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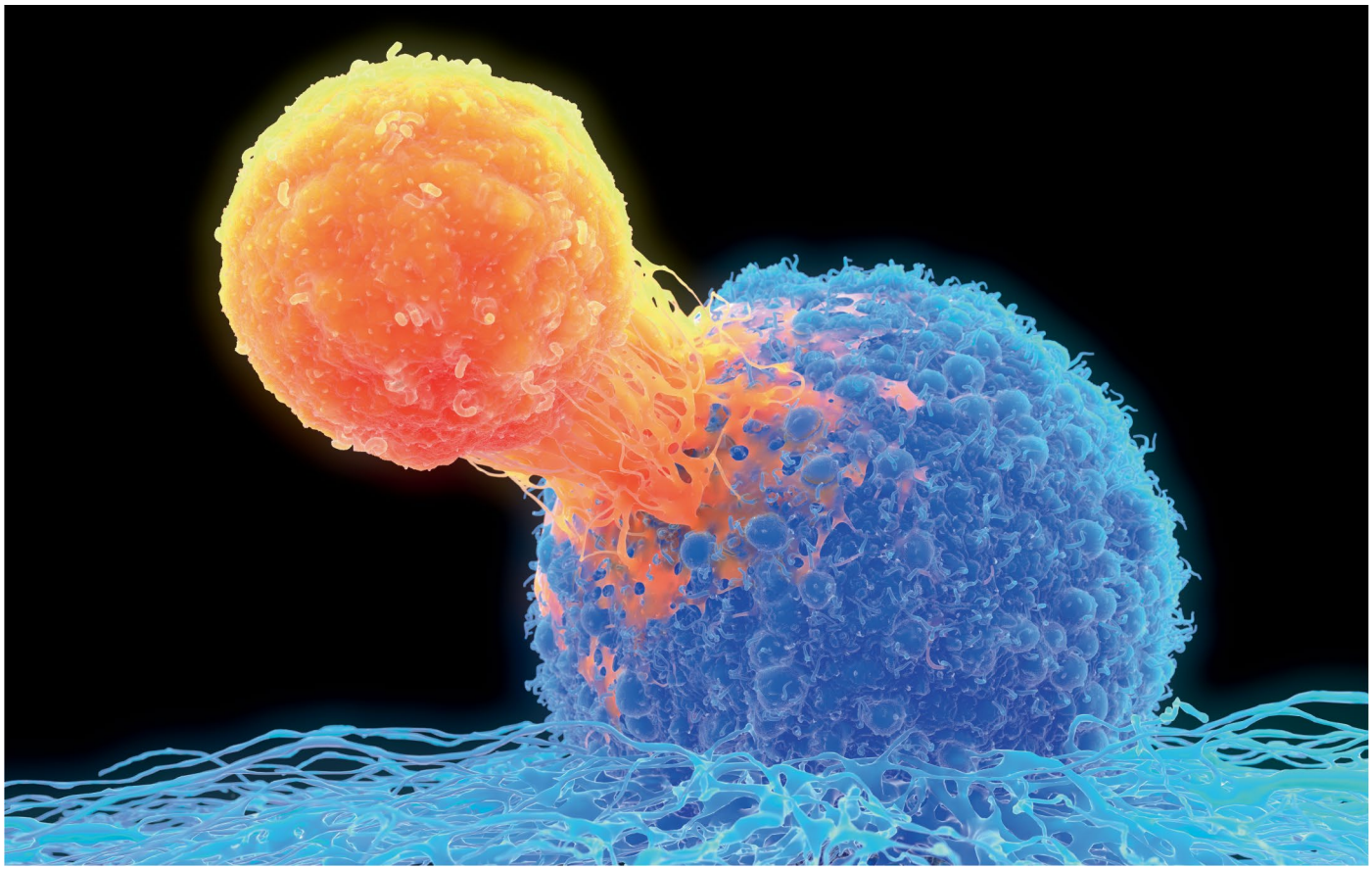


A T cell (orange) attacks a cancer cell (blue).

Closing in on cancer heterogeneity with organoids

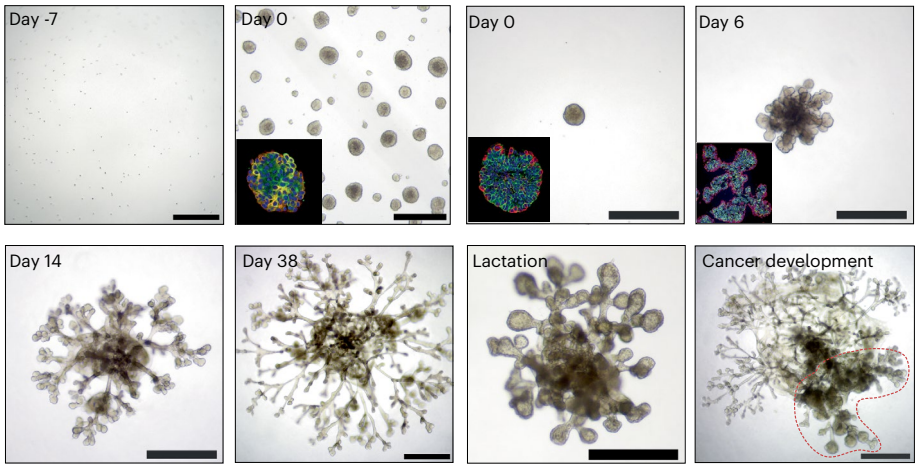
Organoids are an emerging way to model the fraught complexity of tumors.

By Vivien Marx

Tumors are heterogeneous universes and contain cells that can differ in shape, size and gene expression. Immune cells and fibroblasts associated with a tumor are also heterogeneous, as is the tumor microenvironment. Tumors from patients with the same cancer subtype differ. Such heterogeneity likely shapes the widely different responses to treatments.

Researchers model this heterogeneity with cell lines and mice, and some hope that organoids[1–3], which are three-dimensional structures that can be grown from cells or tiny tumor samples, can help to study how heterogeneity shapes cancer biology. "The field of organoids holds immense potential for creating in vitro organ systems that faithfully mimic the functionality of organs in our bodies," says Shang Cai from Westlake University in Hangzhou, China. Cai and colleagues have developed mouse organoids that model mammary gland development and breast cancer[4]. Takanori Takebe and his teams at Cincinnati Children's Hospital and Osaka University generate liver organoids from stem cells to better understand liver physiology and liver disease, such as the precancerous condition abbreviated as MASH.

Organoids aren't uniform structures, which is due to factors such different media used to culture organoids or the different ways source tissue is processed. Scientists want to be able to make organoids more reproducibly and grow, maintain and manipulate organoids at scale.



The Cai lab has generated a mouse mammary mini-gland that mirrors the physiology of the organ in vitro. Next, the team is working on a human mammary gland. Adapted from ref. 4, Springer Nature.

The challenges related to organoids aren't improving just yet, says Matthias Lütolf, a researcher at École Polytechnique Fédérale de Lausanne and the Institute of Human Biology, which is affiliated with Roche. The potential of organoids motivates researchers to chip away at the challenges. Given that the field is still in its early stages, it's not necessarily bad that one has different versions of the same organoid. "This kind of diversity is interesting and valuable in academic research," he says. But reproducibility and standardization are needed to use organoids in quantitative assays in pharmaceutical research and development. Cancer organoids let you do side-by-side comparisons and benchmark with the real thing: the patient's tumor, says Lütolf.

Unlike when using patient-derived cell lines, 3D organoid cultures let you study interactions between tumor cells and other cells in the tumor microenvironment, says Riyue Bao, a cancer researcher at UPMC Hillman Cancer Center and the University of Pittsburgh Department of Medicine. To recapitulate the complex tumor microenvironment, multiple organoids can be linked into assembloids.

An ethics advantage with organoids is reduced animal testing, says Bao. Animal models enable studies of human cancer biology in a living organism, but gene-editing tools can be more readily used in organoids and assembloids. Beyond patient-derived xenograft mouse models (PDX), scientists can use PDxOs, which are organoids derived from PDX models. They can experimentally cycle between in vivo and in vitro systems. "I believe PDxOs serve as a bridge," she says, to refine treatments and study cancer biology in ways that mirror the clinical scenario more closely than before, "But I am an optimist!"

Modeling cancer's phases

Organoids could help decode cancer's earliest stages. Takebe and his team, who developed hepatobiliary-pancreatic organoids[5], use liver organoids to detail the process of liver fibrosis in MASH, which can progress to liver cancer. They applied gene editing to manipulate metabolically relevant genes in liver organoids and worked out genetic factors that influence metabolic changes involved in MASH[6].

Lütolf likes how optogenetic approaches activate oncogenic driver mutations in a spatially and temporally controlled manner. Such methods, combined with bioengineered scaffolds, promise organoid models that model cancer's earliest phases. He and his team are developing a scaffold that integrates healthy tissue, tumor organoids and the tumor microenvironment.

Using their mammary gland organoid, the Cai team can assess the cell–cell communication that takes place at the inception of cancer development. "By inducing oncogene expression, we can observe and study the initial cellular responses that occur," he says.

It's been gratifying to the team to successfully culture and image these organoids and capture mouse mammary development, maintenance and pathogenesis. Over a lifetime, the gland changes with hormonal shifts during puberty, pregnancy and breastfeeding. In vitro the team can model signaling similar to that taking place when the mammary gland is in the body.

The scientists are now developing human mini-glands to study human mammary physiology "in ways that are not feasible within the human body," says Cai. Insights from these organoids, he says, can add to what is known about mammary gland biology and contribute to developing better diagnostics and breast cancer treatments.

Metastasis is tough to assess with organoids. One reason is that in the body, a tumor has both temporal and spatial invasiveness, says Bao. But organoids derived from metastatic tumors could help scientists pin down how cancer cells invade distant organs. It is promising to delve into this with real-time imaging and spatial 'omics technologies applied to organoids and assembloids.

Cancer clock

Cancer rears its ugly head over time. It's challenging to maintain organoids for extended periods to mirror this, but the Cai lab manages this to some extent. With mini-glands, the dynamic culture media mimic the estrous cycle and its oscillating hormones, he says. This hormonal environment drives the proliferation–differentiation cycle. The organoids stay functional for a while and mimic the organ's physiological processes.

The limited lifespan of organoids is indeed a challenge, says Lütolf. But there's progress. One approach he and his team along with colleagues at other institutions have explored is to grow organoids from intestinal stem cells into the shape of intestinal crypts[7]. The tube-shaped organoids have an accessible, perfusable interior. Such intestinal and other epithelial organoids last for weeks or months in some cases. "This opens up the possibility of modeling biological processes that take a long time to manifest themselves," he says. He and his team have been using this approach with colorectal cancer organoids to study tumor biology over several weeks.

Bao advises tracking genomic drift with sequencing and imaging. "This ensures the fidelity of our organoid models to their source tumors over time," she says. One should watch for shape changes and altered expression of markers because tumor organoids can gradually diverge from the original tumor's genomic profile. Repeated passaging can lead tumor organoids to lose their tumorigenic characteristics. "We're actively investigating the mechanisms behind this plasticity, considering factors such as epigenetic changes and microenvironmental influences," she says.

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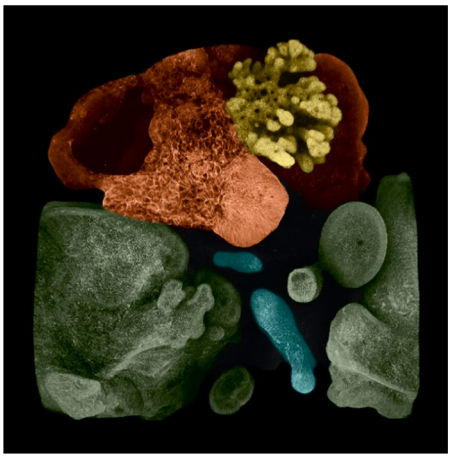
In her lab, organoid cultures can be maintained for over a year, says Anne Rios, a researcher at Princess Máxima Center for Pediatric Oncology in Utrecht, the Netherlands and the Oncode Institute, also in Utrecht. Typically, this happens through 'passaging', when the organoids are transitioned into single-cell suspension every other week and then regrown in a matrix. "These cultures are generally genetically stable, although this stability can vary based on the mutation profile of the patient tumor sample," she says.

One study[8], says Rios, compared various cancer models, including 657 cancer cell lines, 415 patient-derived xenografts mouse models, 26 genetically engineered mouse models and 131 patient-derived organoids. "Overall, they demonstrate that organoids exhibit one of the highest levels of transcriptional fidelity, especially when compared to xenografts mouse models and cancer cell lines," she says.



The Takebe lab developed hepatobiliary-pancreatic organoids, shown here with whole-mount immunostaining, from stem cells.

Protocol vigilance

"Organoids require meticulous attention to media composition and culture conditions to thrive and maintain their relevance to original tumor biology," says Bao. It's about preserving interactions between tumor cells, immune cells and stromal cells that are critical in cancer progression and treatment response. When she and her team work with patient-derived organoids from head and neck cancers, they see high culture purity of tumor organoids. But they faced hard lessons when they turned to develop lung cancer organoids. They consulted other researchers and learned, she says, that healthy lung organoids "might outcompete tumor organoids over time without careful oversight." Indeed, in culture, striking the balance between competing cell types is key, says Takebe. And, he says, it's "still work in progress."

One must be meticulous about protocols when culturing the mammary gland organoid, says Cai. "The mini-gland cannot be developed and maintained using a single medium; it requires a step-by-step induction process," he says. Stem cells are expanded, polarity is established, and symmetry breaking and pattern augmentation occur. He advises following the published protocol. Deviating from the protocol risks compromising "faithfulness of the organoids to the real thing."

The time it takes to establish patient-derived organoids varies, as do growth rates. This can be due to tumor stage, surgery site, metabolic activity and inherent immune characteristics, and it is not yet well understood. "From our point of view, this variability is invaluable for understanding both disease heterogeneity and the diverse responses to treatments observed in the clinic," says Bao.

Says Takebe, much organoid research has been advanced by Japanese scientists, and it's part of Japanese culture to care about fine details. Maybe that is "why we are a little bit poised for this type of very complex methodological development process," he says, laughing. He sees bioengineers increasingly entering the organoid field for its second phase, in which groups work to scale up organoid production and enhance reproducibility. His Cincinnati lab uses robots to culture organoids. Through a commercial venture with Molecular Devices, they license organoids for high-throughput assays at pharma companies.

A realistic in vitro model of cancer, says Lütolf, can't do without recreating key aspects of the complex tumor microenvironment. "We really need to go beyond the culture of cancer cells alone," he says. It's indispensable to capture the functional interaction between the cancer cells and the major cell types in the microenvironment, especially the immune cells, fibroblasts and blood vessels.

What's missing

Unquestionably, says Tak Mak, a cancer researcher at Princess Margaret Cancer Centre in Toronto, organoids are superior to work in cancer cell lines. But an important current shortfall with organoids is the absence of an immune system, he says. Some experimenters try to reintroduce lymphocytes into organoids, which in his view does not adequately address the ways immune cells function in the physiological or pathophysiological setting. This is true of both lymphocytes and myeloid cells, such as macrophages and others. These cells' half-lives, says Mak, especially those of macrophages, are short. To reflect the physiological modus, fresh monocyte infiltration and differentiation are needed. Nerves and blood vessels, which are important in healthy organs, "are missing in organoids," he says. But sensory, parasympathetic and sympathetic nerves play important roles. Blood vessels bring key factors and nutrients into organs.

Organoids certainly complement cancer research done with mouse models, says Mak. Mice can be readily generated. One can access tissues and immune cells in these animals and match the peptide–major histocompatibility complexes and antigen-specific T cells in the mice. But mice and humans differ in many pathophysiology aspects. In mice, telomeres – the DNA sequences at the outermost chromosome tips – are three times as long as in humans. Mice and people have different myeloid cells. For example, PirA and PirB, the mouse immunoglobulin-like signaling receptors on myeloid cells, have five to six human equivalents, including human immunoglobulin-like receptors LILRA and LILRB.

Currently, labs making organoids lack an efficient way to synthetically generate functioning immune cells from stem cells, says Takebe. Coculture with immune cells retrieved from patient's blood is a way to capture an individual's immune profile. But even this, he says, is not obvious to do, and it's, he says, "kind of a dream experiment." The fetal liver environment is a niche for hematopoietic cell and blood cell development processes. One could add such a niche component to a liver organoid to try to orchestrate development and differentiate the lineages. He and his team are developing fetal liver ecosystems in organoid culture to generate systems with model immune systems.

Takebe thinks highly of approaches such as those in the Lütolf lab to build engineered perfusable systems. Another approach he and his group apply is to implant the organoids into animals such as immunocompromised mice to leverage the animals' circulatory and endocrine systems. This extends the lifespan of organoids and lets the scientists characterize a gradually maturing organoid in vivo. The scientists are adding complexities to the liver organoids, such as by adding cell types at the right time in organ development and at the right spatial coordinates. The plan is to translate learned principles to coax immature progenitor cells into a complex tissue.

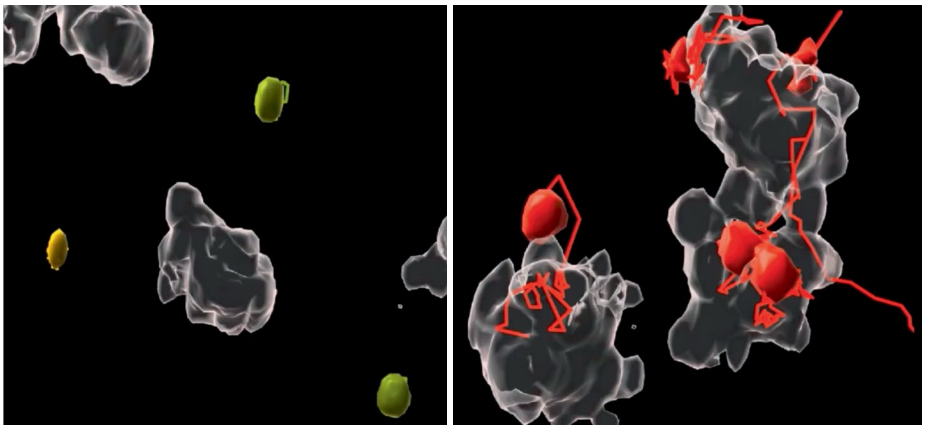
CREDIT: K. IWASAWA, A. KODAKA, T. TAKEBE, CINCINNATI CHILDREN'S

Bao uses patient-derived organoids to study therapies and mechanisms of drug resistance. She and her team explore how well p38 mitogen-activated protein kinase (MAPK) inhibitors combine with immune checkpoint inhibitors. The idea came from a clinical trial in which patients on this regime for whom conventional therapies hadn't worked had cancers go into remission. Both patient-derived organoids and mouse models will help to understand differing responses.

In a perfect world, she says, one would have an organoid "avatar" of every patient's tumor so that one could treat that system and then monitor treatment in patients. She also wants to coculture tumor organoids with immune cells from the tumor microenvironment.

In her work on pediatric cancer, Rios focuses on immune mechanisms and the harsh reality that, in children, cancer is the number one killer from disease. Treatment options for pediatric cancers lag behind those of adult cancers, especially for solid and brain tumors, she says. Unlike adult cancer, childhood cancer stems from embryonic tissues, "but we're still using treatments designed for adults," she says. Pediatric cancers are, compared to breast cancer, relatively rare and receive less funding. She and her team hope to uncover what makes pediatric cancers different by using BEHAV3D[9], which she and her team and colleagues at other universities developed. This imaging-based platform is for assessing engineered T cells used in immunotherapy, which harnesses the ability of T cells to locate and physically destroy cancer cells. BEHAV3D reveals behavioral differences between T cells engineered with different types of T cell receptors. Transcriptomic profiling and analysis of imaging-based readouts indicate which are "super-engager killer T cells."

Engineered T cells have shown some encouraging results in patients with diffuse midline glioma. Alas, the cancer relapses after a few months. The scientists are using BEHAV3D on patient-derived tumor organoids to compare how well several engineered T cells targeting this brain tumor perform. They want to identify the engineered T cell product with the most super-engagers. Once they find the cancer organoid, "they stick to it and don't let go until the organoid is killed," she says. These super-engagers could indicate an immunotherapy approach to these currently incurable pediatric brain tumors. The platform is a way to thoroughly characterize an engineered T cell product's cell composition, function, strengths and pitfalls.



BEHAV3D is a platform from the Rios lab that can reveal the different behaviors of T cells engineered to attack tumors. Some (left) don't attack the tumor organoid and either remain static or die while others (right) are 'super-engagers'. Adapted from ref. 9, Springer Nature.

BEHAV3D, she says, can capture how complexity and composition of individual tumors shape how T cells respond. It can show when treatment stops working – when T cells seemingly get lazy and slow down or even ignore tumor organoids rather than attack them. BEHAV3D has a module for comparing different responses to T cells between tumors from different patients, and it can be used to assess variations within the same tumor. With organoids one can simulate these differences and variations.

Thus the way organoids show biological differences mimics the complexity of individual tumors. Variations can influence T cell therapy effectiveness in terms of timing; perhaps not all organoids are killed simultaneously. Some organoids may be more susceptible to engineered T cells than others. "We are currently studying how this relates to the observed heterogeneity of response observed in patients for such therapy," she says. Future plans, she says, are about broadening the platform's capabilities to include other immunotherapies, such as bispecific antibodies.

Organoids next

Advanced imaging, computational biology and machine learning are greatly changing how useful organoids are in cancer research, says Bao. With high-resolution imaging, researchers can observe cellular behaviors and interactions in real time. Computational models and machine learning algorithms can predict outcomes on the basis of complex data modalities. It's possible to identify patterns and changes in gene expression, cell morphology and movement that are not discernible to the human eye.

"These technologies have opened new avenues for understanding the heterogeneity of tumors and their response to drugs at an unprecedented level of detail," she says. Certainly, organoids present several cultivation and modeling challenges but, she says, "I believe they are indispensable for unraveling the intricacies of cancer biology and personalized medicine."

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