Tumor organoid model of colorectal cancer (Review)

CHI YANG[1*], WANGWEN XIAO[2*], RUI WANG[3*], YAN HU[2], KE YI[2], XUAN SUN[1], GUANGHUI WANG[3] and XIAOHUI XU[1,2]

[1]Department of Gastroenterology; [2]Central Laboratory, The First People's Hospital of Taicang, Taicang Affiliated Hospital of Soochow University, Soochow Medical College of Soochow University, Suzhou, Jiangsu 215400; [3]School of Pharmacy, Soochow Medical College of Soochow University, Suzhou, Jiangsu 215123, P.R. China

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Abstract. The establishment of self-organizing 'mini-gut' organoid models has brought about a significant breakthrough in biomedical research. Patient-derived tumor organoids have emerged as valuable tools for preclinical studies, offering the retention of genetic and phenotypic characteristics of the original tumor. These organoids have applications in various research areas, including *in vitro* modelling, drug discovery and personalized medicine. The present review provided an overview of intestinal organoids, focusing on their unique characteristics and current understanding. The progress made in colorectal cancer (CRC) organoid models was then delved into, discussing their role in drug development and personalized medicine. For instance, it has been indicated that patient-derived tumor organoids are able to predict response to irinotecan-based neoadjuvant chemoradiotherapy. Furthermore, the limitations and challenges associated with current CRC organoid models were addressed, along with proposed strategies for enhancing their utility in future basic and translational research.

Contents

Correspondence to: Professor Guanghui Wang, School of Pharmacy, Soochow Medical College of Soochow University, 199 Renai Road, Suzhou, Jiangsu 215123, P.R. China
E-mail: wanggh@suda.edu.cn

Dr Xiaohui Xu, Department of Gastroenterology, The First People's Hospital of Taicang, Taicang Affiliated Hospital of Soochow University, Soochow Medical College of Soochow University, 58 Changsheng South Road, Taicang, Suzhou, Jiangsu 215400, P.R. China
E-mail: xhxu_tc1988@suda.edu.cn

*Contributed equally

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1. Introduction

CRC is the third most common cancer type in the world (after breast cancer and lung cancer), and the second leading cause of cancer-related death worldwide (after lung cancer) (1). Although the incidence of CRC in individuals aged 65 years and older has decreased in most countries (2), the incidence in individuals under the age of 50 years has been increasing worldwide (3). CRC is a heterogeneous neoplastic disease caused by the malignant degeneration of the external epithelium of the large intestine. Its development follows the sequential accumulation of various carcinogenic mutations, namely the adenoma-carcinoma sequence, with dysregulation of signalling pathways such as WNT/β-catenin, p53 and TGF-β-Smads (4).

Common pathogenic factors of CRC include genetic mutation, poor diet, obesity and sedentary lifestyle (5,6). CRC is usually an asymptomatic disease until it progresses to an advanced stage. As a result, numerous patients with CRC are clinically diagnosed with advanced disease (7). Since adenomas take at least 5-10 years to develop into CRC, early endoscopic screening is crucial for the diagnosis of CRC. In recent decades, although the understanding of CRC has markedly improved at the molecular and genetic levels, it has not brought an equivalent level of clinical benefits to patients. Furthermore, the clinical outcomes and therapeutic responses of patients with CRC differ significantly from the expected outcomes. Numerous drugs have been found to be effective in *in vitro* cancer models but have eventually failed in clinical trials, indicating that there are marked differences between

the existing research model system and clinical practice (8). The current landscape of antitumor drug development relies heavily on traditional models, such as two-dimensional cell lines (2D) and patient-derived tumor xenografts (PDXs). However, these models have inherent limitations that hinder their effectiveness. Cell lines, for instance, fail to preserve the genetic information and heterogeneity of tumors during prolonged passaging. In addition, PDX models experience tumor evolution specific to mice and they differ in genetic characteristics and growth environments from those in human tumor patients. Furthermore, PDX models suffer from a low success rate, high cost and time-consuming procedures (9). Therefore, it is urgent to establish a model that may accurately reflect the genetic diversity and specificity of cancer to better aid in the clinical diagnosis and treatment of colon cancer.

In recent years, tumor organoids have emerged as a highly promising tool in the field of cancer research. Organoids, a 3D structured *in vitro* culture system containing self-renewing stem cells, have organization and function similar to those of an organ. This system overcomes many of the limitations of traditional models. These organoids more closely resemble the cellular composition, behaviour and physiology of natural tissues. Currently, researchers are able to generate cancer-like organoids derived from mouse or human tumor tissues. Under appropriate culture conditions, the organoid forms a three-dimensional structure similar to a mouse or human tumor and mostly maintains the tumor tissue structure, gene profile and heterogeneity observed in the original tumor tissue. Tumor organoids are able to accurately report the drug response of the corresponding patient to determine a more effective treatment plan for individual patients (10,11). In this paper, the history of organoid culture systems, their application in CRC and the challenges they face as preclinical models in CRC research were reviewed.

2. Organoid definition

Currently, preclinical cancer models mainly include cancer cell lines (CCLs), PDXs and organoids (12). CCLs are still widely used models for large-scale drug screening. However, due to their inability to accurately simulate the microenvironment of the original tumor growing in 3D, they do not accurately represent the heterogeneous characteristics of cancer cells *in vivo* (13). There is also a lack of corresponding cell lines derived from normal tissues as a reference control (14). There are significant differences in cell morphology and drug susceptibility between 2D cell lines and 3D cell models (15). PDXs can, to a certain extent, preserve cell interactions and capture tumor heterogeneity in 3D environments; however, they take a long time and have a low success rate (13,16).

Recently, a novel 3D culture technique has facilitated the development of organoid models and numerous researchers have similar definitions of organoids. Organoids refer to stem cells (including embryonic stem cells, adult stem cells, induced pluripotent stem cells, or tumor stem cells) embedded in extracellular matrix (ECM) produced in a specific environment and have specific organ structures and functions (17-20). Organoids may be created from almost any tissue, such as brain (21), intestine (22), thyroid gland (23), mammary gland (24), stomach (25), liver (26), ovaries (27), kidney (28) and oesophagus (29). Successfully constructed organoids may effectively mimic the morphological structure and epigenetics of both normal tissues and cancer tissues. It has been established that the general culture method of organoids involves the use of ECM hydrogels, such as Matrigel or basement membrane extract (BME), the addition of various growth factors to various tissues, the use of a gas-liquid interface, and the coculture of immune cells to mimic the tumor microenvironment to simulate the matrix environment for 3D culture. Organoid culture *in vitro* may be used for intervention studies prior to clinical treatment, such as those for drug sensitivity screening, new drug development, personalized medicine and regenerative medicine (Fig. 1). Of note, intestinal organoids are thought to be superior models to CCLs and PDXs for investigating cancer genetics, cancer processes and antitumor drug activity, since they make up for the inadequacies of existing models (30) (Fig. 2).

3. History of intestinal organoids

The mammalian intestinal epithelium consists of a single columnar epithelium containing resorptive and secretory cells that allow them to absorb nutrients and protect the intestine (31). The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals and the villus-crypt structure is completely renewed every 4-5 days. The villi-crypt structure is the basic building block of the intestinal epithelium, which is proliferated and differentiated by intestinal stem cells at the base of the crypt into mature intestinal functional cells (32,33) (Fig. 3). However, the specific location of intestinal stem cells is not well defined, so it is difficult to study their role. Barker *et al* (34) found that leucine-rich G-protein-paired receptor 5 (Lgr5, Wnt target gene) was a marker gene of intestinal stem cells. Subsequently, Sato *et al* (35) found normal mouse intestinal stem cells at the base of the crypt and differentiated them into self-organized 'mini small intestine', establishing the first 3D organoid. The characteristics of the crypts remained after 8 months of culture (35). They went on to successfully create organoids from normal human cells and human tumor colon epithelial cells, and improve the colon culture system (long-term culture requires niacin amide, a small molecule inhibitor of Alk and p38 inhibitors) (Table I) (36-42). In addition to their presence in the gut, Lgr5+ stem cells may be found in other tissues and organs, e.g., the liver (43), pancreas (44), ovaries (45), stomach (46), kidneys (47) and lung (48,49). At present, almost all human tissues may be cultured *in vitro* to produce organoids (50).

Intestinal organoids may also be derived from the progressive differentiation of human pluripotent stem cells (51,52). Spence *et al* (53) were the first to successfully construct intestinal organoids using human pluripotent stem cells. They first induced PSC differentiation into a defined endoderm by activin A (TGF-β molecule) and then treated them with a medium containing a combination of fibroblast growth factor 4 and Wnt3A to form post intestinal spheres. Finally, this was transferred to cultures that a known to facilitate the formation of organoids, resulting in polarized columnar epithelial cells containing goblet cells, Pan's cells and intestinal endocrine cells (53). Transient activation of bone morphogenetic

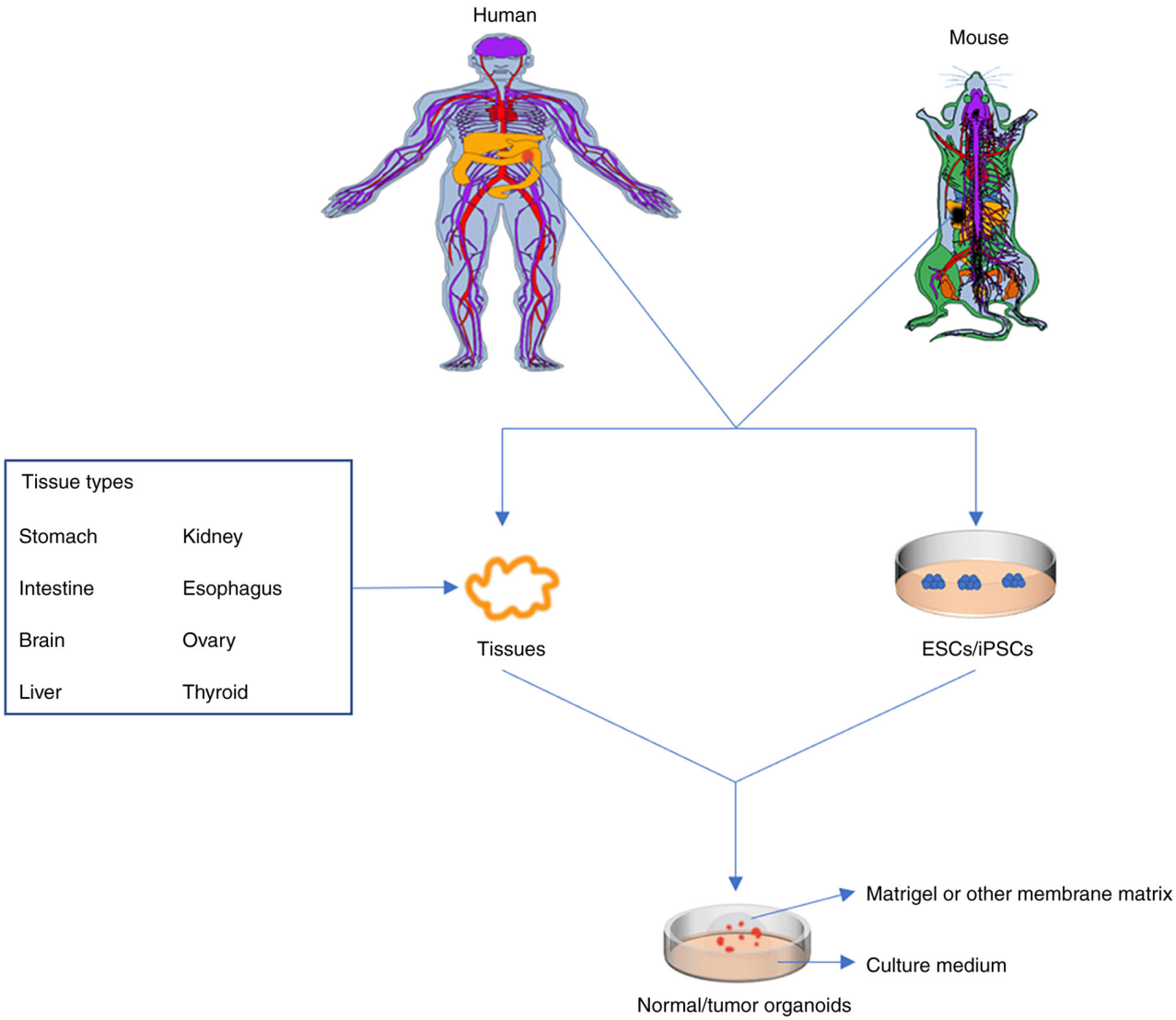

Figure 1. Schematic depicting the establishment of organoid cultures. Tissues, stem cells or activin-treated human pluripotent stem cells from different organs of humans or mice are embedded in a basement membrane matrix and maintained in a medium containing niche factors important for proliferation. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.

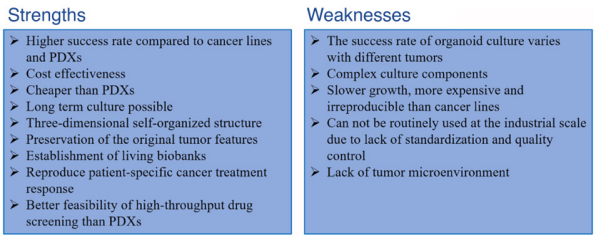

Figure 2. Strengths and weaknesses of tumor organoids. PDX, patient-derived xenograft.

protein signalling is essential for organoid development in the hindgut (54). Of note, CRC organoids have received growing recognition in studies of intestinal organoids.

4. CRC organoid

Sato *et al* (35) successfully established CRC organoids by improving the initially established mouse small intestine organoids and found that Wnt3A conditional medium was not necessary for the long-term culture of CRC organoids. Wnt3A, SB202190 and oxygen concentration have an important impact on the proliferation of CRC organoids and optimized culture conditions may increase the generation efficiency of CRC organoids to 100% (removing unqualified tissues such as pollution) (37,55). As CRC develops according to the adenoma-cancer sequence, the dependence of CRC organoids on ecological factors is reduced and the demand of some organoids for certain factors is optional (37,55-57). To date, a number of studies have successfully constructed CRC organoids, that can accurately mimic the genetic characteristics of tumors *in vivo* (55,58-60). Furthermore, proteomic analysis of CRC has shown that the primary tumor and its derived organoids have comparable proteomic characteristics and organoids from different patients have diverse proteomic characteristics. Relevant research on organoids from various individuals can help to better direct individualized medical care (61).

Patient-derived colorectal carcinoids are mainly derived from surgical resection specimens, biopsies and stem cells.

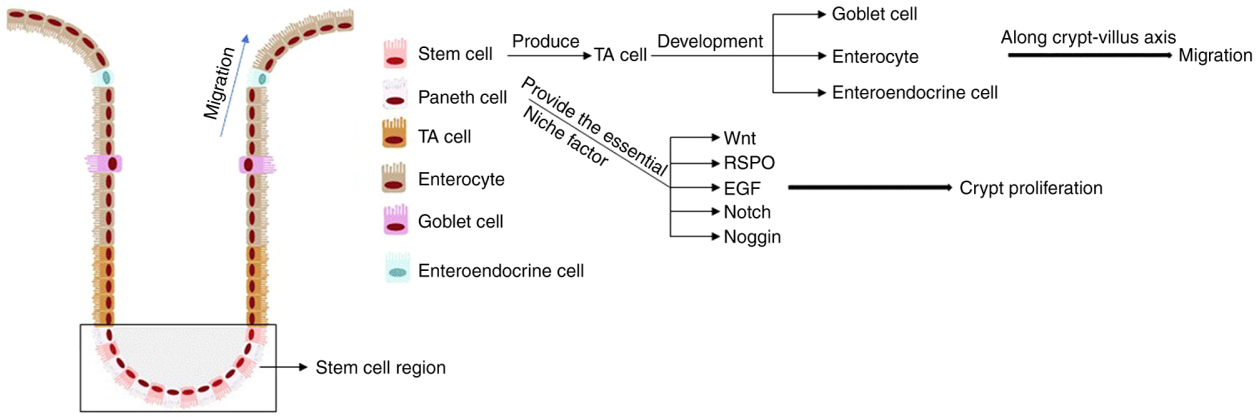

Figure 3. Composition of intestinal crypts. Intestinal stem cells initiate crypt renewal and generate TA cells, and Paneth cells provide essential niche factors [Wnt, RSPO, EGF, Notch, Noggin]. RSPO, R-spondin; EGF, epidermal growth factor; TA cell, transit-amplifying cell.

Organoid culture medium for normal human colon tissue was used with minor modifications for CRC organoid culture (Table I). There are currently no standardized methods for CRC organoid culture The current methods mainly involve the derivation of organoids on polystyrene-coated polydimethylsiloxane (PDMS) microporous matrix or on flat matrix gel. The former method is well suited for high-throughput clonal culture and subsequent retrieval of individual organoids. However, polystyrene-coated PDMS may impair organoid development. The latter method enables imaging and tracking of organoids, similar to the traditional 3D culture environment, which has the disadvantages of low throughput and poor reproducibility (13). Matrix is a prerequisite for cell growth, proliferation and differentiation. In addition, researchers have found that matrix gels are not suitable for high-throughput drug screening and developed type I collagen gels instead of matrix gels for organoid culture (62,63). The combination of patient-derived CRC organoids with the orthotopic transplantation model enables the tumor to grow in the natural colon environment of mice, more accurately simulating the development of CRC and liver metastasis (64). Researchers have successfully introduced mutations into organoids derived from normal intestinal epithelial cells to prepare CRC organoids for disease studies (56). Thanks to the significant improvements of methods in organoid cultures, CRC organoids have been widely applied in preclinical studies of CRC.

5. Applications of organoids for CRC

Currently, organoids are considered to be the best preclinical models, as they may maintain genetic diversity while being capable of long-term stable expansion, cryopreservation and simulation of the link between cell polarity and tissue changes during cancer progression. Organoid models have applications in the study of cell heterogeneity, the construction of disease models, drug screening, drug toxicity testing, the invention of new drugs and the practice of personalized medicine. With the combination of CRISPR-Cas gene editing technology, organoid models have become a superior and unique tool for analysing gene function (65). Intestinal organoids are the first successfully cultured organoids and are currently the most developed and well-researched organoids. Studies based on intestinal tumor organoids are also more extensive. The types of CRC studies that have been conducted using intestinal tumor organoids as models are summarized in Fig. 4 and limitations of the application of CRC organoids are discussed below.

6. Tumor heterogeneity in CRC organoids

Epigenetic and environmental factors are primary determinants of tumor heterogeneity which may be further subdivided into two main categories: Intratumor heterogeneity and intertumor heterogeneity (66). The cancer stem cell theory demonstrates the existence of intron clonal heterogeneity, which further complicates the heterogeneity of CRC tumors (67,68). In recent years, CRC organoids constructed from tissues of different parts of the same tumor or tissues of different malignancy degrees of the same tumor have largely maintained the characteristics of the original samples. These organoids have been studied by cancer genomics (41,69), multiregion sequencing (70), single-cell derived cloning (71,72) and single-cell sequencing (73,74) to reveal the heterogeneity of CRC.

Tumor heterogeneity is specifically responsible for the multiple capabilities and biological characteristics of the tumor, which make it more prone to metastasis, recurrence and drug resistance (75,76). Kim *et al* (77) performed whole-exome sequencing from a multiregion-generating organoid tumor model in 12 patients, revealing intraregional tumor heterogeneity in individual patients. They tested 24 drugs and found that tumors with high intratumor heterogeneity showed elevated expression of oncogenic features, thereby reducing their susceptibility to related inhibitors (77). Jeong *et al* (78) constructed organoid models for different sites of multifocal CRC, performed whole-exome sequencing, evaluated 25 medications to capture patients' intertumoral heterogeneity and identified clonal relationships between multifocal CRCs in terms of biological processes and therapeutic responses. After constructing primary and metastatic CRC organoids, transcriptomics and single-cell sequencing analysis revealed differences in cell composition between primary and metastatic foci (79). Using CRC organoids to study tumor heterogeneity can help reveal possible mechanisms of metastasis of CRC and its potential role in predicting the chemotherapeutic response

Table I. Comparison of intestinal organoid culture media.

First author, year	Reagent	Human			Mouse			(Refs.)
		Small intestine	Colon	CRC	Small intestine	Colon	Tumor	
Sato, 2011	Advanced DMEM/F12 (Basal medium)	+	+	+	+	+	+	(37)
Sato, 2011	HEPES	+	+	+	+	+	+	(37)
Sato, 2011	Glutamax	+	+	+	+	+	+	(37)
Sato, 2011	B-27 supplement	+	+	+	+	+	+	(37)
Sato, 2011; Grabinger, 2014; Ganesh, 2019	N2 supplement	+	+/-	+/-	-	-	-	(37-39)
Sato, 2011	N-acetyl-L-cysteine	+	+	+	+	+	+	(37)
Sato, 2011	Antibiotic	+	+	+	+	+	+	(37)
Sato, 2011; Ganesh, 2019	WNT3A conditioned medium	+	+	+/-	-	+	-	(37,39)
Sato, 2011; Ganesh, 2019	R-spondin-1 (Wnt agonists)	+	+	+/-	+	+	-	(37,39)
Sato, 2011; Ganesh, 2019; Fujii, 2015	Nicotinamide	+	+/-	+/-	-	-	-	(37,39,40)
Sato, 2011; Fujii, 2015	PGE2	+	+/-	+/-	-	-	-	(37,40)
Sato, 2011	EGF	+	+	+	+	+	+	(37)
Sato, 2011; van de Wetering, 2015	Gastrin	+	+	+/-	-	-	-	(37,41)
Sato, 2011; van de Wetering, 2015	FGF-10	+	+/-	+/-	-	-	-	(37,41)
Sato, 2011; Xie, 2016	Noggin (BMP inhibitor)	+	+	+/-	+	+	-	(37,42)
Sato, 2011	Y-27632 (ROCK inhibitor)	+	+	+	+	+	+	(37)
Sato, 2011	SB202190 (p38/MAPK inhibitor)	+	+	+/-	-	-	-	(37)
Sato, 2011	A83-01 (TGF-β type I receptor inhibitors)	+	+	+/-	-	-	-	(37)

CRC, colorectal cancer; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PGE2, prostaglandin E2; EGF, epidermal growth factor; FGF-10, fibroblast growth factor-10; BMP, bone morphogenetic protein; ROCK, Rho-associated kinase; MAPK, mitogen-activated protein kinases.

and clinical prognosis of patients (70,80). Furthermore, gene editing techniques have also been applied to study CRC organoids.

7. Application of organoids to simulate colorectal carcinogenesis

As previously mentioned, in addition to organoids that may be prepared from patients with CRC, with the development of gene editing techniques, organoids derived from normal intestinal epithelial cells with introduced mutations have been developed to model mutations at all stages of cancer *in vitro*. By using CRISPR-Cas technology, Matano *et al* (56) knocked out adenomatous polyposis coli (APC), SMAD4 and TP53 tumor suppressor genes and knocked in KRAS and phosphatidylinositol-4,5-bisphosphate 3-kinase in normal epithelial organoids, allowing five mutant organoids to be cultured in a medium free of ecological factors. In addition, these organoids were transplanted into the renal capsule and spleen of nude mice to form tumors. They were found to have different abilities of tumor formation, invasion and metastasis, but failed to colonize in the liver (56). Drost *et al* (57) used CRISPR-Cas to target APC, P53, KRAS and SMAD4 in human intestinal stem cells. They removed one growth factor from the culture medium to select mutated organoids and then transplanted triple/quadruple mutated organoids into immunodeficient mice to induce tumor growth. Both triple- and quadruple-mutant organoids showed high proliferation rates,

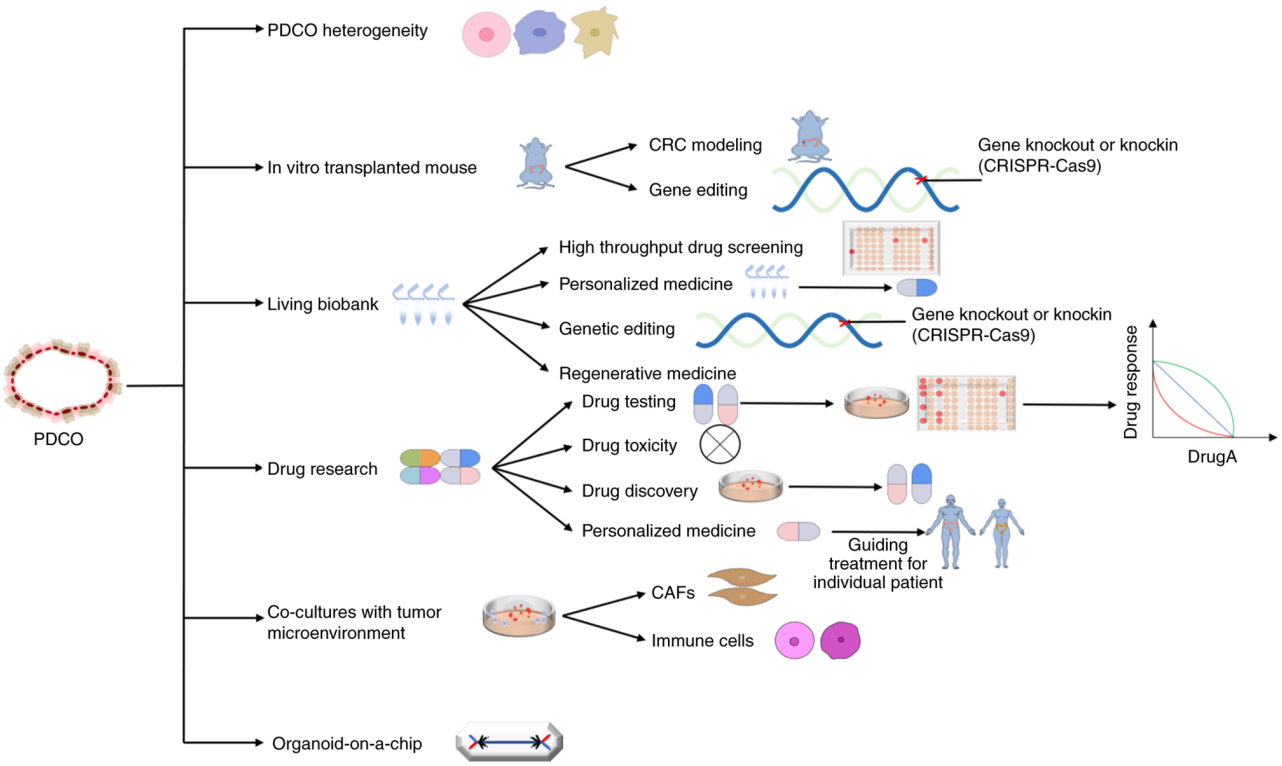

Figure 4. Schematic representation of the uses of normal and tumor organoids for CRC basic and clinical research. CRC, colorectal cancer; PDCO, patient-derived cancer organoids; CAF, cancer-associated fibroblast.

while only quadruple-mutant organoids appeared as solid tumor masses (57). Fumagalli *et al* (81) demonstrated that the continuous accumulation of oncogenic mutations in the Wnt, EGFR, P53 and TGF-β signalling pathways promoted effective tumor growth, migration and metastasis using an *in situ* transplantation model of human colon organoids with different combinations of CRC mutations. Roper *et al* (64) injected lentivirus into the intestinal mucosa of mice to generate CRC organoids of APC, P53 and KRAS and injected these organoids into the colon mucosa of mice to form an *in situ* transplantation model. After 12 weeks, 1/3 of the mice developed liver metastases. Human CRC organoids were then injected into the intestinal mucosa of mice and it was found that the *in situ* transplantation of patient-derived CRC organoids effectively mimicked both primary and metastatic human CRC (64). The establishment of a living biobank significantly facilitates the study of CRC organoids.

8. Establishment of a living biobank of CRC organoids

To date, researchers have established live organoids for various tumors, including locally advanced rectal cancer (82), CRC, liver metastasis of CRC (80), breast cancer (83), bladder cancer (84), nasopharyngeal cancer (85), pancreatic cancer (86), non-small cell lung cancer (87), gastric cancer (88) and glioblastoma (89). For the first time, van de Wetering *et al* (41) successfully established a living biobank for CRC, including 22 tumor organoids and 19 normal adjacent organoids from 20 patients. Genomic analysis has indicated that organoids may display the genomic characteristics of primary colon cancer and mutation analysis has demonstrated that the spectrum of genetic changes in organoids was highly consistent with the large-scale mutation analysis of CRC (41). Vlachogiannis *et al* (90) used 110 fresh biopsy samples from 71 patients in four prospective phase I/II clinical trials to construct a living CRC organoid biobank. Histological evaluation revealed significant morphological similarities between organoids and patient-derived tissues. Organoid sequencing, along with drug responses, demonstrated a high degree of similarity in organoid phenotypes and genotypes to tumors derived from the patient. Molecular profiling of organoids is matched with the results of drug screening, which may predict the clinical response of patients to various medications (90). These results suggest that, compared with traditional biobanks, organoids may better simulate the primary tumor and reduce tumor heterogeneity. It may be used for preclinical high-throughput drug screening, enabling patients to undergo drug testing without participation, screening appropriate medications for patients, forecasting patients' responses to drugs and providing more possibilities for individualized treatment of tumor patients. In addition, organoid biobanks can also be used for gene editing and other research, novel drug development or regenerative medicine (91).

9. Organoids in the treatment of CRC

The advantages of using patient-derived organoids (PDOs) as preclinical models have been extensively studied over the last few years, particularly in four settings: i) Drug screening, ii) drug toxicity testing, iii) drug discovery and iv) individualized therapy.

Drug screening. How to determine the sensitivity of patients to chemotherapeutic drugs is critical to patient treatment and it has been proven that CRC organoids are valuable in individualized drug sensitivity tests (92). Kong *et al* (93) developed organopharmacogenomic data and a web-based calculation method to accurately predict drug response in 144 patients with CRC receiving 5-fluorouracil (5-FU), and they found that the expression levels of components of the 'BH3-only protein' pathway correlated with the sensitivity of CRC organs to 5-FU. Pasch *et al* (94) verified the response of CRC organoids to chemotherapy with different concentrations of 5-FU and oxaliplatin. The results indicated that multiphoton imaging was used to prospectively predict the response to FOLFOX chemotherapy in a patient with metastatic CRC receiving FOLFOX (5-FU, leucovorin and oxaliplatin) chemotherapy, identifying those who did not respond to standard chemotherapy for CRC. Ooft *et al* (95) used 67 colon cancer organoids to accurately predict the drug response of >80% of patients receiving irinotecan. However, they failed to predict the outcome of 5-FU and oxaliplatin, and failed to identify patients who may benefit from treatment. Chen *et al* (96) administered different concentrations of 5-FU and oxaliplatin to CRC organoids to investigate drug susceptibility and the test data were consistent with clinical data. Then, through single-cell sequencing technology, it was speculated that driver genes Stathmin 1, vascular endothelial growth factor A and N-myc downstream-regulated gene 1 and transcription factors [E2F transcription factor 1, breast cancer susceptibility gene 1, MYB proto-oncogene like 2, caudal-type homeobox 1 (CDX1) and CDX2] were potential oxaliplatin resistance targets (96). Lv *et al* (97) used locally advanced rectal cancer organoids to observe whether the sensitivity of irinotecan could predict complete response and survival. The results suggested that patient-derived locally advanced rectal cancer organoids are able to predict response to irinotecan-based neoadjuvant chemoradiotherapy (97).

Toxicity studies. Drug toxicity is a major cause of failure and withdrawal of effective potential drug candidates from the pharmaceutical pipeline (98). Compared to other preclinical models, patient-derived tumor organoids better mimic the original tumor tissue and their toxic response to drugs can help predict drug safety for patients. It also allows the study of drug effects in mice and the subsequent generation of normal and tumor organoids to evaluate their potential systemic toxicity and prioritize their future preclinical assessment (99,100).

Lu *et al* (101) successfully used Ugt1 locus in intestinal epithelial cells ($Ugt1^{\Delta IEC}$) to increase apoptosis in organoid cultures of the small intestine of mice treated with camptothecin-11. Schnalzger *et al* (102) established a quantitative platform for chimeric antigen receptor (CAR)-mediated cytotoxicity to patient-derived CRC organoids and demonstrated that the cytotoxicity of CAR NK-92 can effectively target tumor organoids, providing a valuable tool for the development of novel immunotherapies. Park *et al* (103) found that butyrate may be used as a radiosensitizer. They used the CRC organoid test and found that butyrate was able to protect the normal mucosa, while improving the efficacy of radiotherapy and minimizing the toxicity associated with radiotherapy and that butyrate did not increase radiation-induced cell death or improve the regenerative ability of normal organoids after exposure to radiation (103). De Oliveira *et al* (104) used CRC organoids to test the cytotoxic effects of KAN0438757, a novel 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 inhibitor. They found that KAN0438757 had a significant effect on tumor organoid growth, while normal colon organoids were unaffected (104).

Drug discovery. CRC organoids and living organoid biobanks can provide an accurate summary of the heterogeneous genetic and morphological composition of cancer cells in the initial tumor, and their responses to drugs are highly consistent with patients' clinical responses, providing great hope for the development of novel drugs (105).

Rae *et al* (106) summarized the application of organoids in anticancer drug discovery. Plocabulin is a novel microtubule-destroying marine biology-derived antitumor agent, whose antitumor effects may be tested using CRC organoids. It was indicated that plocabulin is more cytotoxic than the active derivative of irinotecan, SN38, providing additional data for clinical trials and helping to accelerate drug development (107). Zerp *et al* (108) used CRC organoids to investigate the tumor cell killing effect of the second-generation TRAIL agonist APG-880 combined with radiation and the results suggested that this drug has a promising role as a tumor therapeutic. Norkin *et al* (109) developed a high-throughput platform for targeted organoid-sequencing ('TORNADO-seq') for drug discovery in CRC. This platform uses targeted RNA-Seq to monitor the expression of a wide range of genetic markers, isolates drugs rich in differentiated cell phenotypes that can induce differentiation of intestinal wild-type organoids and cancer organoids and are highly effective against CRC (109).

Individualized therapy. Personalized medicine is an evolving concept in oncology. The goal is to determine the most appropriate treatment based on the genetic, transcriptome, environmental and lifestyle features of each patient (110). Research has indicated that personalized medicine may achieve significant improvements in treatment response (111,112). The high consistency between the organoid model and the original tumor in terms of morphology, genotype and mutation characteristics, as well as physiological and pathological transformation, makes it possible to achieve precise personalized therapy.

CRC organoids may be used to predict the clinical outcomes of CRC. Yao *et al* (82) studied the ability of rectal cancer organoids to predict tumor patients' responses to chemoradiotherapy and confirmed that the clinical response of rectal cancer organoids to chemoradiotherapy was highly similar to that of the primary tumor. Hsu *et al* (113) constructed CRC organoids from normal colon, adenoma or tumor tissues of neoadjuvant patients to predict the radiosensitivity of colorectal organoids. The study found that following the development of the normal lining of the colon mucosa into benign tumors, it was able to maintain the colon's inherent resistance to radiation after transformation to colorectal adenocarcinoma. However, CRC develops radiosensitivity at the stage of adenoma to adenocarcinoma transition. A large proportion of patients develop high radiological susceptibility (113). Cho *et al* (114) established organoids in 54 patients with CRC who had never received any treatment (except for one patient) and developed an 'organoid

score' to quantify the comparison between organoids and patients' response to drugs. It was found that patients with higher organoid scores had a worse prognosis than those with lower organoid scores. This method is helpful in predicting the effects of anticancer therapy. To determine the next treatment plan for drug screening, Geevimaan *et al* (115) established a living organoid biobank from patients with advanced CRC and divided organoids into a drug-resistant group and a sensitive group according to their response to oxaliplatin. These groups were compared with patients receiving oxaliplatin treatment. The results indicated that oxaliplatin-sensitive organoids were consistent with patients' responses to oxaliplatin treatment (115). Increasing evidence indicates that patient-derived organoid response to chemoradiotherapy in the clinic is consistent with that of the primary tumor, so the organoid response to chemoradiotherapy in CRC may become a new therapeutic tool (39,116).

In general, the use of organoids to guide the personalized treatment of CRC has promising applications. The establishment of the CRC organoid biobank broadens the diagnosis and treatment vision of CRC, which is helpful in promoting the implementation of personalized diagnosis and treatment.

10. Limitations of CRC organoids and solutions

Organoids are powerful tools for studying human development and disease. However, there are still numerous limitations and challenges.

Single-cell sequencing may provide novel solutions to the question of whether organoids may serve as a true model of human biology, whether organoid models need to be optimized and whether organoids may be used in basic biology (genetic and pharmacological perturbations) and biomedical research (drug development and personalized medicine). Studies have developed a suspension culture technique for cancer organoids. This technique simplifies organoid cell culture and extends organoid applications to include routine use in large-scale perturbation screening (117,118). The efficiency of CRC organoids generation still needs to be further studied.

The success rate of CRC organoid creation is high; however, there is a need for improved efficiency, reduced time and decreased costs associated with organoid generation. One of the main problems is the limited number of cancer cells contained in biopsies. The success rate of culture may be further improved by obtaining multiple biopsies and direct evaluation by pathologists (119). CRC organoids with different histological subtypes have been described to be successfully constructed and clinical parameters, such as tumor size or presence of ulcers, were not associated with the occurrence of contamination (55,80,120). However, the establishment of organoids from tumors with rare CRC histologic subtypes (e.g., poorly differentiated adenocarcinoma, mucinous adenocarcinoma and neuroendocrine carcinoma) is more difficult (33). Furthermore, a limitation of the application is organoid culture.

The organoid culture of CRC is relatively expensive and the Wnt3A conditioned medium is not suitable for long-term storage. As a result of acylation between Wnt and Fzd interactions, the hydrophobic Wnt protein needs to be purified. Janda *et al* (121) developed water-soluble Wnt agonists to replace Wnt3A and they established an economical and clear recombinant culture reagent. Traditional organoid generation methods produce organoids with a closed sac-like structure and uncontrolled self-organization formation, which limits the lifespan, morphology, experimental operation and homeostasis of organoids. The different morphologies of organoids may lead to different responses to drug therapy. To solve this problem, researchers use bioengineering technology to control the shape of organoids (122,123). Most of the Matrigel used for organoid culture is of animal origin. Matrigel is a basal membrane extract purified from mouse sarcoma cells by Engelbreth-Holm-Swarm. However, ECMs of animal origin are characterized by batch-to-batch variability and lack of tissue-specific ECM components (124-126). To overcome the limitations of ECMs of animal origin, hydrogels are being developed to replace Matrigel and BME (127-129). The establishment of living organoid biobanks still needs to be further studied.

Not all organoid biobanks maintain tumor heterogeneity in individual patients and the reason why organoids reproduce tumor heterogeneity in individuals is unknown (130). There are many limitations to the establishment of living organoid biobanks in CRC. First, the generation efficiency of the organoid biobank is low, which may be caused by: i) Pollution of tumor organoids (overgrowth of normal organoids or tissue sample contamination); ii) optimization of medium formula; iii) few tumor cells; and iv) quality control problems. Second, the coculture system with other cell types is not clearly defined, so it is difficult to reproduce the tumor microenvironment. Third, standardized procedures for living organoids have not been defined. Fourth, there are ethical issues, such as informed consent, commercialization, and the manufacture and safety of patentable products (131-134). The toxicity of drugs to the liver is often overlooked.

Researchers commonly utilize normal intestinal organoids as models for studying drug toxicity. However, it is essential to recognize and address the potential role of liver toxicity in chemotherapy, which is often ignored by these studies (104,135,136). Combining normal liver and intestinal organoids with CRC organoids from patients in a single organoid microarray system may provide a better understanding of drug toxicity (137).

Another important limitation of organoid models is that they usually contain only tumor epithelium, which cannot fully reproduce the diversity of cell types in the tumor microenvironment (including nonvalvular cells, such as immune cells and stroma), and the heterogeneity of cell types in the tumor microenvironment has an important impact on the development and treatment of tumors (138,139). Therefore, numerous studies have attempted to develop next-generation tumor organoids by cocultivation with nonepithelial stromal cells, such as cancer-associated fibroblasts (CAFs), enteric nervous system cells, endothelial cells and immune cells. However, a holistic view of these cells *in vivo* is still lacking, limiting their ability to fully mimic the primary tumor. It is difficult to determine the therapeutic efficacy of individual cell populations (140,141).

11. Coculture with fibroblasts

CAFs are a major part of the tumor microenvironment and successful implementations of a coculture system of organoids

and fibroblasts for CRC have been reported. Mosa *et al* (142) constructed a WNT-independent CRC organoid-inflammatory CAF coculture model and found that EMT markers were significantly upregulated, while cancer-associated myofibroblasts restored this phenotype. The results suggested that tumor growth and malignancy are modulated differently by different fibroblast subtypes under the influence of Wnt signalling (142). Naruse *et al* (143) constructed a coculture system of CRC organoids and CAFs, and performed sequencing that showed that the coculture system of CRC organoids and paired CAFs was able to partially replicate the tumor microenvironment. In addition, Luo *et al* (128) found that CAFs were able to maintain the proliferation of CRC organoids cultured in hydrogels and restore the unique biological pathway that was present not only in organoid cultures but also in patient tissues.

12. Coculture with immune cells

The interaction of immune cells with tumors in the tumor microenvironment was examined by coculturing normal or tumor intestinal epithelial organoids with immune cells. Noel *et al* (144) established the first human macrophage-intestinal organic-coculture system to elucidate human innate immune processes and cellular communication, and to examine host-pathogen interactions by accurately representing what happens in human intestinal epithelial cells. Neal *et al* (145) used the liquid-gas interface method to reconstruct the tumor microenvironment and successfully retained a variety of immune cells except for T cells in the organoid culture system. Single-cell sequencing and immunohistochemical analysis demonstrated that organoid tumor-infiltrating lymphocytes accurately retained the original tumor T-cell receptor profile and successfully mimicked immune checkpoint blockade. The organoid-based global proliferation of primary tumor epithelial cells and endogenous immune stroma should enable immuno-oncology studies in tumor microenvironments (145). Dijkstra *et al* (146) constructed an organoid-autologous T-cell coculture system for CRC with mismatch repair defects and found that the system amplified tumor-reactive T cells, providing a possibility for the generation of patient-specific T-cell products. T cells may be used to evaluate the killing efficiency of matched tumor organoids and to allow the establishment of an *in vitro* testing system for T-cell-based immunotherapies at the individual patient level (147). Frenkel *et al* (148) studied the interaction between lymphatic vessels and CRC by coculturing lymphatic endothelial cells (LECs) with CRC organoids and found that LECs formed a permeable vascular structure on the extracellular matrix, resulting in a longer and more stable lifespan of the cocultivation model.

13. CRC organoids on a chip

Patient-derived CRC organoids are usually closed sacs and lack the tissue-tissue interface between epithelial tumor cells and the surrounding vascularis and stroma, which are crucial for cancer control and progression. It is challenging to provide organoid nutrients, supply oxygen and remove waste. Therefore, bioengineered devices or scaffolds are needed to reconstruct organoids that are more representative of the internal environment (149).

Organ chips, also referred to as microfluidic cell culture devices, are developed from microfluidic chips and contain continuously perfused microcavities, in which live cells are used to simulate the functional units of human tissues and organs *in vitro*. In recent years, organ chip technology has been greatly developed, and currently, organ chips of different structures may be constructed, providing a new method for drug screening (150,151). Organoid technology is combined with chip technology to form organ-on-a-chip technology. Combining the advantages of these two technologies, organ-on-a-chip may be used as a more predictive preclinical model that is widely applicable to drug discovery, personalized medicine and regenerative medicine (152,153). However, at present, microfluidic organ chips mostly rely on organoids formed from human pluripotent stem cells, and there are few studies on organ chips for CRC (154,155).

Organ chips open up numerous possibilities for the use of microfluidic devices, such as specifying the shape of organoids, coculture of CRC organoids with tumor microenvironments and drug screening (156). Gjorevski *et al* (122) used microfluidics to control the initial shape of intestinal organoids, helping to control the self-organization of organoids. Frenkel *et al* (148) injected immortalized lymphatic endothelial cells into a microfluidic chip with an independent extracellular matrix to form a perfectible vascular structure, and introduced mouse colon cancer organoids into lymphatic vessels to establish a stable coculture model for studying lymphatic vessel formation and tumor cell metastasis. Rajasekar *et al* (157) successfully designed a microfluidic platform for infusion-vascularized colon organoids that may guide organoid development without the use of physical structures to artificially define and limit biological structure and remodelling, and this system is expected to be used for CRC organoids. Pinho *et al* (158) found that, compared with conventional PDO culture, no significant differences were verified in the organoid response to 5-FU treatment on-chip and on-plate. However, the size and efficiency of colony formation of the organoid were significantly increased.

14. Conclusion

CRC is a worldwide health concern. The lack of good *in vitro* models has been a limitation to improving clinical treatment for a long time. Organoid technology has become a new strategy to solve this issue and may further deepen the understanding of the occurrence and development of CRC. Organoid models, which may be combined with biobanks, CRISPR, high-throughput screening, chip technology and xenotransplantation, can make a significant contribution to the further development of organoids. A promising correlation exists between CRC organoids and patient responses to cancer treatment.

Although CRC organoids have shown some limitations and gaps in their application to cancer modelling and personalized medicine, with the continuous optimization of organoid technology, CRC organoid models will become an indispensable tool for preclinical and clinical research.

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Authors' contributions

CY, WWX and RW were involved in the conception, writing and editing of the manuscript. YH, KY, XS, GHW and XHX critically contributed to the drafting of sections falling within their expertise, and reviewed and corrected the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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