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MEETING REVIEW

Bringing together the organoid field: from early beginnings to the road ahead

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ABSTRACT

From October 12-15th, 2016, EMBO|EMBL held a symposium to bring together those in the scientific community with a shared interest in using three-dimensional (3D) culture methods to study biology, model disease and personalize treatments. The symposium, entitled 'Organoids: modelling organ development and disease in 3D culture', which was organized by Juergen Knoblich, Mina Bissell and Esther Schnapp, was particularly timely as there were otherwise few opportunities for those interested in using 3D culture platforms to interact outside of their organ-specific scientific community. The meeting was a fantastic success, creating a lot of discussion and cross-fertilization of ideas from developmental biologists to bioengineers and biophysicists. This Meeting Review provides a summary of the talks presented and the major themes that emerged from the symposium.

KEY WORDS: Disease modeling, Organoids, Stem cells, *In vitro*, Three dimensional

Introduction

The field of organoid biology is advancing rapidly. An array of different organs and tissues can now be recreated – at least partially – *in vitro*, and hold promise for studying both basic developmental biology and for use in translational research. Despite the diversity of organoids generated and their potential uses, the field as a whole faces some common challenges in order to realize the exciting opportunities ahead. The 2016 EMBO|EMBL Symposium 'Organoids: modelling organ development and disease in 3D culture' brought together an entire community of researchers working in the field of 3D organoid biology, with interests as diverse as salivary gland, tooth and inner ear, as well as better-known organoid systems such as intestinal and cerebral organoids. The symposium, which was organized by Juergen Knoblich, Mina Bissell and Esther Schnapp, provided a fantastic opportunity for scientists using organoid systems to come together and discuss their progress, the challenges that they face, and their hopes for this exciting technology.

It wasn't long into the meeting before it became clear that some researchers use terms such as 'organoids', 'spheroids' and '3D culture' interchangeably. In general, however, the consensus was that organoid culture refers to growing cells in 3D to generate cellular units that resemble an organ in both structure and function – hence the term 'organoid', where 'oid' stems from the Latin 'oides' meaning resemblance. In her opening remarks, keynote speaker Mina Bissell (Lawrence Berkeley National Laboratory, Berkeley, USA) presented a thought-provoking account of the history of 3D culture and its impact in modeling organ development *in vitro*. Her talk also helped to clarify what we mean when we talk about organoids, as opposed to 3D culture generally. One of the earliest reports of organoid culture that demonstrated restoration of organ-specific structure and biochemical function was from Michalopoulos and Pitot, who used floating gels of rat tail collagen to grow hepatocytes that not only recapitulated their correct morphology but also restored secretion of cytochrome P450, a characteristic function of hepatocytes *in vivo* (Michalopoulos and Pitot, 1975; Michalopoulos et al., 1976). Subsequently, similar culture conditions were used by Emerman and Pitelka (Emerman and Pitelka, 1977) and the Bissell laboratory (Bissell et al., 1982) to generate mammary epithelial cells that formed acini-like structures and secreted milk proteins (Emerman et al., 1977). Together, these studies led to the birth of the field of organoids – that is, modeling organ-like growth in culture. It is important to distinguish organoid cultures from suspension (spheroid) cultures, which lack any attachment to extracellular matrix, or tissue slice cultures, a method that was frequently used in the past. Whereas slice cultures do not require growth or morphogenesis, organoid cultures require the ability of cells to expand and self-organize into organ-like units in 3D culture.

During her talk, Bissell presented her seminal discoveries on understanding the relationship between structure and function either using normal mammary epithelial cells or breast tumor cells. She summarized studies in which transformed mammary epithelial cells could be reverted to assume a normal 3D organoid-like structure by interfering with cell-matrix interaction or by reducing other signaling inhibitors, highlighting the need for a reciprocal interaction between the ECM and nucleus in the normal cell, and how this could be restored in malignant cells. She emphasized the importance of laminin 111 and the role it plays in promoting organogenesis, and proposed that a loss in the ability of epithelial cells to respond to laminin 111 might lie at the core of their ability to become quiescent. Bissell also presented a new observation from her laboratory, namely that inhibition of glucose uptake could also revert the malignant cells. This relationship between 3D structure and biochemical function, which she refers to as 'dynamic reciprocity', is a property used by organoids to reach and maintain homeostasis. Lastly, Bissell closed her lecture with eye-catching images of direct cytoskeletal connection between desmosomes and nuclear membrane using focused ion beam scanning electron microscopy and electron tomography. These images provide direct support for the long-held belief that a cytoskeletal connection exists between the outer cell membrane and the nuclear membrane.

In the second keynote lecture, Hans Clevers (Hubrecht Institute, The Netherlands) presented his path to developing organoid models using organ-specific stem/progenitor cells from the intestine, stomach, bladder and lung. He highlighted the role played by Wnt signaling pathways in organogenesis and discussed the interesting finding that intestinal stem cells do not appear to show signs of quiescence or asymmetric division. Using lineage-tracing studies,

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Clevers showed how cells of the intestinal crypt lack any developmental hierarchy, instead exhibiting extensive plasticity whereby almost any cell can assume the role of a stem cell when needed. He highlighted the power of using the organoid platform for both discovery and clinical translation efforts. From a discovery point of view, he presented their studies on single-cell sequencing from intestinal organoids and the identification of Reg4 as a novel marker of a rare population of secretary enteroendocrine cells.

From a clinical translation perspective, Clevers, and a later presentation by Jeffrey Beekman (University Medical Center Utrecht, The Netherlands), provided what is arguably the most powerful demonstration to date of how organoids can be used in the clinic to guide treatment decisions for patients with cystic fibrosis (Dekkers et al., 2016). In this disease, the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene is mutated, leading to a mutant CFTR protein. Rectal organoids generated from subjects with wild-type or mutant *CFTR* genes were used to assess differences in lumen area under steady state or upon stimulation with forskolin, an activator of CFTR channel function. In healthy individuals, forskolin treatment of organoids induces swelling of the lumen. By contrast, organoids derived from cystic fibrosis patients fail to exhibit forskolin-induced swelling. Interestingly, pharmacological restoration of CFTR function was associated with restoration of forskolin-induced swelling. Clevers used this distinction as the basis for an assay to predict whether a particular drug might be successful in treating cystic fibrosis, based on whether it could restore the forskolin-induced swelling in patients with mutated CFTR. Tissues from patients with rare CFTR variants, previously unknown to respond to CFTR restoration therapy, were screened for CFTR-restoring drugs in the organoid assay and identified as candidates for treatment with the drug Ivacaftor. Strikingly, administration of the drug resulted in improved pulmonary function, demonstrating the power of organoid-based screening to match patients with drugs. These results have paved the way for insurance companies to use (and pay for) these services in the context of off-label use.

Pancreas and liver organoids

The meeting brought together a number of efforts focused on the generation of pancreas and liver organoids. In session one, I presented our efforts to induce human embryonic stem cells (ESCs) to differentiate towards the exocrine pancreas lineage (Huang et al., 2015). This system can be used to understand the developmental mechanisms that underpin human exocrine commitment and, in addition, can serve as a platform for modeling human diseases such as pancreatitis and pancreatic cancer. We have expanded our efforts into growing primary human pancreas ductal adenocarcinoma cells from the patient and maintaining them in culture as tumor organoids. I highlighted the fact that our method is complementary to, and not redundant with, those published by Clevers and Tuveson (Boj et al., 2015) because we do not use any Wnt agonists and our medium contains only a simple cocktail of tissue-relevant growth factors. The tumor organoids generated using our growth cocktail tend to maintain both the histological and differentiation characteristics of the matched primary tumor, giving us confidence that we are likely to maintain the patient's tumor cells in their native state in culture. As such, this platform might be well suited for screening therapeutic options. Christine Chio (CSHL, USA) from the laboratory of Dave Tuveson presented their recent studies using pancreas tumor organoids to uncover an unexpected relationship between expression of the redox regulator NRF2 (NFE2L2) and promotion of mRNA translation in *KRAS* mutant pancreatic cancer cells. They went on to discover a synergy between increased reactive oxygen species and inhibition of AKT kinase. Anne Grapin-Botton (DanStem, University of Copenhagen, Denmark) presented her studies on culturing pancreatic progenitor cells from E10.5 mouse embryos. Plating cells as clusters of few cells resulted in the development of branched structures with endocrine cells in the middle and exocrine cells in the periphery, mimicking organogenesis *in vivo*. This system provides a powerful platform for understanding organogenesis involving multiple cell types. It would be interesting to establish whether a similar heterotypic organogenesis could be modeled using human cells.

Although the pancreas and liver share a developmental lineage, generating organoids to model liver hepatocytes has been a challenge for the field. Two talks, one from Meritxell Huch (Gurdon Institute, University of Cambridge, UK) and another from Takanori Takebe (Yokohama City University, Japan) presented ongoing efforts in generating organoids to model the liver and other organs. The Huch lab works on the generation of pancreas and liver organoids from adult organs, both mouse and human. In addition to generating ductal structures from adult liver, they have identified methods to induce them to differentiate into functional hepatocytes that produce bile acid and posses cytochrome activity. They are now extending these studies to grow organoids from disease patients. Takebe presented a sophisticated method of combining multiple cell types under conditions to promote mesenchyme-driven condensation, compaction and self-organization. He discussed how this approach enables organogenesis of complex tissue structures including the liver (Takebe et al., 2013), kidney and pancreas (Takebe et al., 2015), with heterotypic cell types including vascular cells that are able to form networks. He emphasized the power of this method to provide crucial insight into steps regulating organogenesis *in vivo*.

Although it is generally thought that the pancreas does not have the ability to regenerate, Rocio Sancho (The Francis Crick Institute, London, UK) argued that there are some physiological (pregnancy and pancreatitis, for example) and experimental (such as cytokine treatment and transgenic overexpression of transcription factors) conditions under which the adult pancreas exhibits a capacity to remodel. She presented her findings on the role played by the E3 ubiquitin ligase Fbw7 (Fbxw7) in beta cell specification and reprogramming (Sancho et al., 2014). She showed how the loss of Fbw7 in CK19 $(Krt19)^{+}$ adult pancreatic ductal cells *in vivo* resulted in stabilization of the transcription factor Ngn3 (Neurog3), a regulator of endocrine differentiation, which led to reprogramming of ductal cells to insulin-secreting beta cells. They also found cells that were double positive for CK19 and insulin, suggesting the presence of a metastable state that represents both exocrine and endocrine cells. They have now begun to generate ductal organoids from their *CK19-CreERT:Fbw7*$^{fl/fl}$ mice in order to better understand the cell and developmental mechanisms that regulate this reprogramming process.

Intestinal organoids

Our understanding of intestinal development and morphogenesis has benefited greatly from organoid technology. James Wells (Cincinnati Children's Hospital Medical Center, USA) presented his elegant studies using human pluripotent stem cells (PSCs) to model gastrointestinal organogenesis. In particular, he discussed his group's efforts in generating human intestinal organoids from PSCs. Importantly, these intestinal organoids are heterotypic and have the potential to generate enterocytes, goblet cells, Paneth cells, tuft cells and enteroendocrine cells when transplanted under the kidney

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capsule *in vivo*. In addition, he also presented efforts to generate human gastric organoids from PSCs. He conveyed a clear message of the importance of human models of organogenesis for a better understanding of species-specific differences in development and disease modeling. He also outlined his group's efforts to generate certain cell types in the gut that have so far evaded modeling – parietal cells, for example. Wells discussed their efforts to generate gut organoids with increased function by incorporating enteric neurons, which resulted in functional neuroglial cells.

Toshi Sato (Keio University, Japan), a pioneer in the development of gut organoids during his time at the Clevers laboratory, presented his efforts to model early stages of human colon cancer development by combining organoids and CRISPR/Cas9 technology (Fujii et al., 2015, 2016; Matano et al., 2015). A combination of multiple genetic changes associated with human colon cancer and long-term culture of the genetically altered organoids enabled Sato to engineer organoids that develop metastatic disease when transplanted into mice. Sato also discussed his group's efforts to explore the impact of the organ site used for the transplantation of human colon organoids, and highlighted the importance of using orthotopic sites for modeling normal development and disease.

Modeling brain development in a dish: the final frontier

The brain is arguably the most complex of human organs, and one in which we still understand very little regarding how such complexity is generated. Modeling cerebral development using an organoid platform is an exciting topic of research as it opens up amazing opportunities to understanding human brain development and disease. Juergen Knoblich (IMBA, Vienna, Austria) broke away from the *Drosophila* model system and presented his group's ground-breaking work on the generation of cerebral organoid models using mammalian systems. He outlined their efforts in combining precise media conditions with 3D cell culture methods to better control organoid formation. Knoblich showed how this method not only promoted neural commitment and differentiation but also permitted self-organization of neurons to form a cortical plate with a defined ventricular zone and interneurons. The method enables the development of more complex 'mini-brain' structures in culture, opening the door to a greater insight into brain development as well as to the modeling of developmental neurological disorders, an area that is being pursued in the Knoblich laboratory. In an interesting study to understand evolutionary differences in neocortex development, Gray Camp (Max Plank Institute for Evolutionary Anthropology, Leipzig, Germany) discussed his efforts to characterize human PSC-derived cerebral organoids using single-cell RNA sequencing, and showed how the organoids recapitulate fetal neocortex development. Camp went on to compare chimpanzee and human cerebral organoids, and presented the intriguing observation that human neural progenitors show a prolonged prometaphase compared with chimpanzee neural progenitors, a difference that was not observed in non-neural cells (Camp et al., 2015; Mora-Bermúdez et al., 2016).

Bioengineering meets organoids

Inter-batch variation in Matrigel, which is often used as a substrate for plating cells, represents a significant challenge in generating reproducible and efficient organoid cultures. Elly Tanaka (IMP, Vienna, Austria) presented work from a collaboration with Matthias Lutolf (EPFL, Lausanne, Switzerland) aimed at removing the need for Matrigel. They have developed a polyethylene glycol (PEG)-based hydrogel that contains a number of additional components, including extracellular matrix molecules such as laminin-111, perlecan and collagen IV. The hydrogel can be engineered with varying levels of stiffness, and contains additional functionality in that matrix components can be degraded using matrix metalloproteinases. Tanaka discussed how specific formulations of the gel support neuroepithelial cell fate decisions and the development of apicobasal polarization and dorsoventral patterning. In addition, she discussed how this synthetic platform could be used to investigate the role played by Shh, Wnt, BMP and retinoic acid signaling pathways during neural tube patterning. Matthias Lutolf presented his group's efforts to overcome the limitations of animal-derived extracellular matrices for organoid culture. Using intestinal organoids as a model system, Lutolf's team developed a fully defined hydrogel system that affords much greater control over *in vitro* organoid development. By systematically probing intestinal stem cell behavior in response to changes in the biochemical and physical properties of the hydrogel, the researchers discovered that different stages in organoid formation require different matrix properties (Gjorevski et al., 2016). For example, they found that intestinal stem cell fate is strongly influenced by the hydrogel's mechanical properties, and that this was mediated by the Hippo mechanotransduction pathway.

Most organoid systems facilitate the development of spherical or tubular structures, and modeling more complex organ shapes remains a challenge that needs to be addressed. In this context, Mukul Tewary (University of Toronto, Canada) from the Zandstra laboratory discussed their group's use of sophisticated bioengineered platforms for modeling mesoderm development in culture. Zev Gartner (UCSF, USA) presented an elegant method that uses the DNA-programmed assembly of cells to reconstitute the mesenchyme-driven folding of tissue-like structures. This approach offers unique opportunities to understand the complex mechanisms by which epithelial and mesenchymal tissues interact during development.

Organs galore: organoids of the salivary gland, endometrium, inner ear, tongue, kidney, prostate, bladder, breast and thyroid

The true power of organoid technology was on full display as investigators showcased a diverse array of organoids representing multiple organs and tissues. Michael Shen (Columbia University, USA) presented his group's studies on mouse prostate organoids using castration-resistant Nkx3.1-expressing cells (CARNs). They showed that a single CARN cell was able to generate a bilayered (basal and luminal) organoid with a hollow lumen, and also found that organoids could be formed from lineage-marked CK8 $(Krt8)^{+}$ luminal epithelial cells in the normal prostate. In addition, he presented their efforts to model bladder tumor organoids in culture (Chua et al., 2014). Margherita Turco (University of Cambridge, UK) discussed the establishment of a long-term, chemically defined system for culturing human endometrial glands. The endometrial organoids have clonal ability, respond to hormones and contain secretory and ciliated cells. Jason Spence (University of Michigan, USA) presented their studies on modeling human lung development by the generation of lung organoids from human PSCs. He discussed an important role for FGF10 in promoting the maintenance of lung epithelia in the organoids. The organoids were heterotypic, containing cells from the upper airway epithelia, ciliated cells surrounded by smooth muscle and myofibroblasts, and showed similarity to human fetal lung (Dye et al., 2016, 2015).

Eri Hashino (Indiana University, USA) presented her group's efforts to recapitulate the 3D development of the inner ear sensory

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epithelia from human ESCs. Sensory hair cells are limited in number and do not regenerate, highlighting the need for regenerative medicine approaches to correct hearing loss. The approach taken by Hashino's group is an elegant method whereby the human ESCs not only generate functional sensory hair cells but also generate supporting cells and sensory-like neurons with synaptic structures. Using this platform, she presented their recent data on the role of Wnt signaling, where addition of the Wnt agonist CHIR99021 increased the efficiency of generating PAX8/PAX2/FBXO2-expressing otic progenitor cells capable of giving rise to inner ear organoids. Her group is actively pursuing efforts to use human inner ear organoids to model genetic inner ear disorders.

Takashi Tsuji (RIKEN Center for Developmental Biology, Japan) discussed the possible applications of organoid biology in next-generation regenerative medicine. He discussed his group's efforts to investigate and facilitate the regeneration of oral organs such as tooth, salivary or lacrimal gland, as well as hair follicles. He presented elegant studies in which mesenchymal cells were combined with epithelia and tooth germ, leading to new tooth growth in the mouth of mice. Importantly, the newly grown teeth displayed normal histoarchitecture and nerve connections. In addition, Tsuji presented their studies on the fate of these bioengineered organs when orthotopically transplanted into mice. The transplanted organs could connect to the surrounding tissues and recapitulated some physiological functions of the bona fide organ. Hiroo Ueno (Kansai Medical University, Japan) presented his group's efforts in growing lingual (tongue) organoids. Starting from lingual epithelial cells, they identified conditions to generate organoids that contained multilayered keratinized epithelial structures, similar to the mouse tongue *in vivo*. The platform has demonstrated utility for modeling tongue cancer. Melissa Little (Murdoch Childrens Research Institute, Australia) discussed her group's efforts to direct the differentiation of human pluripotent stem cells to complex kidney organoids containing glomeruli, renal tubules, endothelium and renal stroma. Her emphasis was on evaluating the robustness of the protocol and its applicability for disease modeling. Sabine Costagliola (University Libre de Bruxelles, Belgium) presented a thyroid organogenesis model in which transient coexpression of NKX2.1 and PAX8 was sufficient to induce thyroid follicular structures from mouse ESCs.

Makoto Furutani-Seiki (Yamaguchi University, Japan) presented an interesting observation on the role of Hippo/YAP signaling in regulating 3D body shape. While a role for Hippo/YAP signaling in organ size has been well investigated, its role in regulating whole body shape was previously unknown. Furutani-Seiki reported that loss of YAP in medaka (Japanese rice fish) resulted in body and organ flattening due to tissue flattening and dislocation. Analysis of the YAP mutants showed that YAP is required for tissue tension to maintain the tissue shape and to align tissues properly by tissue tension-mediated fibronectin fibril formation. Analysis using human 3D spheroids demonstrated that YAP control of tissue tension is mediated by ARHGAP18, which depolymerizes F-actin, suggesting that F-actin turnover is required for maintaining actin network contraction and hence tissue tension.

Although they have most commonly been used to model development and disease, organoids can also be used to model more subtle, complex phenomenon. In this light, Cyrus Ghajar (Fred Hutchinson Cancer Center, USA) presented his work on modeling tumor cell dormancy using a 3D culture platform. In patients, chemotherapy induces the dissemination of tumor cells, which then lie dormant near microvascular beds. Ghajar showed how plating on a bed of human microvasculature in 3D culture induced dormant behavior in tumor cells by protecting against their death. He made the case that this model has significant implications for understanding dormancy in cancer and, importantly, how these cells are later re-awakened (Ghajar, 2015; Ghajar et al., 2013).

Summary and future directions

The meeting provided an impressive display of the power and utility of organoids in understanding normal development and modeling disease. The field has grown at a startling rate in recent years, and the early 'organoid' pioneers – Michalopoulos and Pitot (Michalopoulos and Pitot, 1975) – would surely be amazed at how far organoid culture has come. But the road ahead is still long, and this meeting highlighted the need to collaborate closely with engineers, physicists, chemists and clinicians in order to expand and exploit this methodology to its full potential. The success of the meeting in bringing together representatives from diverse fields with a shared interest in organoid biology will no doubt help to foster such collaborations, and thus it is with great anticipation that the field awaits the next reiteration of this exclusively organoid-focused meeting.

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Competing interests

The author declares no competing or financial interests.

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