Giving organoids room to grow

These 3D structures derived from human cells can be an improvement over simple cell lines, but organoids can still lack important physiological cues for development. Finding the right in vivo environment can take things a step further.

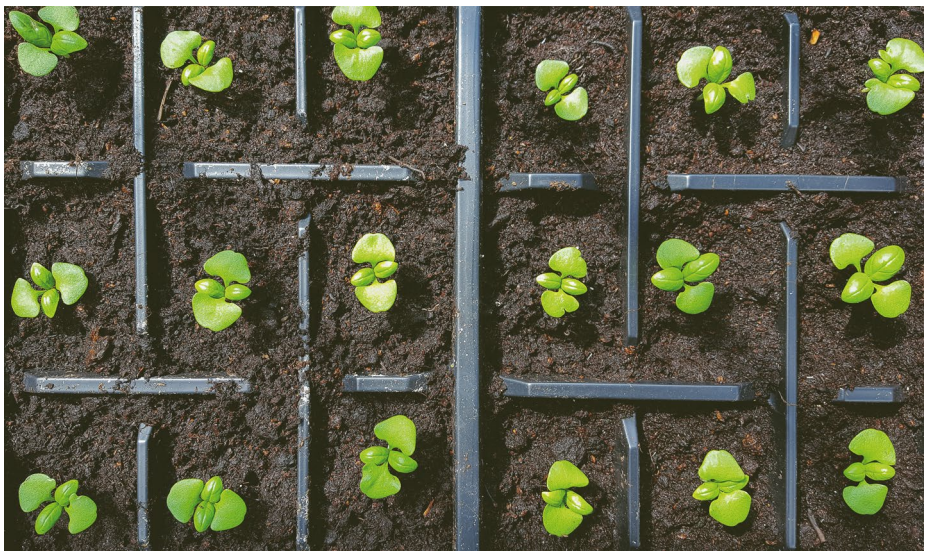
Michael Eisenstein

Pancreatic cancer is a stealthy foe, typically only revealing itself when effective treatment options are limited—or nonexistent. Roughly 90% of patients receive their diagnosis at advanced stages of disease, when surgery is no longer an option. David Tuveson's team at the Cold Spring Harbor Laboratory hopes to flush this disease out of hiding with sophisticated models that can essentially re-enact the earliest stages of onset and progression for actual patients' tumors: 'organoids,' transplanted in vivo.

While cell lines are often the fastest and most economical tool for exploring biological questions, they can also be a poor surrogate for complex living organisms. These cells are separated from their physiological context, grown on flat artificial surfaces, and often contain extensive genetic abnormalities that allow them to grow continuously in such conditions. For cancer research in particular, such cultures bear little resemblance to the diverse and complex cellular ecosystems found within real tumors.

Organoids are 3D tissues formed from patient-derived cells, which are cultivated in a manner that promotes assembly into multicellular structures that mirror key features of their origin tissue. Under the right conditions, researchers can generate organoids that replicate components of the liver, kidney, intestine, and brain—as well as virtually any tumor. "You can get an organoid that looks a lot like a human cancer does under the microscope," says Jatin Roper, who uses these models to study colorectal cancer at Duke University.

Although powerful on their own, organoids grown in a dish can only develop so far without guidance from the symphony of physiological cues produced in the body. To unlock this potential, researchers have been transplanting organoids into rodent hosts. In vivo, the organoids can flourish and offer an unprecedented view of diverse biological processes. "We can recapitulate the early stages of pancreatic cancer, before it invades and spreads," says Tuveson. "That's valuable because we don't really see humans with early pancreatic cancer."



Room to grow. Transplanting organoids into the right environment can help them reach even greater research potential. Credit: Chris Winsor / Moment / Getty

The findings from these models could enable earlier diagnoses or reveal new treatment options. Many other investigators are now turning to similar transplantation systems to study organ development and disease pathology, and even laying preclinical groundwork for organoid-based regenerative medicine. However, for these implants to flourish, researchers must also ensure that the organoids are finding the supportive conditions they need to feel at home within their rodent recipient.

No place like home

Left to their own devices in vitro, organoids can quickly self-assemble into relatively complex structures. For example, Takanori Takebe's team at Cincinnati Children's Hospital routinely converts human pluripotent stem cells into 'liver buds'—highly organized assemblies of multiple cell types that resemble the developing embryonic liver. But as they grow, these organoids are threatened by starvation due to lack of a working circulatory system. "Maintenance is really challenging in vitro," says Takebe, "After 24 days of culture, they're dying off."

Other organoid types can be more resilient, but nevertheless fail to reach their full functional potential outside the context of a living body—for example, tumor organoids comprised primarily of cancer cells may lack essential features that contribute to growth and response to therapy. "The traditional understanding of cancer was that it is a genetic disease driven by signaling pathways," says Roper. "But we're realizing more and more that many other cell types in the body are also responsible for cancer progression." Indeed, there is now considerable research into how the 'tumor microenvironment' formed by a patient's vasculature, immune system, and other surrounding cells influences pathology.

Transplantation models allow researchers to essentially plug immature organoids into living systems, thereby allowing them to develop further and even integrate with their host. "The complexity we observe

is remarkable," says Jason Spence of the University of Michigan, who works extensively with intestinal organoids. "Organoids remodel from a very simple fetal-like structure to a complex tissue with crypts and villi that form after transplantation." However, researchers must overcome a number of technical challenges to ensure the stable integration, or 'engraftment', of these implants.

As with any real-estate deal, location is key. Early in vivo efforts tended to entail implantation beneath the surface of the skin, a site that is easy to access and monitor. Such subcutaneous implants may survive and grow more robustly than they would in vitro, but they are still vulnerable to arrested development due to a lack of tissue-specific growth and developmental cues. Orthotopic transplantation, in which the organoid is placed into its native tissue environment or a near equivalent, tends to yield better, more physiological results, explains Toshiro Sato, a researcher at Keio University who was among the early pioneers of the organoid field. "But orthotopic transplantation is less accessible than subcutaneous transplantation, and thus technically more difficult."

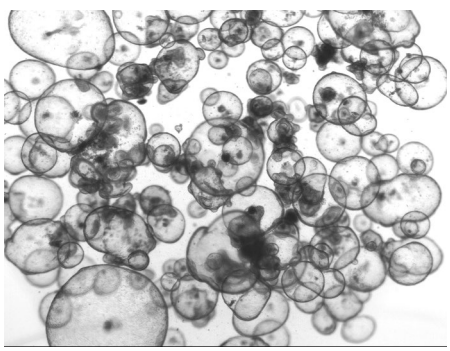
As a postdoc in Ömer Yilmaz's lab at MIT, Roper helped devise a strategy that uses colonoscopy to guide the injection of colorectal organoids at specific sites within the mouse intestine[1], a technique that requires specialized equipment and expertise. But the results are worth the effort. "We can model early tumors called adenomas as well as the metastatic process, from the initiation of the tumor through invasion of the muscle layer and spreading to the liver," Roper says.

Other organoids pose an even more daunting challenge. For example, Hongyan Zou and colleagues at the Icahn School of Medicine at Mount Sinai recently grappled with the implantation of large organoids modeling the human dorsal forebrain in neonatal mice[2]. This required a delicate surgical procedure to ensure that there was room to implant the organoid within the skull without seriously damaging the surrounding tissue. But Zou's efforts ultimately proved successful—"the engraftment rate was close to 100%," she says, noting that they subsequently observed efficient infiltration of mouse blood vessels into the newly transplanted organoids.

That being said, some organoids can still flourish at non-orthotopic sites, if given appropriate support. For example, Takebe has been able to get his liver buds to engraft and mature within the outer layer of the kidney, a tissue that is more accessible than the interior of the liver but also offers a dense network of blood vessels to sustain organoid growth. The lung is another challenging target for transplantation; Spence's group has partnered with bioengineer Lonnie Shea to develop synthetic scaffold materials that allow lung organoids to engraft and even form functional airways when transplanted into deposits of fat cells located within the mouse abdomen[3]. "There's something about the physical environment at the time of transplantation that seems to be important for lung organoids, but we don't quite understand it yet," says Spence.

Even more sophisticated models are also possible. Although many orthotopically implanted organoids can efficiently hook up with the host vasculature and surrounding tissues, they may still lack key attributes of the original organ. Spence notes that the absence of neuronal wiring is a particular shortcoming of many gut organoid models, but his laboratory and others have demonstrated the potential to restore this component of the intestinal tissue by co-cultivating separate gut and neuronal organoids prior to transplantation[4]. "If you mix them together before transplantation, the neurons incorporate into the organoid," he says, "and then you can start to get peristaltic-like motion."

As the organoids are derived from foreign cells, the transplants must also be insulated against immune rejection, regardless of where they end up. For their cerebral organoid experiments, Zou's team used 10-day-old mouse pups—an early age at which the immune system is not fully active, and which allowed the grafted organoids to grow unperturbed for as long as a month. But in most cases, organoid transplants must be performed on animals that have been genetically or pharmacologically rendered immunodeficient. This can be a serious limitation for some cancer studies, given that many contemporary therapeutic strategies exploit the interplay between tumors and immune cells. However, Roper, Yilmaz and colleagues have demonstrated that such features can be safely recapitulated with mice that have been genetically engineered to have 'humanized' immune systems[5].



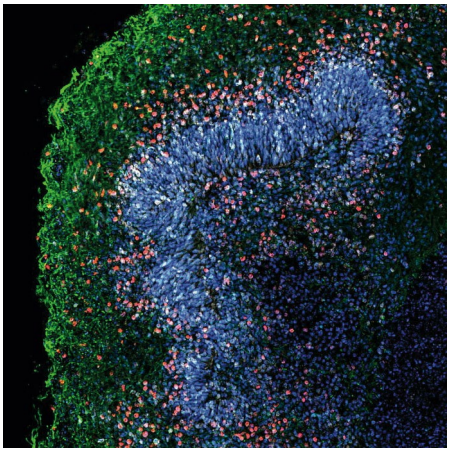
Colon organoids under a light microscope. Credit: J. Drost

Truer tumors

In the realm of cancer research, this new generation of organoid-based models is providing a more accurate picture of how tumors behave in the body. Genetically modified mice, in which researchers selectively 'knock out' genes that protect against cancer or 'knock in' genes that promote tumorigenesis, have long been a mainstay of the oncology world. But Jarno Drost of the Princess Máxima Center in the Netherlands notes that these are also something of a blunt instrument for studying a very complex process, citing his own experience with a knockout mouse strain commonly used to study colorectal cancer. In many cases, Drost notes, these animals develop so many colorectal tumors that they end up with complications such as intestinal blockage, which can force the premature end of a cancer study. "It's very difficult to follow tumorigenesis until the stage of metastasis," he says.

Drost's group, in collaboration with Jacco van Rheenen at the Netherlands Cancer Institute, has demonstrated that orthotopically transplanted organoids show much more localized tumor growth within the intestinal wall[6], like doctors would typically see in a human cancer patient prior to the onset of metastatic spread. These tumors subsequently progress to more advanced stages of the disease. The team is able to employ 'intravital imaging' methods—in which internal tissues are microscopically visualized through a literal window implanted in the mouse—to monitor the tumor over time. "It grows until the stage that it metastasizes to the lung and liver, where these cancers normally spread to," he says.

The efficient and precise genome-editing capabilities conferred by CRISPR technology have also proven a valuable asset for these studies. The methods for genetically manipulating mice are expensive and time-consuming, requiring multiple rounds of breeding and screening to obtain a well-defined strain—and doing so for multiple genes is even more laborious. By using CRISPR, researchers can easily introduce multiple mutations into an organoid or selected cell types within that organoid, and then study the behavior of that genetically modified tissue in vivo. Roper's team is using such an approach to study genetic factors associated with both colorectal



Dorsal forebrain organoid. Credit: H. Zou

cancer onset and normal development of the intestine. "It's a very elegant way to study gene function in a mouse without having to generate the mutation directly in the mouse," he says.

Because tumor organoids are derived directly from biopsy specimens, they are also a promising tool for the identification of patient-specific treatment regimens. For example, Tuveson's group is now routinely generating pancreatic tumor organoids from specimens provided by various hospitals across Long Island. These can be efficiently used for in vitro drug testing of many different regimens for multiple patients in a relatively short period of time. "Using these organoids, we found that we could classify patients into groups that would either be sensitive or resistant to the chemotherapy we use for pancreas cancer," he says.

Transplanted organoids could yield even greater predictive power. Many cancer centers are making use of 'patient-derived xenografts' (PDX), in which surgically excised tumor tissue is directly implanted and allowed to grow within a mouse. These animals can then be dosed with different therapies in an effort to identify an effective tumor-killing strategy. But biopsy tissue is limited and precious—in many cases, just a thin thread of material—and multiple rounds of grafting are typically needed to prepare enough PDX mice for drug testing. As an alternative, Tuveson, Drost and others are using biopsies to generate multiple organoids in vitro, which can then be implanted in many mice in a single go. This can trim weeks off the time required to generate a model, and tends to produce tumors that closely reflect the original cancer both histologically and genetically. For example, researchers led by Nicola Valeri at the Institute of Cancer Research in London, used a combination of in vitro cultured and transplanted patient-derived organoids to test various regimens for treating gastrointestinal cancer[7]. Their results showed that these models had 100% predictive value in terms of identifying treatment approaches that would prove unsuccessful in patients, and an 88% success rate for identifying effective regimens.

Almost human

Organoid transplant models might also help researchers dissect processes that would be challenging or impossible with either human subjects or animal models. For example, Zou is making use of organoid models to study the impact of trauma from hypoxia on early brain development. "Episodes of brief hypoxia can cause a lot of neurological learning disabilities," she says. "This is very difficult to study in human babies, and animal models don't have certain primate-specific neuron progenitor pools." Similarly, by using orthotopically transplanted organoids to study specific sub-populations of human intestinal stem cells, Sato's team has identified important differences in the way these cells divide and behave in vivo relative to their mouse counterparts[8]. "This suggests some data derived from mouse intestinal stem cell studies may not be true for humans," he says. Sato also notes that human organoids could prove powerful for studying pathogens that do not normally infect or cause disease in rodents.

The insights gained from these and future transplantation studies might ultimately lead to rapid strides in regenerative medicine—giving patients ready access to compatible donor tissue to repair damaged or diseased organs. Takebe's team has already obtained compelling evidence that transplanted liver buds can improve survival of animals with liver disease, and he and collaborators in Japan are looking to embark on clinical trials within the next couple years. One possibility would be to use these organoids as a short-term fix for newborns with congenital liver metabolic disorders. "We are thinking of this as a 'bridge', using liver bud transplant until they reach the age of six months and are able to get a full transplant," says Takebe. Sato notes that another group in Japan, led by Mamoro Watanabe at Tokyo Medical and Dental University, is now planning the first human transplantation trial, using gut organoids to repair the damage from ulcerative colitis.

Spence is cautious about clinical applications, and notes that although his team hasn't seen any serious complications or cancer risk after transplantation in rodents, it would be reassuring to have longer term safety data—perhaps including some work in larger models such as pigs—before moving to humans. Confirming safety is likewise at the top of Takebe's agenda; his Japanese collaborators have already established a robust workflow for the reproducible manufacture of liver bud organoids at a scale suitable for use in human patients, and are currently monitoring the long-term health effects of transplanting these organoids into immunodeficient mice. "We are waiting for these safety assessment results," he says, "and then we can go forward with the next step." ❐

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Published online: 17 June 2019
https://doi.org/10.1038/s41684-019-0333-7

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