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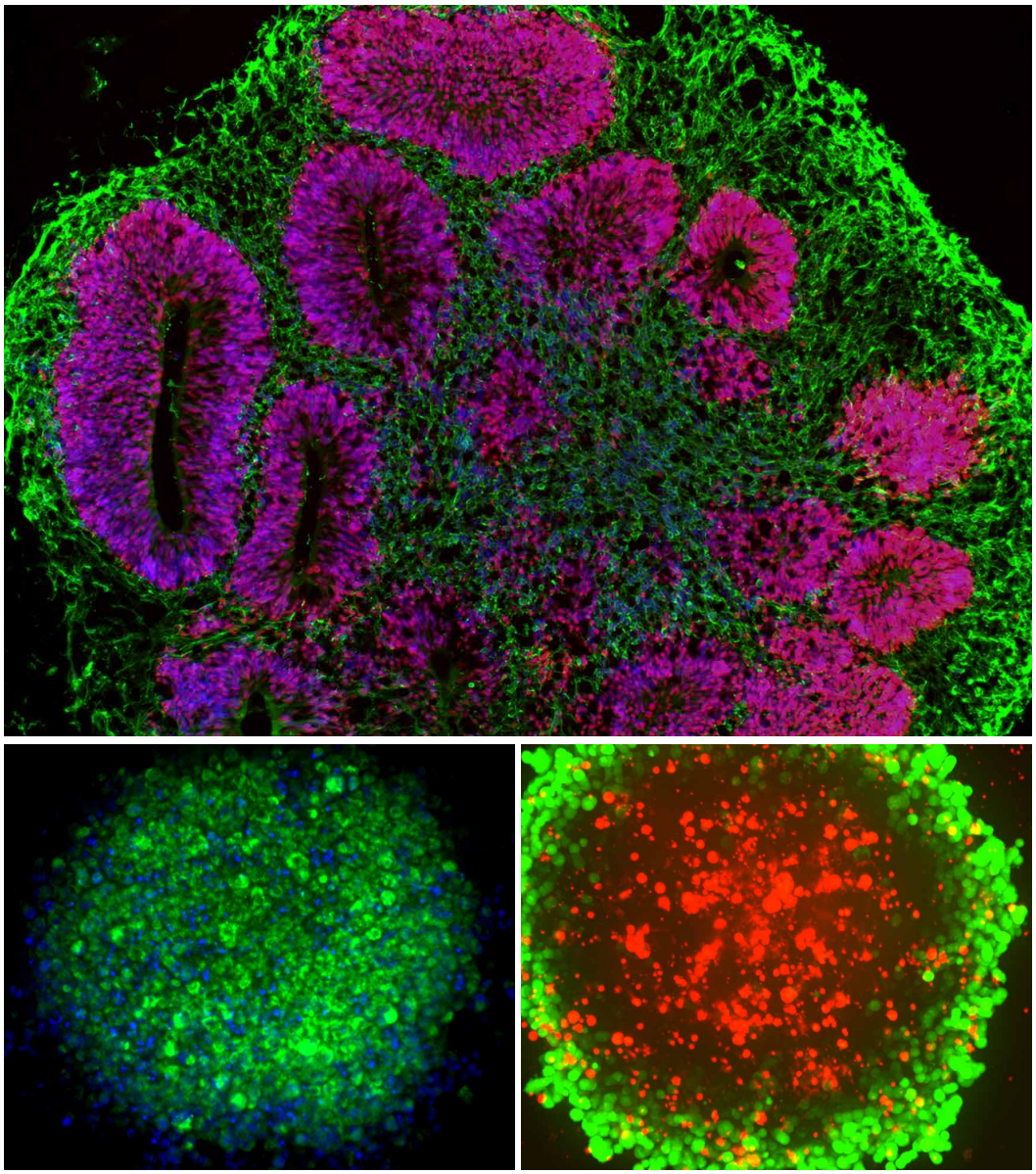
Organoids



Organoid models include three-dimensional (3D) cell culture systems that closely resemble the *in vivo* organ or tissue from which they are derived. These 3D systems replicate the complex spatial morphology of a differentiated tissue, and allow biologically relevant cell–cell and cell–matrix interactions; ideally, sharing similar physiological responses found within *in vivo* differentiated tissues. This is in contrast to traditional two-dimensional (2D) cell culture models that often bear little physical, molecular, or physiological similarity to their tissue of origin.

Although the earliest 3D organoid models were first described over 40 years ago, their utility is only recently exploited further. Early organoid models required large numbers of starting cells, were not amenable to high-throughput screening, and often exhibited limited *in vitro* viability [1]. These drawbacks have now been largely eliminated as advances in multipotent stem and progenitor cell isolation have allowed researchers to develop highly reproducible, long-lived organoids.

The rapid developments in organoid technology, and the wide usage of the term organoid for a variety of both *in vitro* and *in vivo* structures, led Lancaster and Knoblich to suggest a basic definition for organoids. They defined organoid as:

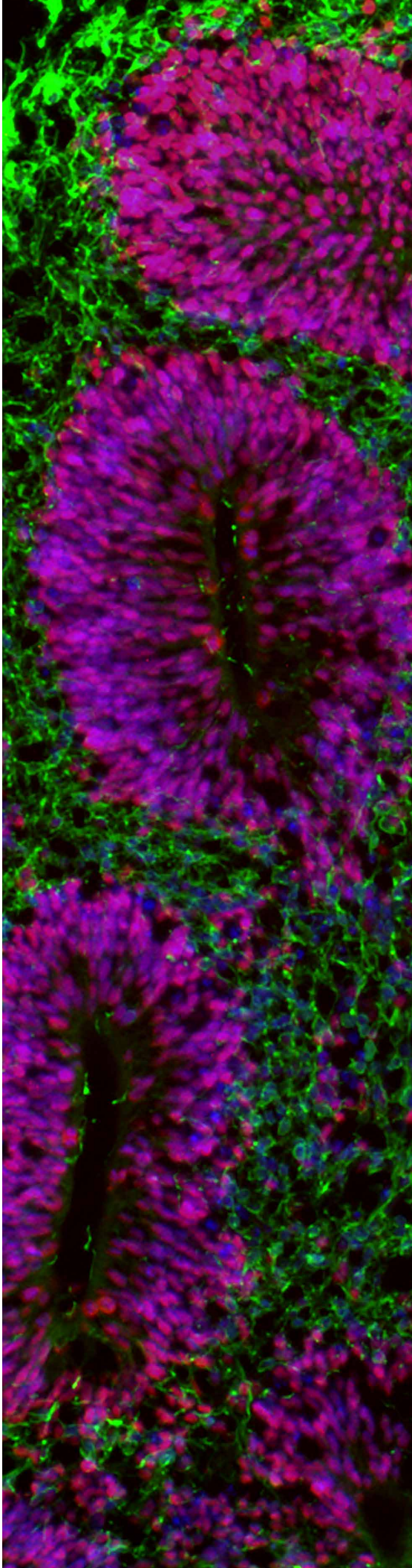
> "A collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*".

According to Lancaster and Knoblich, an organoid should possess several important features characteristic of the respective organs: "(1) it must contain more than one cell type of the organ it models; (2) it should exhibit some function specific to that organ; and (3) the cells should be organized similarly to the organ itself" [2].

In 2009, Clevers and Sato used adult stem cells from mouse intestine to create the first mini-gut organoids from murine cells [3] and later extended their method to human epithelial organoids [4]. These organoids were expected to allow researchers to gain new insights into the biology of gut health and disease, including colorectal cancer.

This method inspired many other scientists to create a variety of organoids from mouse and human tissues. These clumps of cells are small enough to survive without blood supply, yet large and complex enough to teach us something about tissue and whole-organ development and physiology.

A typical organoid protocol starts with isolated embryonic or pluripotent stem cells, which are then cultured in a supporting scaffold (such as Matrigel™ matrix) that enables three-dimensional growth. Organoids comprise multiple differentiated cell types that are found in the relevant organ *in vivo*. For example, all cell types of the intestinal epithelium are represented in the Matrigel matrix–based model described by Sato et al. [3]. The signaling pathways governing organoid formation were found to be identical to those used during *in vivo* organ development and homeostasis; thus, cytokines, growth factors, and small molecules were also included in the culture medium in order to activate or inhibit specific signaling pathways. Even tissues that are closely related, such as the small intestine and colon, require different combinations of signaling molecules in the process of organoid formation [4].



There are different ways to obtain an organoid culture, and some of these are just beginning to be explored. On the following pages you will find some examples of various organoid models that have been developed, with an emphasis on the cytokines, growth factors, and small molecules that were used.

Gastrointestinal (GI) organoids

Historically, preclinical GI medical research has relied entirely on animal models and cancer cell cultures that are of limited relevance to human physiology; thus, the ability to obtain GI organoids from human cells is of great importance [4,5].

The intestinal epithelial layer is made up of tiny, slender projections called villi. The niches formed at the bases of the villi, known as crypts, are home to the intestinal stem cells responsible for constant renewal of the intestinal lining. In the original study that generated murine small intestine organoids, epidermal growth factor (EGF), R-spondin-1, and noggin were included in the medium [3]; whereas a later study that demonstrated the formation of murine colon organoids added Wnt-3a to the above three growth factors. The generation of small intestine and colon organoids from human cells also required two small molecules, TGF-β inhibitor (A 83-01) and p38 MAP kinase inhibitor (SB 202190), in addition to the above-mentioned cytokines [4].

In a 2014 study, human intestinal organoids (HIO) that were produced *in vitro* from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), were engrafted *in vivo* and became functioning mature intestinal tissue. For induction of definitive endoderm (DE), human ESCs or iPSCs were treated with medium containing activin A, followed by a medium containing activin A, FGF-4, and GSK-3 inhibitor, CHIR 99021, to form spheroids. The spheroids were then plated in Matrigel matrix, and maintained in intestinal growth medium supplemented with EGF and noggin to generate the HIO that were later engrafted into immunodeficient mice [6].

Brain organoids

The complexity of the human brain, which made it difficult to study many brain disorders in model organisms, called for the establishment of an *in vitro* model of human brain development.

A protocol for generating 3D brain tissue called cerebral organoids, which closely mimics the endogenous developmental program, used patient-specific iPSCs to form a 3D organoid culture model of microcephaly, a disorder that had been difficult to reproduce in mice. These iPSCs, resulting from reprogrammed patient skin fibroblasts, were incubated in a medium supplemented with FGF-basic, CHIR 99021, and MEK inhibitor (PD 0325901) for 21 days. The outgrowing colonies were picked and passaged on inactivated CF-1 mouse embryonic fibroblasts (MEFs). Later, single cells were plated in media containing low levels of FGF-basic and high levels of ROCK inhibitor (Y-27632). The neuroepithelial tissues that were formed were transferred to droplets of Matrigel matrix, and after a period of stationary growth, these tissue droplets were transferred to a spinning bioreactor containing differentiation medium supplemented with retinoic acid. Analysis of these patient organoids demonstrated premature neuronal differentiation, which could explain the disease phenotype [7].

In a 2016 study, a miniaturized spinning bioreactor was developed to generate forebrain-specific organoids from human iPSCs. Detached human iPSC colonies were transferred on day 1 to a 6-well plate with stem cell medium containing A-83-01. On days 5 and 6, half of the medium was replaced with induction medium containing Wnt-3a, CHIR 99021, and a selective TGF-β inhibitor (SB 431542). On day 7, organoids were embedded in Matrigel matrix and grown in induction medium for 6 more days. On day 14, organoids were dissociated from Matrigel matrix and 10–20 organoids were transferred to wells of a 12-well spinning bioreactor containing differentiation medium. At day 71, the differentiation medium was changed to maturation medium, containing BDNF, GDNF, and TGF-β1. The organoids could grow beyond 110 days in maturation medium with medium change occurring every other day. These organoids were used to study the effects of Zika virus exposure on the brain, and could be employed in the future for drug testing. In addition, this platform was also used to generate midbrain and hypothalamus organoids from human iPSCs [8].

B cell follicle organoids

When naïve B cells encounter antigens, they form clusters of cells called germinal centers in a lymph node or in the spleen; here they proliferate, mutate to produce high-affinity antibodies, and undergo clonal expansion. Recreating this process using 2D cultures *in vitro* has traditionally been difficult.

Instead of using the conventional Matrigel matrix for 3D culture, a gelatin and silicate-nanoparticle mix that mimics the environment of the body's lymphoid organs was developed. Naïve B cells obtained from splenocytes were co-cultured with engineered stromal cells expressing both CD40L and B cell activating factor (BAFF) in a medium containing murine IL-4. The B cells in these organoids matured much faster than in 2D cultures and displayed class switching within days [9].

Liver organoids

Liver development involves an intricate interaction of tissues derived from both the endoderm and mesoderm. The liver is initially derived from endoderm hepatic bud structures, which develop from foregut epithelium. The hepatoblasts derived from hepatic buds contribute hepatocytes and biliary epithelium, whereas liver fibroblasts and stellate cells originate from nearby mesoderm-derived mesenchyme [2,10]. An established approach to generate human liver bud–like tissues employed a mixture of three cell populations, mimicking the early cell lineages of the developing liver: human iPSC–derived hepatic cells, human mesenchymal stem cells, and human endothelial cells. For endodermal differentiation, human iPSCs were seeded on a Matrigel matrix–coated dish in medium containing activin A. Human iPSC–derived endodermal cells were then treated with a medium containing human FGF-basic and BMP-4 for differentiation of hepatic endoderm cells (iPSC-HEs). Human iPSC-HEs were then cultivated with two stromal cell populations: human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (MSCs). The cells spontaneously formed 3D liver buds when mixed at a high density on a layer of Matrigel matrix. When these liver buds were transplanted into mice, they displayed vascularization and showed liver-specific functions, and transplanted mice survived the drug-induced lethal liver failure model [2,11].

Retina organoids

The eye is a highly complex organ and consists of a variety of cells that are combined in an organized 3D fashion. The retina is the light-receptive neural region of the eye and is derived from the neural ectoderm. Two adjacent epithelial layers are formed early in retinal development: the outer retinal pigmented epithelium and the inner neural retina, which eventually become tissue-containing layers of photoreceptors and supportive cell types [2,12].

Optic cup organoids were generated from human ESCs, and were compared to similar organoids generated from mouse ESCs. For retinal differentiation, hESCs were reaggregated in retinal differentiation medium containing Y-27632. Matrigel matrix was added from day 2 to day 18. For optic cup formation, CHIR 99021 (or recombinant Wnt-3a) and recombinant human Sonic Hedgehog (Shh) were added to the differentiation medium from day 15 to day 18. These human retinal organoids shared many characteristics displayed by mouse retinal organoids; however, they showed several human-specific differences. In particular, the human retinal organoids were larger than mouse organoids, developed more slowly, and grew into tissue-comprising multilayers that contained both rods and cones (cone differentiation is rare in mouse ESC culture) [2,13].

Kidney organoids

The kidney differentiates from the intermediate mesoderm (IM) through the interaction of IM-derived metanephric mesenchyme (MM) and a formed ureteric bud (UB). Nephron progenitors derived from the MM are the source of nephrons, while the IM itself is derived from the posterior primitive streak [14,15].

Human ESCs that were cultured on irradiated mouse embryonic fibroblast feeder cells were plated in a Matrigel matrix–coated 96-well plate. After overnight culture, cells were exposed to BMP-4 and activin A, or alternatively to CHIR 99021, in a serum-free medium, then cultured in FGF-9 and heparin-containing medium to induce IM cells. These cells were subsequently incubated in a medium containing FGF-9, BMP-7, and retinoic acid (in the case of cells induced with BMP-4 and activin A), or FGF-9 and heparin (in the case of cells induced with CHIR 99021). For the induction of kidney organoids, hESC-derived kidney cells were dissociated into single cells, spun down to form a pellet, placed onto a filter membrane with a collagen IV coat, and floated on culture media. This study successfully differentiated hESCs under chemically defined culture conditions, using growth factors that participate in normal embryogenesis, and resulted in coordinated generation of UB and MM that formed *in vitro* self-organizing 3D structures, including nephrons [15].

Lung organoids

The lungs, which are responsible for the respiration process, are composed of a variety of specialized cells that construct a complicated branched system [16]. Derived from adult lungs, epithelial stem and progenitor cells, including basal cells, airway secretory club cells, alveolar epithelium type II cells (AEC2s), and embryonic and induced pluripotent stem cells, can be used to derive mouse and human lung organoids [16]. Modeling of lung diseases was traditionally done using two-dimensional (2D) cell lines, genetically engineered mouse models (GEMM), and patient-derived xenografts (PDXs); however, each has its limitations [17]. With the purpose of overcoming these limitations in the study of lung disease, more advanced lung organoids systems were used to develop more valid and relevant disease models. These models have been used to study basic disease development, as well as drug screening for a variety of respiratory diseases, including cystic fibrosis, various lung cancers, and infectious lung diseases, including SARS-CoV-2 [17].

Lung organoids were used in the search for alternative receptors to AEC2s that facilitate infection by SARS-CoV-2. In order to obtain lung organoids, normal lung cells that were obtained from lung resection were dissociated and seeded in growth factor–reduced Matrigel matrix. The cells were further incubated

in a medium containing recombinant human EGF, human FGF-10, human FGF-basic, 10% R-spondin-1, noggin, and 30% Wnt3a [18].

Cancer organoids

Traditionally used patient-derived cancer cell (PDC) and patient-derived xenograft (PDX) cancer models possess several limitations, which can be overcome by using cancer organoids [19,20]. Patient-derived cancer organoids portray characteristics closer to the individual tumor and allow better representation of the heterogeneity of human cancers. A variety of cancer organoids were established, including those for colorectal cancer, lung cancer, pancreatic cancer, breast cancer, and liver cancer [20]. The culture systems that were used for growing cancer organoids comprised a combination of growth factors and small molecules together with extracellular matrix. For example, breast cancer organoids were grown on basement membrane extracts with a medium containing a combination of R-spondin-1, FGF-7, FGF-10, nicotinamide, noggin, primocin, Heregulin-β-1, A83-01, and Y-27632 [20]. Cancer organoids can be employed for a variety of applications, such as high-throughput drug screening, immunotherapy, and translational medicine. However, advancing cancer organoids to clinical use requires navigating the limitations of varied reproducibility, failure to reconstitute the tumor microenvironment, and lack of vasculature [20].

Therapeutic potential of organoids

A wide array of organoids from organs of all three embryonic layers have been studied, and include: endoderm-derived organoids of thyroid, lung, pancreas, liver, stomach, and intestine; mesoderm-derived organoids of heart, skeletal muscle, bone, and kidney; and ectoderm-derived organoids of retina, brain, pituitary, mammary gland, inner ear, and skin [19,21].

The major focus of future organoid studies will continue to include investigating and refining the developmental processes, and most likely, will subsequently advance into disease modeling. Organoids may be useful to further research of developmental disorders, genetic conditions, cancer and degenerative diseases, to name a few [2,19,21].

Utilizing patient iPSCs will allow valuable disease modeling, especially when adequate animal models are not available. Organoids can also be used for more efficient testing of drug efficacy and toxicity by removing discrepancies due to the differences between animal and human cells. Organoid drug testing might also dramatically reduce the use of animals for preclinical trials [2]. The hope is that organoids are another step in the long journey towards *in vitro* construction of tissues and organs for transplantation into patients. However, many obstacles still need to be addressed along the way, such as the dependence on mouse derived extracellular matrix, limited reproducibility, proper maturation, and improved vascularization [21].

Organoids on a chip

When aiming to mimic natural tissues and organs ***in vitro***, besides culturing the tissue-specific cells, environmental conditions such as a gradient of chemical factors, and mechanical and electrical cues, should be taken into consideration [22,23]. The advances in microsystem technology and the development of microfluidic chips have made incorporating these conditions possible for ***in vitro*** experimentation. These microfluidic chips contain miniature tissues allowing controlled flow of tiny volumes of solution through microchannels and often incorporate sensors that enable in-line measurements [22,23].

Organoids on a chip are developed for a variety of applications including development of pharmaceutical and chemical compounds, biomaterial testing, and disease modeling [22].

When fabricating an organoid-on-a-chip device, many design considerations should be considered depending on the functionality required from the system. These design aspects include the level of complexity (single- or multi-organ), material selection, fabrication method, and selection of the biological elements and additional elements that might be required, such as incubators, mechanical stimulators, and sensors [22].

Organoid-on-a-chip models can provide a lot of valuable information; however, organs constantly interact with other organs. Thus, multi-organ chips were developed in order to better resemble physiological conditions and to study multi-organ interactions such as in drug testing. Unfortunately, culturing different tissues on the same chip proves to be quite the challenge [22].

Organ-on-a-chip technology is turning into a widely used platform for ***in vitro*** studies of human physiology; however, challenges remain before this technology can be expanded further. The next step is to establish a body on a chip to better mimic the complicated activities and interactions of the various organs in the human body by combining several organoids into a single chip: brain, lung, bone, gut, heart, liver, pancreas, and kidney [23].

Related products

Product	Cat. No.
Human/Murine/Rat Activin A	120-14
Human BAFF	310-13
Human/Murine/Rat BDNF	450-02
Human BMP-4	120-05
Human BMP-4 (*E.coli*-derived)	120-05ET
Human BMP-7	120-03P
Murine CD40 Ligand	315-15
Human DKK-1	120-30
Animal-Free Human EGF	AF-100-15
Human/Murine/Rat Activin A (*E.coli*-derived)	120-14E
Human/Murine/Rat Activin A (CHO-derived)	120-14P

Product	Cat. No.
Human FGF-4	100-31
Human FGF-9	100-23
Human GDNF	450-10
Murine IL-4	214-14
Human Noggin	120-10C
Human R-Spondin-1	120-38
Human Sonic Hedgehog (Shh)	100-45
Murine Wnt-3a	315-20

Related GMP cytokines

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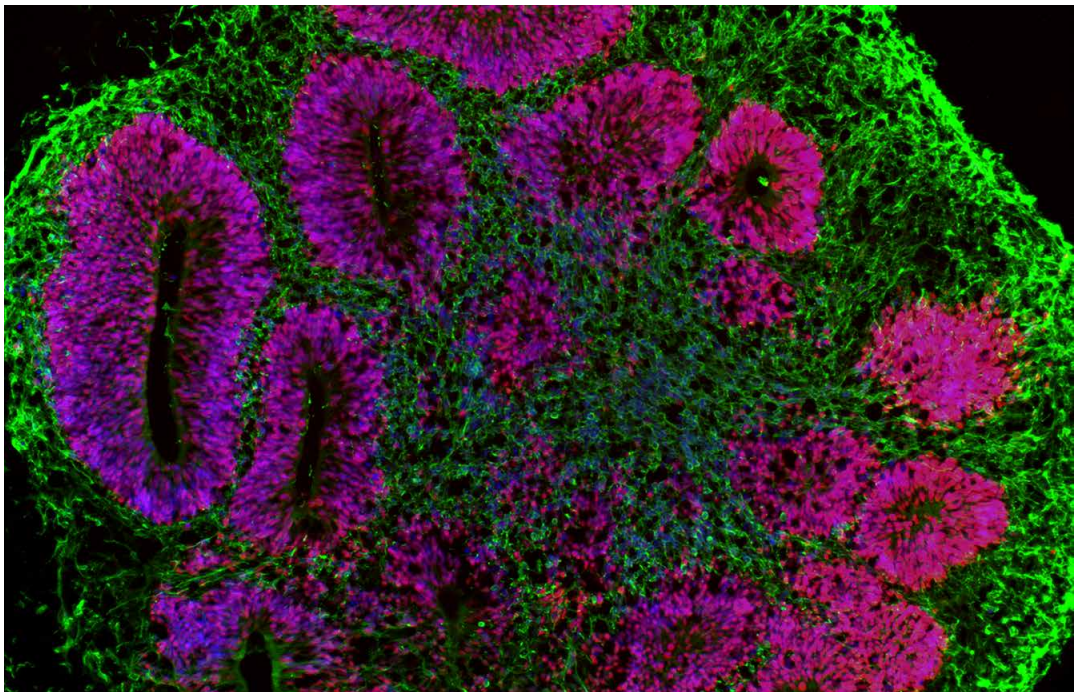

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