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Next-Generation Modeling of Cancer Using Organoids

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In the last decade, organoid technology has become a cornerstone in cancer research. Organoids are long-term primary cell cultures, usually of epithelial origin, grown in a three-dimensional (3D) protein matrix and a fully defined medium. Organoids can be derived from many organs and cancer types and sites, encompassing both murine and human tissues. Importantly, they can be established from various stages during tumor evolution and recapitulate with high accuracy patient genomics and phenotypes in vitro, offering a platform for personalized medicine. Additionally, organoids are remarkably amendable for experimental manipulation. Taken together, these features make organoids a powerful tool with applications in basic cancer research and personalized medicine. Here, we will discuss the origins of organoid culture, applications in cancer research, and how cancer organoids can synergize with other models of cancer to drive basic discoveries as well as to translate these toward clinical solutions.

Cancer is one of the leading causes of mortality and morbidity in the world (Sung et al. 2021). It is a highly heterogeneous disease, arising from different organs and harboring complex genomic and phenotypic landscapes that are unique to each cancer case. The complexity of cancer has been difficult to model using traditional preclinical models of cancer research. Cancer cell lines grown in vitro are rapid, but in general are highly clonal and poorly recapitulate in vivo phenotypes and genomics (Masters 2000). Patient-derived tumor xenografts (PDTXs), in which patient biopsy specimens are grafted into immunocompromised mice, are an improvement from cancer cell lines (Bleijs et al. 2019). However, PDTXs carry a high financial burden and are time and labor consuming. PDTXs can offer insight into tumor–stromal interactions by mimicking cancer growth in vivo but cannot effectively model tumor–immune interactions (Aparicio et al. 2015). Lastly, PDTXs can undergo mouse-specific tumor evolution, thus reducing chances of successful translation to the clinic (Ben-David et al. 2017). Genetically engineered mouse models (GEMMs) of cancer have been pivotal in advancing cancer research by enabling the study of cancers in their native immune-competent environment in a controlled genetic background (Dranoff 2012; for review, see Murphy and Ruscetti 2023). Although extremely powerful, the generation of GEMMs is costly and time consuming. Additionally, not all human cancers are

Editors: Katerina Politi and Cory Abate-Shen
Additional Perspectives on Modeling Cancer in Mice available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a041380
Cite this article as *Cold Spring Harb Perspect Med* 2024;14:a041380

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currently recapitulated by GEMMs (Bleijs et al. 2019), and there are notable differences in the human and the mouse genome (Yue et al. 2014), immune system (Mestas and Hughes 2004; Bjornson-Hooper et al. 2022), and transcriptional regulation (Odom et al. 2007; Lin et al. 2014).

Organoids, a recent advance in three-dimensional (3D) cell culture technologies, resolves several of the constraints of classical cancer models. Organoids are primary cell culture models grown in a 3D protein matrix, which mimics the mechanical and chemical properties of the extracellular matrix (ECM) by providing key signaling cues via basement membrane ligands such as nidogens, type IV Collagen, Laminin, and heparan sulfate proteoglycans (Ma et al. 2021), which is overlayed with a defined medium. They can be derived from adult tissues or generated via directed differentiation of induced pluripotent stem cells; although these types of organoids will not be discussed in detail in this review, they are discussed in McCauley and Wells (2017). Additionally, organoids can be seeded in an air–liquid interface coculture with mesenchymal stromal cells acting as a growth factor source, although this method will not be discussed in detail in this review (Ootani et al. 2009). Organoids can be established from benign and malignant cells from a wide variety of both murine and patient-derived tissues including but not limited to the small intestine (Sato et al. 2009), colon (Sato et al. 2011), pancreas (Huch et al. 2013; Boj et al. 2015), liver (Huch et al. 2015; Nuciforo et al. 2018), stomach (Barker et al. 2010), mammary gland (Sachs et al. 2018), endometrium (Boretto et al. 2019), prostate (Chua et al. 2014; Gao et al. 2014; Karthaus et al. 2014), and salivary gland (Maimets et al. 2016). Organoids self-organize to recapitulate the organ structure from which they originate, and, importantly, organoids can be expanded and passaged long term without immortalization and remain genetically stable (Clevers 2016).

The first organoid culture was established from the small intestine, an organ with rapid turnover and a well-defined adult stem cell (ASC) population (i.e., a somatic cell with the capacity to self-renew and give rise to the terminally differentiated cells of the organ in which they reside), marked by leucine-rich-repeat-containing G-protein-coupled receptor 5 (LGR5) (Barker et al. 2007). Single $Lgr5^+$ small intestinal stem cells were able to generate all cell types and generated crypt and villus like domains in vitro using three factors, epidermal growth factor (EGF), roof plate-specific spondin (R-SPONDIN), and Noggin, an inhibitor of bone morphogenic protein (BMP) signaling. This EGF/NOGGIN/R-SPONDIN (ENR) combination effectively replaced the mesenchymal niche and allowed for "indefinite" expansion of $Lgr5^+$ stem-cell-derived organoids (Sato et al. 2009). Based on ENR, many different organoid culture methods have been developed.

Remarkably, most organoids rely on a similar combination involving activation of WNT signaling through WNT and R-SPONDIN ligands, signaling through receptors of the ERBB and fibroblast growth factor receptor (FGFR) family members, inhibition of BMP and TGF-β signaling, signaling by the eicosanoid prostaglandin E2 (PGE_2), nicotinamide, a vitamin B3 precursor thought to increase NAD^+ levels to aid catabolism but also a SIRTUIN inhibitor (Bitterman et al. 2002; Avalos et al. 2005), and organoid-specific requirements. Additionally, to improve establishment efficiency from single cells, rho kinase inhibitor is added to cultures to inhibit anoikis (Kretzschmar and Clevers 2016), as well as a p38 MAPK inhibitor to prevent activation of cell stress responses (Jung et al. 2011). Notably, many organoid culture methodologies are still being refined (Fujii et al. 2018; Karthaus et al. 2020; He et al. 2022). Medium optimization is of particular importance for human organoids as it is more complex compared to murine medium composition, and the interplay between signaling pathways and their effects on organoid culture is not yet fully understood often resulting in suboptimal organoid conditions.

Organoids derived from organs with rapid turnover and well-defined ASCs, like the small intestine, colon, and stomach, closely resemble in vivo cellular hierarchies and dynamics. However, in organs with slow turnover and unclear cellular hierarchy, such as the liver, pancreas, and prostate, organoid culture mimics a regenerative response. Indeed, mesenchymal-derived growth

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factors involved in androgen-driven prostatic regeneration strongly overlap with the prostate organoid culture condition (Karthaus et al. 2020). To achieve full differentiation in these "regenerative" organoids, culture conditions often need to be adapted by exchanging or removing key mitogens (Huch et al. 2015).

Cancer organoid cultures are grown in similar medium conditions; however, depending on mutational status, some cancer organoids can be cultured in the absence of growth factors. For instance, colorectal cancer (CRC) organoids harboring mutations hyperactivating cell-autonomous WNT signaling often do not require WNT or RSPONDIN ligands for growth (Sato et al. 2011). In other cases, cancer organoids can be enriched by selectively killing benign cells, exemplified by the selection of P53-mutant cells by addition of the MDM2-stabilizing drug nutlin-3 (Drost et al. 2015) and RB-mutant cells by the CDK4/6 inhibitor palbociclib (Chan et al. 2022). These selectable qualities allow for robust establishment of cancer organoids.

Cancer organoids have been established from many sources including primary tumors (Broutier et al. 2017), metastatic lesions (Weeber et al. 2015), circulating tumor cells (Gao et al. 2014), and ascites fluid (Seino et al. 2018). Importantly, cancer organoids accurately recapitulate the genomics, phenotypes, and cellular heterogeneity observed in vivo.

In summary, organoids retain key aspects of in vivo biology, but have the flexibility and accessibility of an in vitro system. Organoids have been used in a myriad of ways to drive basic and translational cancer research summarized in Figure 1. In this review, we will explore several applications of organoids in translational and basic cancer research, discuss the strengths and weaknesses of this methodology, and offer a future perspective.

APPLICATIONS OF ORGANOIDS IN BASIC CANCER RESEARCH

Modeling Cancer Initiation and Progression in Organoids

Using the rapidly expanding clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 toolbox (for review, see Sanchez-Rivera and Dow 2023), deletions, point mutations, and translocations have been engineered into organoids. Delivery of these gene-editing tools can be achieved using lentiviral transduction with high efficiency (up to 70%) (Koo et al. 2011; Karthaus et al. 2014; Chan et al. 2022); additionally transient expression can be achieved using electroporation albeit at lower efficiency (1%–5% of organoids edited) (Table 1; Drost et al. 2015; Matano et al. 2015; Fessler et al. 2016).

CRISPR-Cas9-mediated genetic editing has been used to model critical steps of tumor initiation of CRC (Drost et al. 2015; Matano et al. 2015; Fessler et al. 2016), cholangiocarcinoma (Artegiani et al. 2019), pancreatic cancer (Seino et al. 2018), mammary cancer (Dekkers et al. 2020), and prostate cancer (PCa) (Karthaus et al. 2014) in organoids. Drost et al. (2015) sequentially engineered *APC*, *KRAS*, *TP53*, and *SMAD* mutations in benign human colon cells, creating an isogenic system that generated invasive colorectal carcinoma when xenotransplanted. Using a similar strategy, Matano et al. (2015) edited *PIK3CA* mutations in addition to the aforementioned alterations to produce nonmetastatic colorectal carcinomas. Strikingly, macrometastases were generated by one edited line, which displayed spontaneous chromosomal instability (CIN), indicating that the complexity of cancer was not fully recapitulated by the induced genetic edits. An important observation noted across edited organoids was the stepwise acquisition of independence of growth factors and niche factors (i.e., APC mutation leading to hyperactivation of the WNT pathways allowed mutant organoids to survive in the absence of WNT-signaling activating ligands and WNT and R-SPONDIN), allowing for efficient selection for edited cells by removal of niche factors from the medium, reminiscent of organoid establishment from in vivo cancers.

Adams et al. (2019) studied the effects of mutations in the Wing2 domain and the DNA contact residue R219 of the pioneer transcription factor FOXA1 that are frequently found in PCa (Robinson et al. 2015) in an isogenic organoid system. When introduced, the chromatin accessibility changed drastically, and each mutation

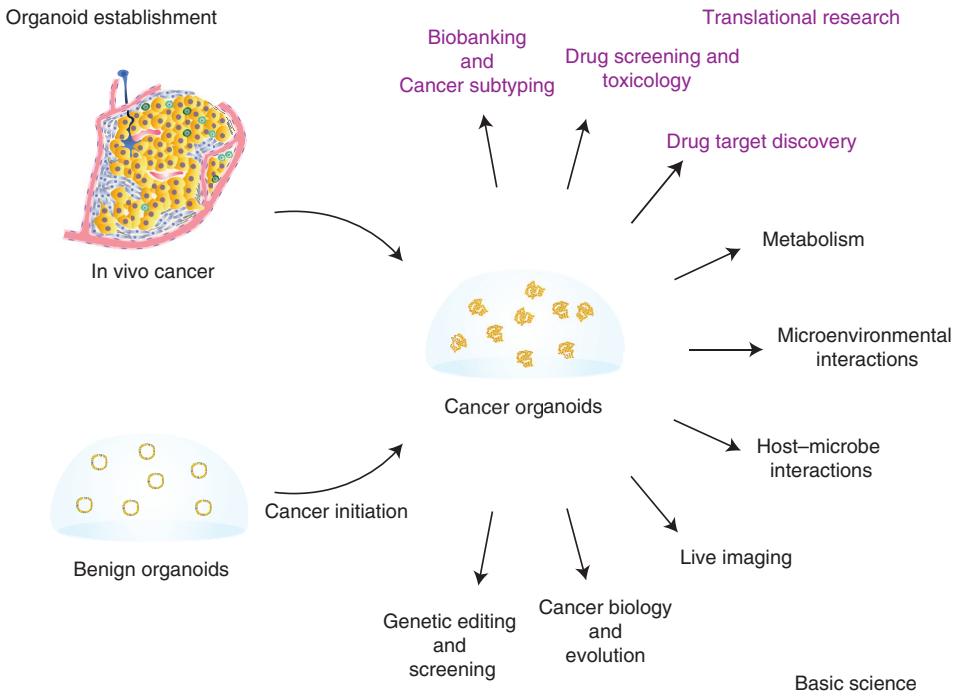

Figure 1. Applications of organoids in basic and translational cancer research. Cancer organoids can be derived from two sources. They can be directly established from in vivo human and murine cancers, or organoids derived from healthy tissue can be transformed using genetic editing tools such as clustered regularly interspaced palindromic repeats (CRISPR)-Cas9. Cancer organoids are highly amendable for experimentation and have been used in a myriad of ways in basic research to study fundamental questions in cancer biology concerning tumor initiation, evolution, and microenvironment. As cancer organoids derived from patient material recapitulate genomics and phenotypes of their in vivo counterparts, they also hold great promise as a tool in translational science. Currently, several biobanks of patient-derived organoids have been established, capturing the spectrum of cancer genomic and phenotypic heterogeneity, thus creating a platform for drug discovery, compound screening, and toxicity, which can be directly translated to a clinical application.

induced a unique alteration in chromatin accessibility profile. Additionally, the phenotypes of the organoids changed, with the majority of *FOXA1* mutations driving a hyperluminal, androgen receptor (AR)–positive phenotype. Interestingly, the R219S mutation drove a nonluminal phenotype, corresponding to the observation that this mutation is enriched in patient samples of castration-resistant prostate cancer (CRPC) with neuroendocrine differentiation.

In summary, these examples show that organoids can provide a great platform to study a variety of cancer-associated mutations, including deletions, amplifications, translocations, and point mutations, in an isogenic system. Additionally, they allow for rapid evaluation of potential novel oncogenes and tumor-suppressor genes and noncoding DNA regions. For example, using CRISPR-Cas9, Li et al. deleted a DNA response element for the Ets-related gene (ERG) from the *Trp63* promoter region in murine-derived tumor organoids (MDTOs). This led to the discovery that the *TMPRSS2-ERG* fusion gene, a common PCa oncogene, directly suppresses basal cell fate in PCa through *TP63* gene repression (Li et al. 2020).

Apart from modeling single mutations, cancer organoids have also been used for high-throughput genetic screening using CRISPR-Cas9 and genome-wide sgRNA libraries (Michels et al. 2020; Ringel et al. 2020). Ringel et al. performed a forward genetic screen with a genome-

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Table 1. Overview of development key applications of organoids in cancer research

Application	Significance	References
Lineage tracing	Tracking of cancer stem cells in patient-derived tumor organoids (PDTOs) reveals colorectal cancer (CRC) tumor growth is driven by $Lgr5^+$ cells via self-renewal or differentiation, and the induced ablation of $Lgr5^+$ cells is not curative. Administration of an anti-epidermal growth factor receptor (EGFR) antibody concurrent with $Lgr5^+$ cell deletion prevents tumor growth.	Shimokawa et al. 2017
	Genetically engineered human CRC organoids with a fluorescent reporter for the persistent cell state are used to identify dormant cancer cells, which remain in a dormant state until cessation of therapy.	Ohta et al. 2022
CRISPR-Cas9 screening	Genome-wide clustered regularly interspaced palindromic repeats (CRISPR) screen in wild-type (WT) and APC-mutant human intestinal organoids identifies genetic and epigenetic alterations, which mediate TGF-β resistance.	Ringel et al. 2020
Organoid cocultures	Identification of the mechanism by which CRC tumor cells acquire immune-specific markers in CRC organoid cocultures, which contribute to the immunosuppressive tumor microenvironment.	Shin et al. 2021
	Cocultures of engineered chimeric antigen receptor (CAR-natural killer (NK) cells and human CRC organoids reveals efficient on-target antitumor effects of EGFR-VIII targeting CAR-NK, while other putative CAR-NK cells showed significant cytotoxicity in healthy organoids—demonstrating the utility of preclinical models in evaluating cytotoxicity.	Schnalzger et al. 2019
	Prolonged microinjection of genotoxic pks^+ *Escherichia coli* into colorectal organoids induces a unique mutational signature found in a cohort of CRC patients, demonstrating for the first time the role of this bacteria in inducing oncogenic mutations.	Pleguezuelos-Manzano et al. 2020
Drug screening	Large-scale functional screen of dual targeting bispecific antibodies in patient-derived CRC organoids identifies candidate therapies that specifically target an $LGR5^+$ cancer stem cell subset that does not respond to current CRC immunotherapies.	Herpers et al. 2022
	Drug responses of PDTOs derived from heavily pretreated metastatic gastrointestinal cancer patients predict clinical outcomes with high accuracy.	Vlachogiannis et al. 2018
Monitoring natural tumor evolution	Fluorescent tagging of the *HIST1H2BC* gene in CRC organoids enabled live tracking of chromosomal evolution at the single-cell level, revealing the intermittent and gradual evolution of the CRC genome.	Bollen et al. 2021
	Temporally induced deletion of *Trp53* and *Rb1* in murine prostate organoids reveals the mechanism by which these cells acquire plasticity, and pharmacological targeting of genes up-regulated during progression to the plastic state resensitizes organoids to growth-inhibiting therapies.	Chan et al. 2022

Continued

Table 1. *Continued*

Application	Significance	References
Cancer subtyping	Genomic and epigenomic evaluation of several prostate cancer PDTOs uncovers further complexity of prostate cancer subtypes and reveals potential drug targets for a group of advanced cases.	Tang et al. 2022
	Identification of functional differences between prognosis-predictive gene-expression signatures in pancreatic ductal adenocarcinoma (PDAC) subtypes via single-cell sequencing and cancer-associated fibroblast (CAF)-PDAC PDTO cocultures.	Seino et al. 2018
Generation of lineage reporter organoids	Introduction of lineage reporters using nonhomologous end joining (NHEJ).	Artegiani et al. 2020
	Introduction of lineage reporters using homologous recombination.	Sun et al. 2021
	Introduction of lineage reporters using in-trans paired nicking (ITPN).	Bollen et al. 2022

wide sgRNA screen to identify genes involved in TGF-β-mediated growth suppression in wild-type and APC mutant adenoma organoids. By withdrawing the ALK inhibitor A83-01 and adding recombinant TGF-β, organoids harboring sgRNAs that drive resistance to TGF-β-mediated growth selection could efficiently be selected. The authors identified sgRNAs targeting several components of the SWI/SNF complex enriched in TGF-β-treated organoids, finding a crucial role for this chromatin-remodeling complex in evasion of growth suppression (Ringel et al. 2020).

Additionally, although it has not been done yet, as organoids closely resemble the in vivo cellular architecture, CRISPR-Cas9 in vitro screens can be coupled with a microscopy-based readout to identify regulators of multicellular behavior and cellular patterning in cancer organoids.

Next to the more conventional use of CRISPR-Cas9 in organoids, fluorescent reporters can be introduced readily into endogenous loci to create high-fidelity reporters of cellular lineages and signaling events. Initial protocols relied on CRISPR-Cas9-mediated nonhomologous end joining (NHEJ) or homologous recombination of introduced DNA templates carrying homology arms and subsequent clonal outgrowth of organoids (Artegiani et al. 2020; Sun et al. 2021). Recently, reporter organoids have been created using Cas9 D10A-mediated in-trans paired nicking (ITPN) and DNA templates carrying homology arms. Importantly, this method does not create double-strand breaks and off-target integrations, circumventing the need for clonal outgrowth of organoids (Bollen et al. 2022).

Using homologous recombination, Shimokawa et al. introduced a tdTomato fluorescent reporter coupled to a synthetic death gene iCaspase 9 into the *LGR5* locus of human CRC organoids (Shimokawa et al. 2017). They showed that, in line with the murine model of CRC, ablation of $LGR5^+$ cells by inducing dimerization of iCaspase9 with AP20187 led to a strong reduction in growth of CRC organoids grafted in mice, suggesting $LGR5^+$ cells drive CRC growth.

In addition to forced cancer initiation by genetic editing, oncogenesis driven by environmental factors can also be studied in organoids. Microbes have long been implied as a causative agent of malignant transformation and cancer progression (Sepich-Poore et al. 2021). Notable examples are *Helicobacter pylori* and Pks^+ *Escherichia coli* bacteria, which are strongly associated with cancers in the gastrointestinal system (Allen and Sears 2019). Pks^+ *E. coli* produce a genotoxin, colibactin, which is known to initiate double-strand DNA breaks (Nougayrède et al. 2006). Pleguezuelos-Manzano et al. microinjected these Pks^+ *E. coli* or *Pks*-deficient *E. coli*

into lumens of normal colon organoids and cultured them for a prolonged period. The authors identified a distinct mutational signature in organoids cultured with *Pks*$^{+}$ *E. coli*, which could be found in CRC genomes as well as genomes from urinary cancers and head and neck cancers. These correlations may indicate a causative role for these microbes in oncogenesis (Pleguezuelos-Manzano et al. 2020). Potentially, transformation driven by oncogenic viruses (Gillison and Lowy 2004) can also be studied in organoids, shown by the infectability of oral mucosa organoids with human papillomavirus 16 particles (Driehuis et al. 2019). In a different study, murine and lung organoids were exposed to the mutagenic chemicals, ethyl methanesulfonate (EMS), acrylamide (AA), and 7,12-dimethylbutylamine (DMBA), and tested for their capacity to generate tumors upon grafting in murine hosts (Naruse et al. 2020). The authors showed a stark increase in tumorigenic potential in organoids treated with these chemicals.

Genomic and Phenotypic Evolution in Organoids

Genomic instability is a hallmark of cancer, driving continuous evolution of the cancer genome and leading to a heterogeneous cancer cell population with many unique genetic alterations found in single cells, promoting tumor progression, evolution, and drug resistance. However, the rate of genomic instability in cancers and the relative contribution of the various driver mutations is not fully understood. This is due to significant differences in the genomic organization between mice and humans and the poor representation of the cancer genome by conventional cancer cell lines.

Bolhaqueiro et al. studied CIN, one of the most common forms of genomic instability in cancers responsible for driving aneuploidy and copy number alterations, in CRC patient-derived tumor organoids (PDTOs) via introduction of green fluorescent protein (GFP)-tagged HISTONE 2B to enable nuclei visualization (Bolhaqueiro et al. 2019). They showed CIN is a widespread phenomenon in human CRC, irrespective of the genomic background. However, differences were observed in the tolerance of CIN, with P53 deficiency identified as a key mechanism for CIN tolerance. Bollen et al. subsequently combined this live imaging approach of CRC organoids with single-cell whole-genome sequencing (Bollen et al. 2021). Using CRISPR-Cas9-mediated homologous recombination, they generated knockin of Dendra2, a green-to-red photoconvertible fluorophore, at the carboxyl terminus of the *HIST1H2BC* gene. By positionally marking cells in the organoids using Dendra2 photoconversion and combining spatial information with the single-cell whole-genome sequencing data, the authors were able to reconstruct chromosomal evolution in CRC on a single-cell level, showing that the CRC genome evolves in both punctuated and gradual patterns. An important conclusion from these papers is the continuous evolution of cancer genomes in these organoids will result in genetic drift and could potentially select organoid-specific mutations that could serve as a confounding variable affecting reproducibility.

Chan et al. (2022) used this natural evolution of cancer organoids to their advantage to study the onset of lineage plasticity in PCa. Lineage plasticity is postulated to drive resistance by a cancer cell differentiating into a cellular phenotype that is independent of the drug target. The phenomenon is strongly associated with the resistance to therapies targeting AR signaling in CRPC. In clinical samples, coinactivation of both *TP53* and *RB1* is frequently observed. Indeed, GEMMs of PCa harboring these mutations readily recapitulate features of lineage plasticity; however, the temporal onset is poorly understood. Using a murine organoid model where *Trp53* and *Rb1* (PR) loss could be temporally controlled using Cre recombinase, the onset of lineage plasticity could be followed on daily basis. PR knockout in cancer organoids induced gradual evolution from an AR-dependent state to a completely dedifferentiated AR-independent state within 4–6 wk, mimicking a specific subtype of lineage plastic CRPC, stem-cell like CRPC(-SCL). Acquisition of lineage plastic features was enhanced by pharmacological inhibition of AR by enzalutamide. By teasing apart the transcriptional profile across the time course us-

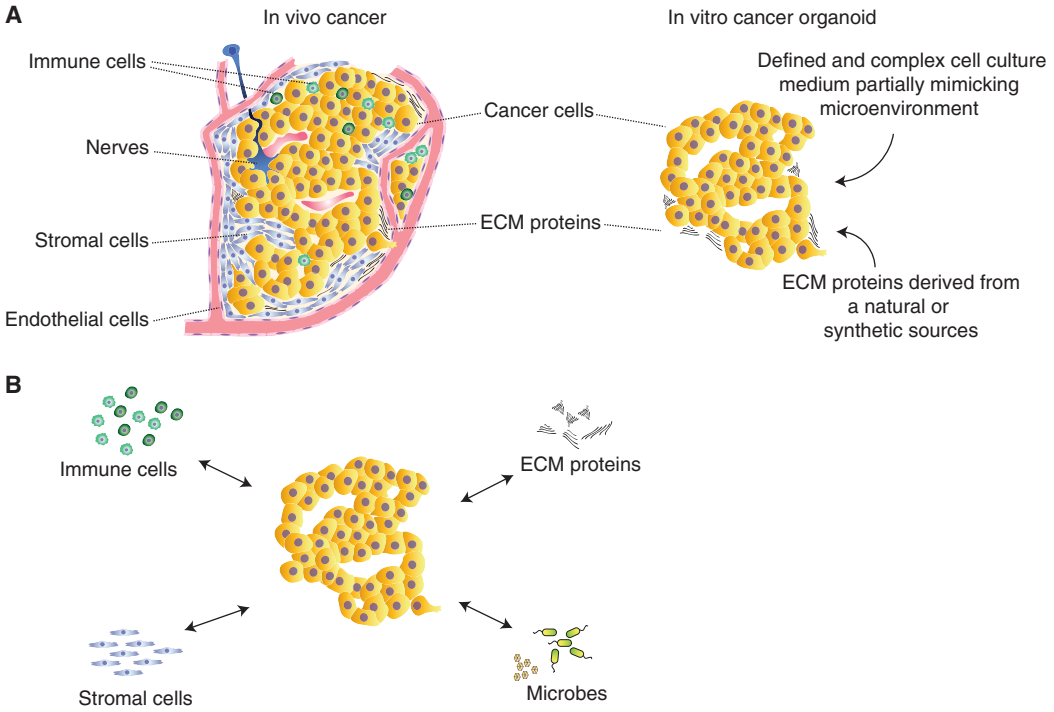

Figure 2. Microenvironmental interactions modeled in organoid research. (*A*) Schematic representation of the complex cancer microenvironment found in vivo and the reduced complexity in the cancer organoid culture. The microenvironment is in part mimicked by the complex, yet defined, cell culture medium supplemented by a variety of nutrients and paracrine growth factors. Additionally, the 3D protein matrix serves as a basement membrane or extracellular matrix (ECM). However, until now, the exact composition of the in vivo cancer ECM is not recapitulated by natural or synthetic sources of ECM. (*B*) Schematic overview of microenvironmental interactions that have been studied in isolation using coculture systems of cancer organoids and immune cells, fibroblasts, or bacteria and viruses. Additionally, some effects of in vitro ECM variations, such as mechanical stiffness, porousness, and ECM protein composition have been tested on cancer organoids.

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ing single-cell transcriptomics, the authors identified Janus kinase (JAK) signaling and FGFR signaling as key regulators of a specific subset of lineage plasticity, CRPC-SCL. Combined treatment with a JAK inhibitor (ruxolitinib) and an FGFR inhibitor (erdafitinib) could revert these CRPC-SCL phenotypes to an AR^+ and enzalutamide-sensitive state, provided AR expression was present. Importantly, similar JAK-STAT and FGFR transcriptional programs were identified in murine models with similar genetics, patient-derived biopsies, as well as CRPC-SCL organoids. An additional important observation in this study was that in vitro PCa organoids did not acquire any neuroendocrine features, and only did so when orthotopically transplanted, implying an important role for microenvironmental regulation of lineage plasticity that is not fully captured by organoid technology.

Minimal Modeling of Microenvironmental Interactions in Organoids

The cancer microenvironment is an important driver of cancer evolution and mediator of anticancer drug responses. Since upon establishment organoids are de facto stripped of their native microenvironment (Fig. 2A), studies of the microenvironment have focused on specific interactions of organoids and single-cell types. When used in parallel to in vivo models this approach can be very powerful.

For example, studying the promotion of intestinal tumorigenesis by cancer-associated fibroblasts (CAFs), Roulis et al. identified PGE_2 as a mesenchymally secreted growth factor. By coculturing organoids with CAFs in parallel to GEMMs, they uncovered that stromal-derived PGE_2 promotes Yap dephosphorylation, nuclear translocation, and transcriptional activity in Sca-1^+ dormant stem cells and was shown to be indispensable for adenoma formation (Roulis et al. 2020). In another study, Seino et al. (2018) used CAF-pancreatic ductal adenocarcinoma (PDAC) organoid cultures to identify one of three functional subtypes of PDAC based on their dependency of CAF-secreted WNT ligands.

Another study using CRC organoids described a novel method to enable high-dimensional single-cell analysis of cell-type, cell-state, and posttranslational modifications in a multiplexed experiment using mass cytometry (Qin et al. 2020). Murine macrophages and immortalized CAFs were separately expanded and cocultured with colorectal wild-type or cancer organoids, which revealed that oncogenic mutations in cancer epithelial cells mimic signaling networks normally induced by stromal cells.

Ohta et al. (2022) used organoids to identify a crucial interaction between an ECM protein, COL171A, and CRC stem cells, which drives maintenance of the persistent cell state during drug treatment. Human CRC organoids were genetically engineered to harbor fluorescent reporters for $LGR5^+$ cancer stem cells and a negative regulator of the cell cycle (p27/*CDKN1B*) to uncover noncycling, persistent CRC cells during drug treatment. Additionally, a CreERT2 recombinase was introduced at the 3′ of the endogenous *LGR5* locus to enable temporally controlled lineage tracing. The authors showed that $LGR5^+$ $p27^+$ dormant cancer stem cells are preexisting and are responsible for tumor regrowth when anticancer therapies are removed. The dormant state in cancer cells is maintained by ECM protein COL17A1-dependent repression of the FAK-YAP pathway. In organoids lacking COL17A1, inhibiting YAP resulted in p27 re-expression, indicating that YAP-pathway inactivation plays a crucial role in persistence in CRC.

Driven by the excitement around the promising clinical results of cancer immunotherapy, wherein the patient's immune system is unleashed to eliminate cancer cells, cocultures of cancer organoids and various immune cells have been developed to study interactions between the immune system and cancer and elucidate mechanisms of interaction and evasion that are reviewed in detail elsewhere (Bar-Ephraim et al. 2020).

Dijkstra et al. (2018) developed a tumor organoid-based method to study tumor-reactive T cells in patients with mismatch-repair-deficient CRC and non-small-cell lung cancer by establishing tumor organoids and coculturing them with autologous peripheral blood lymphocytes. Cocultures were supplemented with Interferon γ, Interleukin 2 (IL-2), anti-PD1 antibodies, and plate-bound $CD28^+$-specific antibodies to promote tumor-reactive T-cell expansion. Importantly, cancer organoids effectively induced antigen-specific stimulation of autologous T cells and showed that cytotoxic T cells (CTLs) expanded from cocultures effectively killed cancer organoids without inducing cytotoxic effects in healthy cells of the same patient.

Schnalzger et al. (2019) describe an in vitro assay for monitoring chimeric antigen receptor (CAR)-engineered human natural killer (NK) cell-mediated cytotoxicity in patient-derived CRC organoids. Luciferase-based assays and 3D live cell imaging revealed CAR-NK-92 cells against EGFRvIII, a neoantigen found in several cancer types, efficiently killed EGFRvIII-expressing tumor organoids without cytotoxic effects on normal cells. However, a second set of experiments with CARs against the WNT receptor, a putative target in a subset of CRC cases, demonstrated significant off-target effects on healthy organoids. Jacobs et al. (2020) established a living biobank of glioblastoma PDTOs and showed that when cocultured with EGFRvIII-specific CAR-T cells, cancer cells specific but incomplete killing of tumor cells was observed, in concordance with cellular heterogeneity and neoantigen expression observed in vivo. Thus, the methods described provide a strategy for testing the efficacy and safety of solid tumor cancer immunotherapy with CAR-engineered lymphocytes.

These coculture systems also enable live tracking of tumor and immune cell interactions. Shin et al. (2021) identified the presence of tu-

mor cells harboring immune-specific protein markers contributing to the immunosuppressive microenvironment in murine and human CRC models. Sophisticated live imaging of organoid cocultures revealed that the expression of these markers is achieved by trogocytic cancer cells, and the findings were later validated in patient-derived xenograft (PDX) models of CRC and head and neck cancer. Dekkers et al. (2023) developed a live-imaging platform combined with scRNA sequencing to uncover the transcriptional programs underlying the different modes of T-cell interactions and killing of patient-derived breast cancer organoids.

These studies show that despite the lack of a complex microenvironment in vitro, cancer organoids can be used efficiently to study interactions with a single cell type, and discoveries in this setting can often be validated in in vivo xenografts or GEMMs.

Xenografting Organoids to Study in Vivo Aspects of Cancer

As PDTO culture is an in vitro model, several key aspects of cancer evolution such as invasiveness and metastasis cannot be studied directly. Therefore, as a surrogate, organoids are often xenografted in mice to enable in vivo study. Orthotopic engraftment has been applied to many different types of cancer organoids including colon (O'Rourke et al. 2017; Roper et al. 2017; Fumagalli et al. 2018; Lannagan et al. 2019), pancreas (Boj et al. 2015), breast (Sachs et al. 2018; Dekkers et al. 2021), prostate (Chan et al. 2022), and bladder (Lee et al. 2018) to query different aspects of cancer biology and the influence of the complex in vivo microenvironment. Most of these orthotopic transplantation techniques rely on direct injection of organoids into the homeostatic organ. However, in some cases, organoids can also efficiently be engrafted upon damage (Yui et al. 2012; Huch et al. 2015; O'Rourke et al. 2017; Ganesh et al. 2019). A concern of this modeling approach is that cancer organoids graft randomly and do not represent a natural evolution of cancer in situ. However, this approach can be especially powerful for studying human cancer biology in vivo for cancers in which no or few GEMMs are readily available, but cancer organoids are. Indeed, for breast cancer (Sachs et al. 2018; Dekkers et al. 2021), orthotopic transplantation of human cancer organoids provides a viable alternative, enabling in vivo interrogation of complex cancer questions previously difficult to address due to the limited number of GEMMs for this cancer with multiple distinct subtypes.

Ganesh et al. orthotopically transplanted patient-derived rectal cancer (RC) organoids into immune-compromised mice where the colon was transiently damaged using dextran sulfate sodium, a method previously used to transplant genetically edited murine cancer organoids in syngeneic models (O'Rourke et al. 2017; Ganesh et al. 2019). These RC organoids were engrafted with 100% efficiency and in 20%–100% of cases they formed gross cancers. In 22–30 wk, grafted RC formed an invasive front that resembled stage I and II RCs in human, and subsequently these RCs metastasized to the lungs and liver. Importantly, engrafted tumors responded heterogeneously to chemotherapy akin to the observations in the clinic.

Apart from orthotopic transplantation, organoids can be xenografted subcutaneously and in many other sites of the soma. To study cell-intrinsic properties driving metastasis in pancreatic cancer, Roe et al. transplanted matched pairs of organoids derived from primary and metastatic cancer into mice using various methods (Roe et al. 2017). The authors found that metastatic-derived organoids had enhanced metastatic and growth potential irrespective of grafting method. The authors identified enhancer reprogramming driven by elevated levels of the pioneer transcription factor Foxa1 as a key driver of metastatic potential.

All these studies indicate that xenotransplantation of organoids can effectively be used in cancer studies to overcome several of the shortcomings of this in vitro methodology.

APPLICATIONS OF ORGANOIDS IN TRANSLATIONAL CANCER RESEARCH

For several cancer types, organoids can be established with high efficiency from a large patient

Table 2. Overview of cancer organoid types and notable biobanks derived from human cancers

Cancer type	Study	References
Biliary tract cancer	Extrahepatic biliary tract cancer biobank consisting of six gallbladder carcinoma and one extrahepatic cholangiocarcinoma patient-derived organoid (PDO)	Wang et al. 2021
Colorectal cancer (CRC)	Organoid biobank-derived normal and cancerous murine and human colon tissue; first established colon-derived organoid culture method	Sato et al. 2011
CRC	Organoid biobank of 55 colorectal tumor organoid lines of CRC, including rare subtypes	Fujii et al. 2016
CRC	Organoid biobank consisting of >20 CRC and matched normal organoid lines	van de Wetering et al. 2015
CRC	Paired cancer and normal organoids from 20 patients enriched in microsatellite-stable, early-onset CRC	Yan et al. 2020
Gastric cancer	Establishment of 37 patient-derived gastric cancer organoids	Nanki et al. 2018
Gastric cancer	Organoid biobank containing 17 normal and 46 gastric cancer organoid lines encompassing all known molecular subtypes of gastric cancer	Yan et al. 2018
Pancreas	Establishment of protocol for murine and human-derived pancreatic cancer and generation of eight pancreatic cancer organoid lines	Boj et al. 2015
Pancreas	Organoid biobank of 66 pancreatic ductal adenocarcinoma lines	Tiriac et al. 2018
Pancreas	Organoid biobank consisting of 39 pancreatic ductal adenocarcinoma lines	Seino et al. 2018
Rectal cancer	Living biobank with 80 tumor organoids was derived from treatment-naive rectal cancer patients	Yao et al. 2020
Gastrointestinal neuroendocrine	Organoid biobank of 25 cancer organoid lines derived from human gastroenteropancreatic neuroendocrine neoplasms	Kawasaki et al. 2020
Gastrointestinal metastatic	Organoid biobank containing 49 PDOs derived from metastatic, heavily pretreated colorectal, and gastroesophageal cancer patients recruited in phase 1/2 clinical trials	Vlachogiannis et al. 2018
Liver cancer	Organoid biobank containing 10 hepatocellular carcinoma PDO lines	Nuciforo et al. 2018
Liver cancer	Organoid biobank containing eight hepatocellular carcinoma and cholangiocarcinoma human tumor organoid lines	Broutier et al. 2017
Bladder	Development of a culture system for the mouse urothelium and establishment of a living biobank with organoids derived from more than 50 bladder cancer patients	Mullenders et al. 2019
Bladder	Organoid biobank consisting of 22 patient-derived bladder cancer organoid lines	Lee et al. 2018
Prostate	Generation of seven prostate cancer organoid lines from metastasis or circulating tumor cells	Gao et al. 2014
Prostate	Establishment of human and murine prostate cancer organoid culture methods	Karthaus et al. 2014
Prostate	Organoid biobank derived from 20 advanced prostate cancer xenografts	Beshiri et al. 2018
Breast cancer	Organoid biobank of 87 breast cancer lines with a focus on triple-negative breast cancer patients	Bhatia et al. 2022

Continued

Table 2. *Continued*

Cancer type	Study	References
Breast cancer	Establishment of murine and human mammary organoid culture and generation of human breast cancer biobank with >100 primary and metastatic organoid lines	Sachs et al. 2018
Cervical cancer	Establishment of cervical organoid culture conditions and generation of 12 cervical tumor organoid lines	Lõhmussaar et al. 2021
Endometrial cancer	Organoid biobank containing >60 PDOs from varied stages of endometrial disease	Boretto et al. 2019
Ovarian cancer	Establishment of ovarian cancer organoid culture conditions and generation biobank containing 56 PDO lines	Kopper et al. 2019
Ovarian cancer	Organoid biobank of 33 lines derived from patients with high-grade serous ovarian cancer	Hill et al. 2018
Glioblastoma	Organoid biobank of >70 glioblastoma organoid lines	Jacob et al. 2020
Neuroblastoma	Organoid biobank of six neuroblastoma organoid lines	Fusco et al. 2019
Head and neck squamous cell carcinoma (HNSCC)	Establishment of culture conditions for head and neck squamous cell carcinoma cancer organoids and generation of organoid biobank containing 31 HNSCC lines	Driehuis et al. 2019
Kidney cancer	Establishment of protocol for clear cell renal carcinoma and establishment of biobank containing 15 normal and 10 cancer PDOs	Grassi et al. 2019
Kidney cancer	Organoid biobank of > 50 normal and cancerous lines from childhood renal cancer patients	Calandrini et al. 2020
Lung cancer	Establishment of culture conditions for healthy, hereditary diseased, and cancerous lung organoids	Sachs et al. 2019
Lung cancer	Organoid biobank containing 80 lung cancer PDOs	Kim et al. 2019

population. This inspired the generation of so-called "living biobanks" containing collections of PDTOs that capture disease heterogeneity observed in the clinic. An overview of notable PDTO biobanks is given in Table 2; these are predominantly comprised of epithelial cancers of the gastrointestinal system, but recently several other biobanks of other cancer types have been established.

These PDTO biobanks have many uses, including screening of drugs or therapies for efficacy and toxicity, accelerating drug discovery, and reducing costs associated with clinical trials. Van de Wetering et al. (2015) generated a biobank from CRC specimens in parallel with matched benign organoids. Importantly, all major CRC subtypes were represented (Dienstmann et al. 2017). The CRC organoids recapitulated the in vivo architecture and whole-exome sequencing of biopsied specimens, and matched PDTOs confirmed that somatic mutations are largely maintained in CRC organoids. Last, the authors performed an unbiased high-throughput drug screen, showing that in vitro PDTO drug responses in combination with cancer genomics data can be used to examine the underlying molecular mechanisms of clinical drug responses. When establishing another CRC organoid biobank, Fujii et al. (2016) noted several culture conditions such as the presence of WNT signaling activators (Wnt3A, R-spondin1) or p38 inhibitors, and oxygen concentration as factors that influenced establishment efficiency. This discrepancy is likely due to differences in tumor mutational profiles and phenotypes, which are often unknown at the time of organoid establishment. Consequently, organoids that were initially seeded in eight combinatorial conditions yielded nearly 100% tumor organoid establishment efficiency, showing there is not a single culture condition that is optimal for all cancer organoids. And multiple medium compositions should be used when establishing novel cancer organoid cultures.

Similarly, a breast cancer PDTO biobank has been established comprised of >100 tumor or-

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ganoids derived from primary and metastatic tumor lesions representing all major subtypes of breast cancer (Sachs et al. 2018). Significantly, the majority of organoids retained their in vivo histopathology, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) receptor status. Drugs targeting HER2 signaling were incorporated into a proof-of-concept screen, and the in vitro response typically aligned with the HER2 status of the cells.

Vlachogiannis et al. (2018) explored the ability of PDTOs to recapitulate patient drug responses in vitro. Fifteen PDTOs were established from metastatic, heavily pretreated colorectal and gastroesophageal cancer patients enrolled in phase 1/2 clinical trials. The PDTOs were subjected to a compound library of approved and investigational drugs, and drug response of PDTOs was compared to clinical responses of patients. Strikingly, in vitro data showed that the predictive value for drug efficacy was 88%, while the predictive value for drug inefficacy was 100%. This work demonstrated that PDTOs can accurately recapitulate patients' responses in clinical trials, and further emphasizes the potential of PTDOs in advancing personalized medicine approaches.

Recently, PDTO biobanks have been generated from rare and difficult-to-model cancers. Kidney tumors are one of the most common solid tumors found in children, with several distinct subtypes associated with varied prognoses (Brok et al. 2016). Calandrini et al. (2020) established the first pediatric cancer organoid biobank, capturing several renal cancer subtypes including Wilms tumor, malignant rhabdomyoma, renal cell carcinoma, and congenital mesodermal nephroma. In addition to patient/PDTO-specific drug responses, the authors found the organoids derived from patients who had received standard-of-care chemotherapies showed reduced sensitivity compared to cultures derived from untreated patients, thus showing that in vivo drug resistance is maintained in patient-derived organoid (PDO) cultures.

Kawasaki et al. (2020) generated a PDTO biobank of gastroenteropancreatic (GEP) neuroendocrine neoplasms (NENs), a group of rare cancers arising from the digestive epithelium with neuroendocrine features that are associated with extremely poor prognosis. The biobank encompasses 25 GEP-NEN PDTO cultures from multiple gastrointestinal sites including the liver, pancreas, colon, and stomach. The histopathological features and phenotypes observed in clinical tissue samples were conserved in GEP-NEN organoids and xenotransplanted organoids, thus offering a platform to study this rare cancer subtype.

PDTO biobanks can also be used for drug development. Herpers et al. used a paired CRC and healthy organoid biobank to perform a high-content, image-based screen to identify dual-targeting bispecific antibodies for WNT signaling components and receptor tyrosine kinase targets. They identified an antibody, MCLA-158, targeting $LGR5^+$ cancer stem cells and EGFR as an effective inhibitor of CRC organoid growth, and at the same time showing little toxicity toward benign $LGR5^+$ stem cells (Herpers et al. 2022).

PDTOs cannot be efficiently established from all cancer types. For instance, human PCa organoids have only been established at low frequency, driven by variable biopsy quality and overgrowth of benign epithelial cells upon establishment (Gao et al. 2014; Karthaus et al. 2014). Nonetheless, by analyzing the available human PCa organoids in conjunction with other preclinical models using RNA sequencing and ATAC sequencing (assay for transposase-accessible chromatin combined with sequencing), Tang and colleagues further stratified CRPC into four subtypes. In addition, they identified clinically actionable targets for these CRPC subtypes (Tang et al. 2022).

In all, PDTOs accurately recapitulate the genomics and phenotypes observed in patients. As they allow for the study of individual patients' cancer in a controlled environment and for prospective drug screening, PDTOs hold great promise for personalized medicine. Additionally, these models could potentially be further used to uncover new subtypes of cancer.

CONCLUSION AND PERSPECTIVE

Overall, organoid technology has greatly accelerated both basic and translational cancer research.

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Figure 3. Reciprocal interactions between genetically engineered mouse models (GEMMs) of cancer, cancer organoids, and the clinic. In combination, organoids and mouse models of cancer can synergize and facilitate rapid progress. Generation of GEMMs is complex and time consuming; therefore, rapid investigation of cancer biology is often hampered. Cancer organoids can be directly established from the GEMM, allowing questions in a relevant cell culture system. Additionally, organoids are amendable to a variety of experimental techniques unavailable to the GEMM (see Fig. 1) and allow the study of phenomena in a simplified model system. Cancer organoids, notably patient-derived cancer organoids, can form a bridge between murine models of cancers and the clinic. Additionally, cancers that are not well modeled by GEMMs can be established as organoids from clinical samples. However, as the complex and "natural" microenvironment of the cancer is lacking, part of the cancer biology is missed in vitro. Orthotopic transplantation of organoids can be used to study cancer organoid biology in vivo; alternatively, an "organoid-informed" orthogonal GEMM can be generated.

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It is important to note that in nearly all studies, cancer organoids have been used in parallel to GEMMs or have been xenotransplanted into hosts. This shows that organoids are not a stand-alone model, but for now serve as a powerful hybrid system combining "the best" of in vivo and in vitro models. Cancer organoids can be used as a model to study questions too complex to recapitulate in GEMMs. Additionally, PDTOs can serve as an orthogonal model to translate results from GEMMs to a clinical application. In the reverse direction, cancer organoids can be used for initial exploratory studies and inform the basis for in vivo studies by xenografting or the generation of new GEMMs (Fig. 3).

Recently, the Modernization Act 2.0 from the Food and Drug Administration was approved by the United States congress, removing the mandate of animal testing for drugs. This certainly will drive researchers to further leverage the powerful translational properties of PDTOs to generate a rapid, personalized medicine platform for clinical applications.

Despite significant advances, organoid methodologies still face several key challenges due to their novelty as a cancer-modeling technique. Organoid culture is comparatively slower than conventional cell culture, and because organoids rely on a 3D protein matrix for growth, performing high-throughput work is extremely laborious.

One approach to tear down this barrier is the development of a microfluidics protocol enabling rapid generation of many individual cancer organoids encapsulated in an ECM droplet, allowing for various high-throughput applications, including drug screening (Ding et al. 2022; Wang et al. 2022).

The protein matrix used in organoid culture is another major drawback of this methodology. The most used commercially available gel scaffolds are derived from ECM secreted from Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells. However, these suffer batch-to-batch variability and poorly defined chemical composition leading to inconsistencies in long-term growth and limiting the reproducibility of organoid research. Additionally, the patterning of organoids is stochastic and most likely driven by local differences in matrix composition. Bioengineered matrices that can be printed in organ-like shapes, could provide an alternative, and have been used to generate more in vivo–like villus and crypt-like structures using murine small intestinal organoids (Gjorevski et al. 2016, 2022). However, these matrices have not yet been applied to cancer organoids and are currently not widely available.

Another difficulty is the highly variable establishment efficiency of human cancer organoid cultures between cancer types. This is caused in part a variety of factors, including tissue availability and quality prior to establishment, imperfect medium compositions due to lack of understanding of cancer requirements, and lack of suitable reagents. Recent advances in synthetic biology have helped in improving organoid culture conditions. Miao and colleagues designed a water-soluble, next-generation surrogate WNT (NGS-WNT) to replace the stromal cell–conditioned medium containing the hydrophobic, palmitoylated WNT ligands in organoid culture (Miao et al. 2020). NGS-WNT greatly improved establishment and growth for various human and mouse organoid types. Last, contamination of benign cells in biopsies that outcompete cancer cells in organoid culture pose a major challenge in cancers where benign cell outgrowth cannot efficiently be selected against, exemplified by the frequent overgrowth of benign cells seen during attempted establishment of PCa (Gao et al. 2014; Karthaus et al. 2014) and lung cancer organoids (Dijkstra et al. 2020).

Additionally, considering the ongoing evolution of cancer genomes in PDTOs, it is key to continuously evaluate clonal growth patterns and assess for potential organoid-specific genomic events compared to the original in vivo cancer and early established organoids, especially when PDTOs are expanded long term.

Last, although cancer organoids have been used to study microenvironmental interactions in a minimalist system, recapitulating the complex ecosystem of cancer in vivo has not yet been achieved in vitro. Moreover, invasive and metastatic behavior can only accurately be assessed in vivo. Currently, these studies need to be supple-

mented by xenografting organoids into hosts or by use of an orthogonal GEMM.

In all, in spite of several challenges ahead, organoids provide a flexible and powerful model for basic and translational cancer research that in combination with GEMM and clinical data supercharge cancer research.

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Cold Spring Harb Perspect Med 2024; doi: 10.1101/cshperspect.a041380 originally published online September 21, 2023