REVIEW

Cancer modeling meets human organoid technology

David Tuveson[1,2]* and Hans Clevers[3,4]*

Organoids are microscopic self-organizing, three-dimensional structures that are grown from stem cells in vitro. They recapitulate many structural and functional aspects of their in vivo counterpart organs. This versatile technology has led to the development of many novel human cancer models. It is now possible to create indefinitely expanding organoids starting from tumor tissue of individuals suffering from a range of carcinomas. Alternatively, CRISPR-based gene modification allows the engineering of organoid models of cancer through the introduction of any combination of cancer gene alterations to normal organoids. When combined with immune cells and fibroblasts, tumor organoids become models for the cancer microenvironment enabling immune-oncology applications. Emerging evidence indicates that organoids can be used to accurately predict drug responses in a personalized treatment setting. Here, we review the current state and future prospects of the rapidly evolving tumor organoid field.

Techniques for culturing functional human breast epithelium in three-dimensional (3D) matrices have been championed for more than 30 years by Mina Bissell (*1*, *2*). Additionally, around a decade ago, Sasai and colleagues pioneered pluripotent stem cell (PSC)–based technology to create organoids that mirror specific parts of the central nervous system (CNS) (*3*, *4*). Lancaster and colleagues modified this technology and provided particularly notable examples of "mini-brain" structures (*5*). Although PSCs can be used to model everything ranging from tissues to organismal development, adult stem cells (ASCs) can also be isolated to generate organoid models of the primary tissues in which they reside. Specific growth factor cocktails allow long-term expansion of ASC organoids by mimicking the organ stem cell niche, as first established for mouse (*6*) and human (*7*) intestine and airway epithelium (*8*) [reviewed in (*9*)]. The organoid structures generated from PSCs and ASCs reflect crucial tissue features in terms of overall architecture, the collection of differentiated cell types, and tissue-specific function. Organoids thus represent a model system that can be compared to traditional genetically engineered mouse models (GEMMs), cell lines, and patient-derived xenografts (PDXs) (Table 1).

Besides being used to examine normal development, organoids have also been used to study tumorigenesis. In most organoid studies in the cancer field, primary carcinoma samples have been generated under ASC-organoid conditions. However, CRISPR mutagenesis technology has been applied to PSC-based organoids to generate cancer-causing mutations, for example, to model human brain tumors (*10*). In addition, Fine and colleagues have explored PSC-derived mini-brains as an environment for growing patient-derived glioblastoma cells (*11*).

The combination of R-spondin-1 (a Wnt amplifier acting through Lgr5), epidermal growth factor (EGF), and the bone morphogenetic protein (BMP) inhibitor noggin in a serum-free 3D matrix (Matrigel) supports the seemingly indefinite expansion of Lgr5$^+$ mouse crypt stem cells as 3D intestinal epithelial structures (*6*). Human gut organoids require additional components: Wnt, the transforming growth factor–β (TGFβ) inhibitor A83-01, and the p38 inhibitor SB202190. This growth factor cocktail also supports the propagation of patient-derived tumor organoids representing colorectal cancers (CRCs) (*7*). Small modifications allowed expansion of other epithelial tissues, such as pancreas and prostate (*12*, *13*), and carcinomas derived thereof (*14*–*16*), enabling the creation of "living biobanks" (*17*, *18*), where organoid cultures representative of disease diversity in terms of pathological subtypes and mutated-gene frequencies can be stored and disseminated to other investigators.

Two approaches to determine drug sensitivity in patient-derived samples have been widely exploited, namely the short-term culture of tumor sections (*19*) and xenotransplantation of the tumor into immunodeficient mice (i.e., PDXs) (*20*). Short-term culture allows for rapid in vitro screening at a reasonably large scale but is constrained by the limited proliferative capacity of the cultures. Xenotransplantation allows for in vivo screening but is slow and resource-intensive. Tumor organoid technology may bridge these two approaches. Initial studies have demonstrated the feasibility of medium-throughput drug screening on patient-derived organoids (PDOs) (*16*, *17*). Confirmation of in vitro observations on PDOs can be obtained in the PDX setting, as tumor organoids are readily transplantable into immunodeficient mice (*14*, *16*).

R-spondin–based culture conditions to propagate various other human carcinomas are now established, for example, for hepatocellular carcinoma and cholangiocarcinoma (*21*) and gastric (*22*, *23*), breast (*24*), bladder (*25*, *26*), esophageal (*27*), ovarian (*28*, *29*), lung (*30*), and pediatric

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Table 1. Properties of cancer model systems.
GEMM, genetically engineered mouse model; MDO, murine-derived organoid; MDOX, murine-derived organoid transplantation; CLs, cell lines; PDX, patient-derived xenograft; iPS, inducible pluripotential stem cell; PDO, patient-derived organoid; PDOX, patient-derived organoid transplantation.

	GEMM	MDO	MDOX	CLs	PDX	iPS	PDO	PDOX
Wild-type cell culture	+	+	+	–	–	+	+	–
Preinvasive cancer models	+	+	+	–	–	+	+	+
Invasive cancer models	+	+	+	+	+	+	+	+
Metastatic cancer models	+	+	+	+	+	+	+	+
Cost	$$$$	$$	$$$	$	$$	$$	$$	$$$
Time	++++	+	++	+	++++	+++	++	+++
Success rate	high	med	med	med	med	low	med	med
Throughput therapies	low	med	low	high	low	high	med	low

+ denotes 1 month or less; ++, 1–2 months; +++, 1–6 months; ++++, oftentimes more than several months.

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kidney (*31*) cancers. Organoid cultures have enabled several observations: (i) Interpatient variation is captured and maintained. (ii) Organoids can typically be derived from patient material with high efficiency and can be xenotransplanted. (iii) Tumor organoids can faithfully report the drug response of the corresponding patient. (iv) Drug sensitivities of PDOs can be recapitulated in PDX settings.

Creating tumor organoid biobanks

The large majority of samples analyzed by cancer consortiums, such as the International Cancer Genome Consortium and The Cancer Genome Atlas, represent surgical specimens of primary tumors, whereas metastases typically represent the lethal stage of cancer. Theoretically, PDOs allow expansion of small tumor samples, enabling the analysis of cancer at any stage. Indeed, several studies have reported that organoids can be established from needle biopsies taken from, for example, liver cancer (*32*), pancreatic cancer (*33*, *34*), or from liver metastases of CRC (*35*). Gao *et al.* (*16*) have demonstrated the feasibility of growing organoids from circulating tumor cells in a prostate cancer patient. Studies have also reported the generation of healthy organoids from cells in urine (*31*) and from bronchial lavage material (*30*). It remains to be established whether these approaches will allow the outgrowth of tumor organoids as well. Thus, PDO biobanks greatly expand the types of patient samples that can be propagated and studied in the laboratory.

Most biobanking studies have confirmed that PDOs reflect the characteristics of the primary tumor, at least at the level of bulk tumor DNA sequence (*35*). However, it is less clear whether intratumoral heterogeneity is captured in organoid cultures. Another unaddressed variable is the clonal drift of "bulk" organoids over prolonged culture times. At least for a few studied CRC and ovarian cancer PDOs, this clonal drift appears to be relatively small (*18*, *29*).

PDOs established from single cells obtained from CRCs and from adjacent normal crypts (*36*) have allowed molecular and functional analyses that are not possible at the single-cell level. Such analyses revealed that CRC cells exhibit extensive mutational diversification and carry several times more somatic mutations than normal stem cells. Most mutations result from de novo mutational processes. The diversification of DNA methylation and transcriptome states in each tumor is stable and follows the phylogenetic tree of DNA mutations in that tumor. However, anticancer drug responses are markedly different between even closely related cells of the same tumor, indicating that pharmacological heterogeneity likely reflects epigenetic changes that alter gene expression among single cells in a tumor.

Several initiatives aim to make well-characterized PDO biobanks available to academia and industry. In addition to the technical challenges of banking living material, the ethics and informed consent–issues surrounding such biobanks are complex (*37*). The nonprofit HUB (www.hub4organoids.eu) establishes, characterizes, and distributes organoid biobanks. The Human Cancer Models Initiative (HCMI, https://ocg.cancer.gov/programs/HCMI) is a collaborative international consortium generating tumor-derived culture models annotated with genomic and clinical data. HCMI aims to make the developed models and related data available as a community resource.

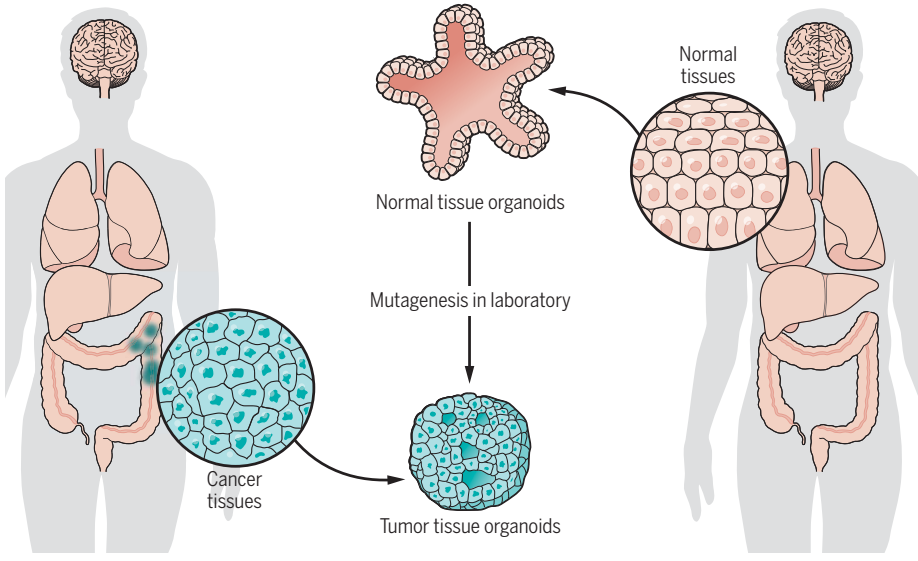

Fig. 1. Methods to generate a human cancer organoid biobank. A biobank of human cancer organoids can be generated directly from neoplastic tissues (left) or by genetic modification of organoids developed from normal tissues (right).

Organoids allow modeling of human carcinogenesis in a dish

Since Fearon and Vogelstein proposed the adenoma-to-carcinoma progression to be the result of an ordered series of specific oncogenic mutations, CRC has become the showcase example of cancer progression (*38*). Organoids can routinely be derived from normal human epithelia, allowing the in vitro mutational modeling of all stages of malignancy (Fig. 1). Sato and colleagues demonstrated the feasibility of growing various types of premalignant colon neoplasia in vitro. Whereas organoids at all stages were independent of Wnt/R-spondin because of activating Wnt pathway mutations, dependency on other niche growth factors was lost specifically at the adenoma-carcinoma transition (*18*). Two independent studies used CRISPR technology for the stepwise recreation of the adenoma-carcinoma progression starting from healthy, wild-type human colon organoids (*39*, *40*). CRCs carry recurrent mutations in members of the WNT, TGFβ, TP53, and phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3K/MAPK) pathways. Organoids with mutated genes could be selected by removing individual growth factors: loss of the adenomatous polyposis coli (*APC*) gene led to Wnt/R-spondin independence; mutating *KRAS* led to EGF independence; mutating *SMAD4* led to noggin independence; and mutating TP53 led to nutlin-3 resistance. Quadruple mutants grow independently of all stem cell niche factors. Upon xenotransplantation into mice, quadruple mutants grow as invasive CRCs. A follow-up study demonstrated that these sequential oncogenic mutations facilitate efficient tumor growth, migration, and metastatic colonization, implying that the ability to metastasize is the direct consequence of the loss of dependency on specific niche signals (*41*).

> ***"...PDO biobanks greatly expand the types of patient samples that can be propagated and studied..."***

Meltzer and colleagues derived organoids from Barrett's esophagus patient biopsies (*42*). To document the role of APC in the malignant transformation of Barrett's esophagus, *APC* knock-out (APC^{KO}) organoids were created by CRISPR genome editing. APC^{KO} organoids displayed histologic atypia as well as higher proliferative and replicative activity, recapitulating the critical role of aberrant Wnt/β-catenin signaling activation in neoplastic transformation of Barrett's (*42*).

A more recent study used sequential CRISPR-mediated editing to create an organoid model for serrated CRC, a subtype associated with activating mutations in *BRAF*. After introduction of the activating mutation $Braf^{V600E}$ into normal colon organoids, inactivating mutations were introduced sequentially in *Tgfβr2*, the Wnt-inhibiting *Rnf43*, *Znrf3* ubiquitin ligases, and *P16/Ink4A*. Orthotopic transplantation of the compound mutant organoid lines, but not

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Braf^{V600E} alone, generated adenocarcinoma with serrated features (*43*). In a similar approach, cancer progression was studied in wild-type human pancreas organoids by the sequential mutation of *KRAS* (K), *CDKN2A* (C), *TP53* (T), and/or *SMAD4* (S). Xenotransplantation into the subcutaneous space of immunodeficient mice showed that KC organoids failed to engraft, whereas KT organoids formed subcutaneous tumors resembling pancreatic intraepithelial neoplasia, yet only when co-transplanted with cancer-associated fibroblasts. KCTS organoids engrafted without cancer-associated fibroblasts (CAFs) and yielded pancreatic ductal adenocarcinomas (*15*).

Gut organoids can be subcloned after CRISPR modification, which allows the detailed study of cancer gene function. When key DNA repair genes are deleted by CRISPR and the resulting mutant organoid clones are subcloned after a period, specific mutational signatures appear that result from defective DNA repair. These signatures can be compared to known cancer-associated signatures. Indeed, mutation accumulation in organoids deficient in the mismatch repair gene *MLH1* correctly modeled mutation profiles of mismatch repair–deficient CRCs. Mutation of the cancer predisposition gene *NTHL1*, encoding a base excision repair protein, revealed a mutational signature previously observed in one breast cancer patient, who was then found to carry a germline *NTHL1* mutation (*44*). In another example, an activating *KRAS* mutation was introduced in a CRC organoid line that did not harbor Ras pathway mutations. Direct comparison of this isogenic pair of organoids revealed a marked effect of the *KRAS* mutation on drug response (*45*).

A major advance in mouse stem cell studies has been the creation of knock-in alleles of stem cell marker genes to allow the visualization, selective killing, and lineage tracing of the marker^{+} cells. Following similar strategies, Sato and colleagues have created Lgr5 knock-in alleles through CRISPR-driven modification in human colon cancer organoids, followed by xenotransplantation. Lineage-tracing experiments with a tamoxifen-inducible Cre knock-in allele also revealed the self-renewal and differentiation capacity of human LGR5^{+} cells in the xenografted CRC organoid–derived tumors. Selective ablation of LGR5^{+} tumor cells using an LGR5-iCaspase9 knock-in allele led to transient tumor regression. A KRT20 knock-in reporter marked differentiated cancer cells with only a limited life span and these cells reverted to LGR5^{+} self-renewing tumor cells upon LGR5^{+} CSC ablation, implying that human CRC growth is fueled by LGR5^{+} cancer stem cells as well as dedifferentiated tumor cells that can facultatively replace the cancer stem cells pool (*46*). De Sauvage and colleagues performed similar experiments using mouse organoids, and they specifically noted that Lgr5 cell ablation leads to primary tumor stasis whereas Lgr5^{+} cells were essential for liver metastasis (*47*).

Current ASC-based organoid technology does not support growth of normal CNS tissues, but PSC-derived cerebral organoids fill this gap. PSC-derived CNS organoids recapitulated brain tumorigenesis by introducing clinically relevant oncogenic mutations, helping to define the specific mutation combinations that result in glioblastoma-like tumors and primitive neuroectodermal tumor–like tumors. These transformed organoids were xenotransplantable and amenable to drug screening (*10*).

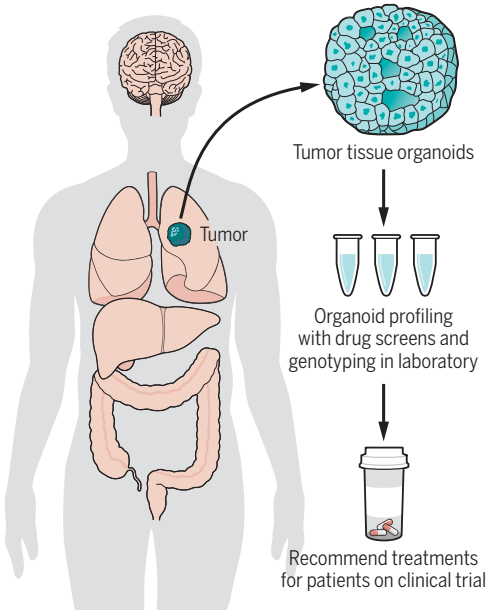

Fig. 2. Personalized medicine using human cancer organoids. Human cancer organoids can be used to rapidly determine drug sensitivities and molecular aberrations, and in clinical trials this information can be evaluated for its predictive potential.

Therefore, the combination of CRISPR and human organoid technology yields a versatile toolbox with which to build human cancer models in a stepwise fashion, generating isogenic sets of progressively more malignant organoids. In their design, they resemble GEMMs, and organoid engineering can expedite the generation of additional GEMMs as well as human cancer transplantation models.

Organoid cocultures identify tumor microenvironment characteristics and immune therapies

The tumor microenvironment (TME) modifies tumor progression and therapeutic response, yet it is challenging to characterize because it is challenging to maintain viably in tissue culture and manipulate ex vivo. Tumor organoid models generally lack an intact microenvironment. However, recent findings show that organoid cocultures with TME cells provide a new method to characterize some aspects of the TME. For example, the coculture of pancreatic stellate cells, a resident mesenchymal cell population, with pancreatic cancer PDOs produced the desmoplastic stroma typical of pancreatic carcinomas and directly led to the discovery of pancreatic CAF subtypes, including one that secreted interleukin-6 to support organoid proliferation (*48*). Additional investigations with PDO-CAF cocultures identified biochemical pathways responsible for the different CAF subtypes and thereby revealed methods to alter CAF composition in tumors (*49*). Nonetheless, these coculture systems are still being developed and a current unmet goal is to determine whether such PDO-CAF cocultures impart resistance to traditional and investigational drugs and whether they can be used to optimize therapeutic response ex vivo.

Immune cells are another common TME cell type, and PDO cocultures have shown early promise in evaluating this feature of human cancer. For example, a modification of traditional ASC organoid approaches introduced by Kuo and colleagues is to use air-liquid interface (ALI) cultures that include the typical Wnt-dependent media used in classical PDOs (*50*). Using such approaches, PDOs with immune and fibroblastic components can be propagated from primary tumor fragments for several weeks, and these ALI cultures display T cell clonal diversity that mirrors the T cell diversity in the patient's peripheral blood. ALI cultures have been applied to the analysis of immune checkpoint therapies in several human tumors that have variable clinical responses, including melanoma, lung cancer, and renal cell carcinoma, and these preliminary assessments have shown that a similar response rate occurs ex vivo (*50*). In addition, cocultures of PDOs generated from tumors with high mutational burden, such as microsatellite unstable CRC and tobacco-related non–small cell lung cancer, can be cultured with peripheral blood lymphocytes from that patient to generate CD8^{+} T cell clones that proliferate owing to the presence of putative neoantigens (*51*). In principle, such cocultures could be used to optimize the response of effector T cells against that patient's neoplastic cells or to generate a large number of effector T cells targeting the neoplastic cells for adoptive cell transplantation.

Organoid cultures for personalized medicine approaches

An important goal for organoid research is to determine whether they may represent, by analogy to infectious diseases, a "bacteriology test" for an individual's cancer, and the U.S. Blue Ribbon Panel for the Cancer Moonshot has proposed this as an objective (www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative). To date, organoids derived from a variety of human tumors have shown a spectrum of responses to conventional and investigational drugs (Fig. 2). In limited cohorts of patients that were retrospectively analyzed, the response of PDOs to

therapies has largely mimicked the initial response of those patients to the same agents (*25*, *28*, *34*, *52*, *53*). These early studies have revealed multiple examples of exceptional responders to targeted drugs, where expected genetic alterations representing driver oncogenes or synthetic lethal pathways were logically matched to that therapy. The PDOs also provide models to develop drugs that circumvent innate or acquired resistance, and this has been shown to be particularly pertinent in the assessment of DNA repair pathways and replication fork stability in ovarian cancer PDOs (*28*). Importantly, the relative sensitivity of PDOs to cytotoxic drugs, which have a narrow therapeutic index in vivo compared with many targeted agents, may also reflect the clinical response of patients to those drugs (*34*, *52*, *53*).

Beyond the empiric potential of PDOs to help choose therapies for individual patients, large panels of PDOs representing one type of cancer have been used to develop biomarkers predictive of drug response for substantial numbers of patients. A recent study that used 66 pancreatic cancer PDOs compared standard cytotoxic drug responses to the gene expression of those PDOs and thereby derived a transcriptional signature of common responders to different chemotherapies (*34*). Although it is unknown whether the gene-expression signature reflects differences in drug pharmacology or drug response, when applied to an independent set of patient samples, this signature correctly identified a large group of patients with an improved response to that therapy (*34*).

To develop PDOs as a clinical test that can guide prospective cancer patient management, initial clinical studies should be designed that can measure the sensitivity and specificity of empiric PDO responses to a large number of the same patients treated with identical drugs. In parallel, other potential predictive biomarkers of therapeutic response, such as chemotherapy sensitivity gene expression signatures, can be assessed in a similar fashion. If the PDO empiric testing reflects patient responses in a large number of patients, PDOs should be further developed as a laboratory test and evaluated appropriately in a clinical trial. Currently, PDOs can be used to choose second-line or adjuvant therapies, because the time required to generate and test the PDO is on the order of 4 to 6 weeks. To shorten PDO development and drug testing to 1 week will require innovation but will also enable PDOs to be evaluated as a prospective test for cancer patients.

Current challenges in organoid research

Several challenges need to be addressed to improve organoid models, including the generation of cancer models that are currently not represented (for example, see Fig. 3), increasing the efficiency and decreasing the time for organoid outgrowth in current models, lowering the costs of organoid generation, and developing methods to perform high-throughput drug and immunotherapy screens (Table 1). Including TME elements besides immune cells and fibroblasts, for example, vascular and neural populations, will open additional research directions for organoid models. As these organoid models increase in sophistication, the genomic and epigenomic heterogeneity inherent in neoplastic cells will become important to consider as these are properties of cancer in humans that establish the behavior of the tumor and the response to therapies.

Outlook

By generating organoids and mimicking the TME, all phases of human cancer progression, including normal cells, preinvasive carcinomas, and invasive and metastatic cells, can be studied in the laboratory and stored in biobanks for worldwide dissemination. These cellular constructs can be interrogated for cancer biology and will undoubtedly continue to be a source of basic discoveries. For example, in certain early-stage human cancers, PDOs can be used to identify molecular aberrations that may serve as biomarkers and prevention targets. Additionally, PDOs are showing early promise in drug development, and clinical trials involving organoids should be designed to determine whether organoids are accurate mimics of a patient's cancer that may empirically predict their response to therapies. Organoids serve as a complement to other traditional cancer models, and the merits and deficits of each model system should be weighed when considering which one to use in cancer biology and medicine (Table 1). Organoid modeling is rapidly evolving, and although current challenges need to be addressed, the prospect that this approach will have a positive impact for basic cancer research and clinical advance is palpable.

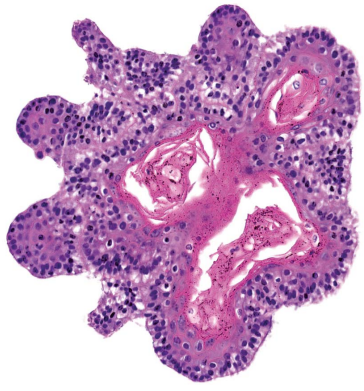

Fig. 3. An example of a new organoid model. Hematoxylin and eosin–stained section of an organoid grown from a squamous cell carcinoma of oral mucosa.

IMAGE: ELSE DRIEHUIS

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