Expense	+	+	++	++	++
Time consuming	+	+	+++	+	+
Culture success rate	+++	+++	+	+++	+++
Genome editing	+++	+++	-	+++	+++
Organization structure	-	+	+++	++	++
Tumor biological property	+	++	+++	+++	+++
Intra-tumor heterogeneity	-	+	+++	++	++
Tumor microenvironment	-	+	+++	++	++
Gene stability	+	+	++	++	++
High throughput screening of drugs	+++	+++	-	+++	+++
Personalized treatment	-	-	+++	+++	+++

PDTX: xenotransplantation model of tumor from patients; GETO: genetically engineered tumor organoids; PDTO: tumor organoids model from patients. „+" indicates a low score, „++" a medium score, „+++" a high score, and "-" unsuitable.

results reveal a predominantly Ki67-positive cell population, aligning with the potent inhibitory influence of Wnt on enterocyte differentiation. H&E staining of the organoid slices demonstrates a clustered cell distribution, a high nuclear-to-cytoplasmic ratio, and notably heterogeneous nuclei, in keeping with the morphological attributes of tumor cells. Notably, the colorectal cancer organoids exhibit a passage number exceeding 30, attesting to their robustness, and demonstrate resilience against multiple freeze-thaw cycles [17, 19, 20].

Establishment of CRC Organoid Biobank and Organoids-on-a-Chip

In 2016, Fujii et al. [18] further optimized the organoid culture method and established a biobank of colorectal tumor organoids, encompassing 55 different histological subtypes and clinical stages, which faithfully recapitulated the clinical characteristics of patients. Organoid technology has proven to be valuable in supplementing existing CRC models, especially for studying sporadic early-onset CRC (EOCRC), a type of CRC with a poor prognosis that cannot be effectively represented by cell line models. The EOCRC organoid biobank facilitated research by Yan et al. [21], who investigated key gene mutations and transcriptome changes, revealing the unique genetic characteristics of EOCRC.

To better control the cultivation and regulation of organoids *in vitro* and mimic the dynamic microenvironment in vivo, researchers have integrated organoid culture with chip technology to create „organoids-on-a-chip" models [22]. Organoid chips are miniature cell culture devices that employ microfluidic chip technology to simulate the functional elements of organoids *in vitro* [23]. By applying microfluidics to organoids and integrating external inputs such as electrical or mechanical cues, as well as in situ monitoring of crucial parameters, these chips offer effective models of human organs [24]. Organoid chips allow precise regulation and control of organoid cells, including blood perfusion, mechanical force, and other microenvironment components that are often lacking in traditional culture systems [25, 26]. Furthermore, organoid chips establish interactions between tissues and multiple organoids, reduce variability in the structure and function during the development of the chip, and enable high-throughput processing [26-28]. However, it is worth noting that these models still need further exploration to address the dynamic changes in organoids and their environment during organogenesis [29].

APPLICATION OF ORGANOIDS IN CRC

Transplantation of PDTO for CRC Modeling

Disease modeling aims to replicate primary and metastatic diseases in vivo for preclinical research and clinical applications. Orthotopic transplantation of PDTO preserves the original tumor characteristics, minimizes the tumor microenvironment, and accelerates the establishment of disease models. This transplantation approach allows PDTO to faithfully recapitulate the molecular, genetic, and pathological features of the primary tumor in vivo (Fig. 2) [30].

Transplantation of CRC organoids has been shown to result in the formation of invasive colon cancer, with liver metastasis observed in 25% of mice at 8 days post-transplantation, increasing to 45% at 12 days post-transplantation [30]. In situ transplantation of colorectal organoids, performed using gene editing techniques, has provided valuable insights into the adenoma-adenocarcinoma-spontaneous metastasis process for gene and preclinical research [31]. Compared to previous approaches involving patient-derived cell lines and genetically engineered mice, in situ transplantation of CRC organoids has demonstrated higher success rates, shorter time consumption, and accurate recapitulation of the histological characteristics of primary CRC epithelium and matrix [31].

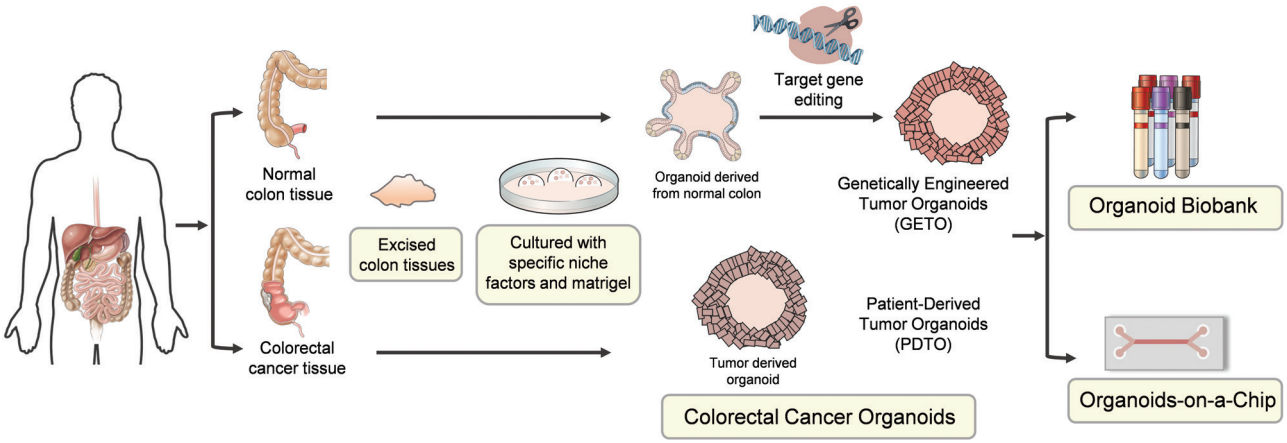

Fig. 1. Establishment of colorectal cancer organoids, organoid biobank and organoids-on-a-chip. Tissues from normal colon or colorectal cancer are excised and cultured with specific growth factors and matrigel to generate organoids. With target gene editing (e.g. introducing mutations in KRAS, APC, SMAD4, and TP53 by CRISPR-Cas9 technology), normal colon tissue-derived organoids can be genetically engineered to generate Genetically Engineered Tumor Organoids (GETO). Colorectal cancer tissue-derived organoids can be cultured to establish patient-derived tumor organoids (PDTO). The two type of tumor organoids can model human colorectal malignant cancer. The colorectal cancer organoids derived from different patients can be preserved to establish biobank. The biobank contains a certain number of patient organoids, encompassing different histological subtypes and clinical stages. Additionally, the colorectal cancer organoids can be cultured with chip technology to create „organoids-on-a-chip" models, which can mimic the dynamic microenvironment in vivo and enable high-throughput processing.

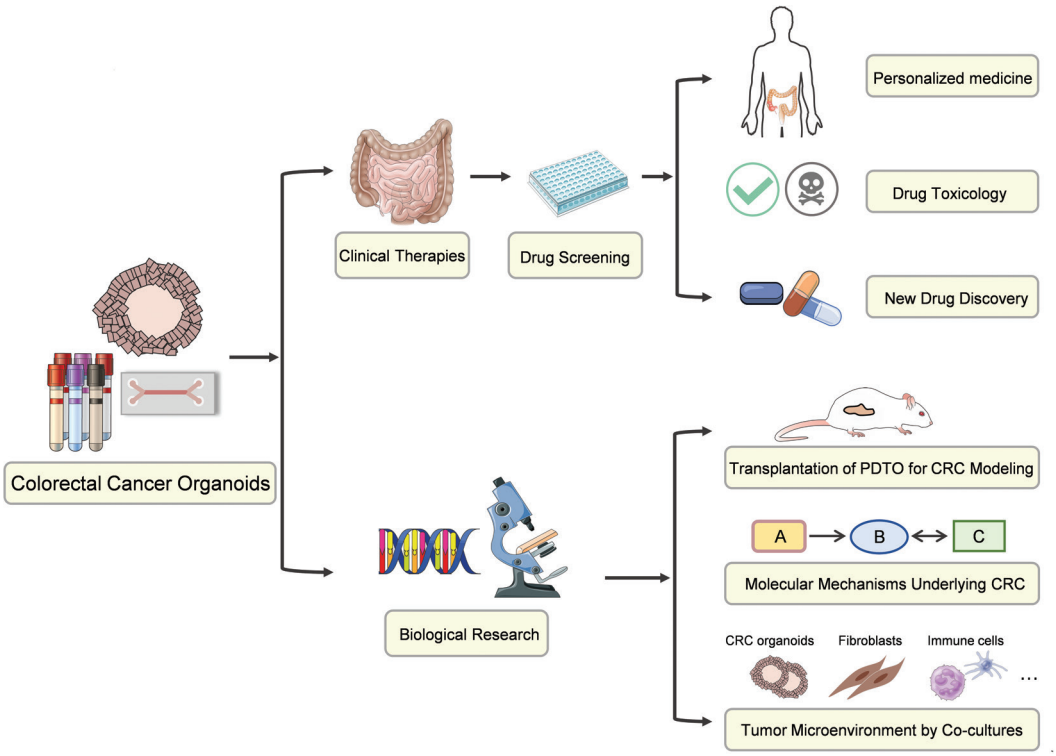

Fig. 2. Application of colorectal cancer organoids in clinical therapy and biological research. The CRC organoids are widely used for clinical therapy by high-throughput drug screening. For personalized medicine, CRC organoids can faithfully simulate patient responses to drugs, providing valuable information for designing personalized treatment strategies. CRC organoids are also suitable for drug development, as they can be used to test drug activity and toxicity, improving the speed and success rate of new drug discovery. In addition, CRC organoids can be used directly in basic cancer biology research. Tumor organoids can be transplanted into mice for CRC modeling, faithfully recapitulating the molecular, genetic, and pathological features of the primary tumor in vivo. CRC organoids are also a suitable tool for exploring the molecular mechanisms underlying colorectal cancer. Besides, combining CRC organoids with fibroblasts, immune cells and other matrix components in the microenvironment has allowed for the characterization of the tumor microenvironment, better for exploring immunotherapy and precision cancer treatment.

Exploration of the Molecular Mechanisms Underlying CRC

The pathogenesis of CRC involves gene mutations in intestinal epithelial cells, leading to aberrations in various signaling pathways such as Wnt, mitogen-activated protein kinase (MAPK), transforming growth factor-β (TGF-β), P53, and phosphoinositide 3-kinase (PI3K), which contribute to adenoma-adenocarcinoma invasion and metastasis [32]. Lineage tracing and sequential mutagenesis studies using Adenomatous polyposis coli (APC)-deleted organoids have revealed oncogene activation in established colonic adenomas [30]. In vivo transplantation of engineered tumor organoids by Fumagalli et al. [33] identified that the accumulation of at least four genetic mutations, including APC, KRAS, SMAD Family Member 4 (SMAD4), and P53, enables tumor cells to grow independently, leading to distant metastasis, colonization, and growth. This model provides insights into the contribution of individual genetic mutations to tumor progression, including the early loss of p53, which facilitates CRC progression. Loss of P53 function allows cells to continue proliferating, while also enabling the accumulation of additional genetic mutations due to chromosomal instability. Moreover, the inactivation of SMAD4 plays a crucial role in late-stage migration, metastasis, and proliferation of CRC [33].

Organoids derived from normal intestinal epithelium can be genetically engineered using CRISPR-Cas9 genome editing technology to introduce various driver pathway mutations, simulating CRC evolution and elucidating the role of these mutations in tumorigenesis [16]. These studies have revealed that driver pathway mutations alone are insufficient for the malignant progression of tumors, and additional molecular mechanisms, such as chromosomal instability, copy number variation, or epigenetic changes, contribute to the aggressive behavior of CRC [16]. Bionic organoids have been employed by Ukai et al. [34] to identify the role of KHDRBS3 in maintaining the stemness of CRC tumor cells and promoting multidrug resistance, potentially serving as a marker for predicting chemotherapy response and patient prognosis. Nagai et al. [35] reported the miRNA profile of exosomes in colorectal adenoma and CRC organoids, revealing the potential involvement of upregulated miR-1246 in adenoma cell proliferation and carcinogenesis.

Tumor organoids offer an ideal model for studying the molecular mechanisms underlying CRC-related ectopic differentiation. Yamazaki et al. [36] discovered that APC spontaneously inhibits the ectopic differentiation of colon epithelial cells into lysozyme-positive cells, a phenomenon

typically observed only in CRC. Lähde et al. [37] found that overexpression of R-Spondin 1 in APC mice resulted in increased cell apoptosis, decreased adenoma cell proliferation and Wnt signaling, and reduced tumor incidence. Furthermore, the addition of R-Spondin 1 to adenoma-like organoids inhibited their growth and promoted the proliferation of intestinal stem cells expressing the APC protein, suggesting a potential strategy for increasing R-Spondin 1 expression in the treatment of intestinal adenomas. Tumor organoids also serve as powerful tools for studying dynamic cellular processes in CRC, facilitating a better understanding of tumor behavior at the cellular level [38]. Bolhaqueiro et al. [39] utilized patient-derived CRC organoids for three-dimensional live-cell imaging, revealing chromosome instability as a common feature in CRC. Cell tracers unveiled tumor tolerance to mitotic errors, and single-cell karyotype analysis confirmed the heterogeneity of copy number variations in organoids derived from human tumors. These findings highlight the contribution of chromosome instability and tolerance to mitotic errors to tumor heterogeneity [39].

The occurrence and development of inflammatory bowel disease (IBD)-related CRC are associated with intestinal barrier dysfunction [40]. Epithelial barrier function relies on the integrity of intestinal epithelial cells, with epithelial proliferation maintaining the continuity of cell layers to form a barrier, while epithelial damage leads to barrier dysfunction [40]. Organoids derived from fetal tissue are employed to study intestinal development and injury repair, offering insights into necrotizing enterocolitis (NEC) [41, 42]. Li et al. [19] quantified the number of proliferating and dead cells in cultured organoids, demonstrating the value of the organoid model in testing the effects of drugs on the proliferation and survival of intestinal epithelial cells. Zha et al. [43] explored the potential mechanism by which IL-22 promotes mucosal healing, identifying that IL-22 promotes the proliferation of migrating expanded cells through the JAK/STAT3 pathway while reducing the survival rate of Lgr5+ stem cells by inhibiting the Notch and Wnt signaling pathways. Thus, the ability of IL-22 to promote mucosal repair may depend on its influence on transitional expanded cells or stem cells [43]. Co-culturing intestinal organoids with gut microorganisms allows for the examination of the relationship between the host and microbes in vitro, enabling a more precise regulation of experimental conditions [44, 45]. This approach also facilitates a better understanding of the mechanisms underlying microbe-induced CRC *in vitro* [46].

Characterization of the Tumor Microenvironment by Organoid Co-culture Technology

Tumor organoids provide a valuable platform to study tumor cell-extracellular matrix (ECM) interactions and the response of tumor cells to chemotherapy. Compact ECM structures have been found to inhibit tumor cell growth, protect tumor stem cell populations, and reduce chemotherapy response [47]. Luo et al. [48] developed a co-culture strategy to maintain the viability of organoids and tumor-associated fibroblasts (CAFs) derived from CRC patients. This co-culture model has proven useful for evaluating drug efficacy and personalized medicine for tumors, even without the use of growth factors traditionally used to support PDTO culture. Co-culture models of hepatocellular carcinoma and endothelial cells have been employed to understand the interplay between angiogenesis and the immune milieu [49].

Combining tumor organoids from patients with immune cells and other matrix components in the microenvironment has allowed for the characterization of the tumor microenvironment and exploration of immunotherapy and precise cancer treatment [50,51]. The use of patient-specific tumor organoid-immune co-culture models has the potential to enhance the effective use of expensive immunotherapeutic medicines by accurately predicting patient-specific responses [52]. Tumor organoid-on-a-chip platforms provide valuable tools for examining how tumors control the tumor microenvironment to evade anti-tumor immunity and resist immunotherapy. These platforms are highly valuable for investigating the intricate relationship between cancer and the immune system [53]. Neal et al. [50] introduced a gas-liquid boundary method to co-culture tumor epithelial cells and tumor-infiltrating lymphocytes (TILs) from patients, successfully simulating the tumor immune microenvironment in vitro. The TILs in these organoids accurately retained the T-cell receptor spectrum and simulated immune checkpoint blockade using PD-1 and/or PD-L1 antibodies, resulting in the amplification and activation of lymphocytes expressing tumor-specific antigens and exhibiting tumor-killing effects. This co-culture system maintains the in vivo connection between TILs and tumor cells, providing a valuable cancer immunotherapy model. However, it is important to consider that epithelial-immune interactions also involve components of the peripheral immune system, which are not fully captured by the tumor microenvironment alone [50]. Consequently, Dijkstra et al. [54] established and validated a novel platform capable of simulating two-way communication between tumor and surrounding tissues and inducing tumor-reactive T cells in a personalized manner to assess tumor-killing efficacy. The co-culture of autologous tumor organoids with peripheral blood lymphocytes enriches tumor-reactive cells from patients‘ peripheral blood and allows the analysis of the cytotoxic effects of these T cells on matched tumor organoids, enabling the evaluation of tumor sensitivity to T-cell-mediated attacks on an individual level [54].

Drug Screening

Tumor heterogeneity has been a major challenge in translating basic research into clinical applications for anti-cancer drug development. The organoid model overcomes the limitations of previous models and accurately simulates tumor heterogeneity [55]. As individual cells within a tumor population undergo genetic and phenotypic alterations during expansion, the organoid model offers the capability to measure the functional changes in single-cell-derived clones [56]. Studies by Roerink et al. [57] involving the characterization and analysis of multiple single-cell-derived organoids from CRCs and adjacent normal intestinal crypts demonstrated that CRC cells exhibit extensive mutation diversity, with a significantly increased somatic mutation rate compared to normal cells. Moreover, cells within the same tumor exhibit different responses to anticancer drugs [57], emphasizing

the importance of studying tumor heterogeneity using the organoid model.

Colorectal tumor organoids from patients have been employed to evaluate the synergistic effect of hyperthermia and chemotherapy, with hyperthermia enhancing the efficacy of reteplase [58]. 5-Fluorouracil (5-FU) is the first-line drug for CRC treatment but often leads to tumor recurrence after chemotherapy [59]. Studies have shown that 5-FU can promote Wnt3 transcription through p53, activating the Wnt/β-catenin pathway and inducing tumor stem cell activation. The combination of a Wnt inhibitor with 5-FU effectively inhibits the activation of tumor stem cells and reduces tumor growth after chemotherapy [60]. Inhibition of the Wnt signaling pathway has shown promise in CRC development, as demonstrated by the Wnt signal inhibitor IWP-2, which inhibits the growth of PDTO. This biological library of PDTO can be used to screen for Wnt pathway inhibitors for high-throughput treatment of CRC [20].

Organoid models derived from induced pluripotent stem cells from patients with Familial adenomatous polyposis (FAP) exhibit increased Wnt activity and epithelial proliferation, consistent with APC gene mutation characteristics. By using this model as a platform for drug testing, Crespo et al. [61] discovered that geneticin (G418), a ribosome-bound antibiotic, effectively targets the abnormally activated Wnt pathway and reverses abnormal epithelial cell proliferation, providing a new therapeutic strategy for FAP patients with APC mutations and serving as a foundation for drug development in the context of colorectal diseases [62].

Personalized Medicine

Accurately predicting a patient's response to treatment is crucial in cancer therapy and is a fundamental requirement of personalized medicine [63]. Tumor organoids derived from patient samples can faithfully simulate patient responses to drugs, providing valuable information for designing personalized treatment strategies and offering alternative options for patients who have failed standard treatments [13, 64]. PDTO has proven beneficial in cancer precision immunotherapy and enables the detection of chimeric antigen receptor (CAR)-mediated cytotoxicity and tumor specificity [65]. The genetic variation spectrum of the PDTO CRC biological library has been found to align with previous large-scale studies on CRC gene mutations [66, 67]. Therefore, PDTO is suitable for high-throughput drug screening to explore the relationship between patient genes and drug efficacy [68].

While biomarkers alone may not fully meet clinical needs, the use of PDTO has allowed researchers, such as Ooft et al., [69] to identify patients who would not respond to standard chemotherapy, thus preventing them from receiving ineffective treatments. In a prospective clinical study, PDTO was used to evaluate the sensitivity of CRC patients to chemotherapy, particularly irinotecan-based chemotherapy, preventing patients from receiving ineffective treatment. However, PDTO was not effective in predicting the outcome of treatment with 5-FU plus oxaliplatin [63, 69]. Bolhaqueiro et al. [39] employed three-dimensional live-cell imaging to study colorectal tumor organoids, proposing the use of organoid reservoirs to investigate whether the instability of chromatin levels is associated with individual treatment sensitivity. Integrating chromatin instability levels into the drug screening process may be a possibility in the future [39, 71, 72].

Overall, the application of organoids in CRC research has proven to be a valuable and versatile tool, enabling the study of tumor biology, molecular mechanisms, tumor microenvironment, drug screening, and personalized medicine. The use of patient-derived organoids in disease modeling and drug testing offers promising prospects for advancing cancer research and improving patient outcomes.

Limitations

The current organoid models do have certain limitations. One of the major concerns is the reliance on animal-based matrix extracts, commonly in the form of Matrigel or Basement Membrane Extract, for organoid growth. These extracts suffer from batch-to-batch variability in their composition, which may impact the repeatability and reliability of research findings. One potential solution is the use of clinical-grade collagen for culturing, which has shown effectiveness in growing colon organoids. However, further adjustments are required for the long-term expansion of intestinal organoids and the expansion of non-intestinal organoids [73]. Additionally, standardized control is required for all organoid culture methods, including the processing of cancer tissue and medium formulations [11]. While some studies have developed Matrigel-free methods, they are still limited to a small number of species of organoid cultivation [74-77]. The expenses associated with organoid culture, primarily due to the costly growth factor cocktails and animal-based matrix extracts, surpass those incurred in standard 2D cell culture. This discrepancy in costs poses a potential constraint on the cost-effectiveness of conducting drug response studies using colorectal cancer (CRC) organoids. While CRC organoids can be generated relatively quickly, the process of expanding these organoids to a scale suitable for extensive drug testing may demand a more extended period (2-4 weeks) and greater resource allocation compared to traditional tumor cell lines. This extended timeline in drug development could impede the swift identification of effective treatments for colorectal cancer. Moreover, the intricacies of CRC organoids mandate the use of specialized equipment, consumables, and specific skills for manipulation. This specialized requirement contributes further to the overall costs, particularly in the context of clinical trials. The cumulative impact of these factors highlights the need for a judicious evaluation of the trade-offs between the unique advantages of CRC organoids and the economic considerations inherent in their utilization.

CONCLUSIONS AND PERSPECTIVES

Organoid models hold immense promise in advancing the study of CRC, with applications ranging from investigations of stem cell function and cell signaling pathways to drug responses, translational medicine, and regenerative medicine. Compared to traditional two-dimensional cell culture and animal models, organoid systems offer significant advantages in accurately mimicking the biological characteristics of tumors, facilitating drug development, screening, and

personalized medicine. Nevertheless, current organoid models have their limitations, particularly in fully recapitulating the patient's tumor microenvironment. While current technology can provide insights into some aspects of the tumor microenvironment, such as the immune microenvironment, the co-culture of quasi-organoids, blood vessels, nerves, and intestinal flora is still lacking [78].

Furthermore, the lack of standardization and uniformity in the organoid culture system, with varying culture conditions used in different laboratories, is a significant challenge. Integrating new technologies such as gene editing can help in exploring the molecular mechanisms underlying tumor occurrence and development. Co-culturing epithelial cells and immune cells can effectively simulate the tumor immune microenvironment in vitro. Microfluidic chip technology offers the potential to accurately mimic the organoid microenvironment. It is anticipated that in the near future, organoid models will overcome current limitations and play a more prominent role in mechanistic research, drug screening, and precision medicine for CRC. The ongoing advancements in organoid technology are likely to pave the way for improved understanding and treatment of CRC, ultimately benefiting patients with this disease.

Conflicts of interest: None to declare

Authors' contribution: J.Z., N.D. and W.H. conceived the study. Q.B., H.L., M.L., X.J., X.G., M.L., S.G., Q.L., W.H., N.D., and J.Z. revised the literature, collected data and drafted the manuscript. Q.B. and J.Z. prepared the figures and revised the literature. Q.B. and H.L. contribute equally to this work. All authors approved the final version of the article and approved the submitted version.

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