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SPOTLIGHT

The hope and the hype of organoid research

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ABSTRACT

The recent increase in organoid research has been met with great enthusiasm, as well as expectation, from the scientific community and the public alike. There is no doubt that this technology opens up a world of possibilities for scientific discovery in developmental biology as well as in translational research, but whether organoids can truly live up to this challenge is, for some, still an open question. In this Spotlight article, Meritxell Huch and Juergen Knoblich begin by discussing the exciting promise of organoid technology and give concrete examples of how this promise is starting to be realised. In the second part, Matthias Lutolf and Alfonso Martinez-Arias offer a careful and considered view of the state of the organoid field and its current limitations, and lay out the approach they feel is necessary to maximise the potential of organoid technology.

KEY WORDS: Disease modelling, Organoids, Stem cells, Therapies

Organoids: the promise and hope of an emerging field

Meritxell Huch and Juergen A. Knoblich

Introduction

Ever since the times of Aristotle, human beings have been fascinated by understanding the biology of their own body, ranging from how organs and organisms are formed to how their malfunction gives rise to disease. As most human tissues are inaccessible for scientific analysis, theories about organ development and function were initially based mostly on speculation. Only in the past century has our understanding of human development and organ function significantly improved (Horder, 2010). This is mainly due to the use of model organisms, ranging from the fruit fly *Drosophila* and the worm *C. elegans* to vertebrate models such as the mouse and the zebrafish. While these models have elucidated the general principles of human development, they fail whenever differences between humans and other animals emerge. In fact, the need for additional and more physiologically relevant human models is exemplified by the resounding failures of translating effective therapies from cell or mouse models into humans. For decades, however, developing *ex vivo* human models had been an insurmountable challenge.

Fortunately, this is beginning to change with the recent establishment of three-dimensional (3D) cell culture methods, which allow embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) or tissue-resident stem/progenitor cells to recapitulate many aspects of their respective differentiation programmes *in vitro*. Stem cells can now be used to grow structures that resemble an organ in culture and therefore have been termed ‘organoids’. Being able to mimic human tissue in a dish, from its early development to its organogenesis or adult stage, represents a key breakthrough in modern biology. Growing human-derived tissues *ex vivo* has opened the possibility to study human development, to model human disease directly from individual patients or to test therapeutic compounds in a personalised medicine approach. Also, the ability of stem cells to self-renew and differentiate in a dish has even raised hopes for future therapies involving the growth and transplantation of solid organs such as the liver, lung and pancreas, thus replacing the need for organ donations.

Before diving into the enormous opportunities arising from the new and exciting organoid field, we would first like to define an organoid as a 3D structure derived from either pluripotent stem cells (ESCs or iPSCs), neonatal or adult stem/progenitor cells, in which cells spontaneously self-organise into properly differentiated functional cell types, and which recapitulates at least some function of the organ (Lancaster and Knoblich, 2014a,b; Huch and Koo, 2015). In all scenarios, self-assembly and differentiation are key aspects of organoid formation and are the result of instructive signalling cues given to the cells by the extracellular matrix (ECM), the medium and also, once the 3D structure assembles, by the cell types present in the organoids themselves.

Organoids as disease models and their medical applications

One of the greatest potential applications of organoid models is in the analysis of human-specific disease mechanisms. Human organoids have allowed the analysis of tissues as diverse as the small intestine (Jung et al., 2011; Sato et al., 2011; Spence et al., 2011), prostate (Gao et al., 2014; Karthaus et al., 2014), brain (Lancaster et al., 2013) and liver (Huch et al., 2013, 2015; Takebe et al., 2013). The possibility of generating human 3D cultures that resemble specific organs has opened up enormous possibilities for using organoids as a source for cell therapies, and as a potential alternative for whole-organ transplantation. Also, the huge expansion potential of some types of organoids makes it possible to design autologous cell therapy strategies and to establish biobanks of organoids (van de Wetering et al., 2015) for potential allogeneic cell therapy transplantation. Furthermore, combining organoids with novel genome-editing tools such as CRISPR/Cas9 technology opens up the possibility of performing gene correction and to select the corrected clones prior to autologous transplantation. Indeed, it was recently shown that cystic fibrosis

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patient-derived organoids could be readily corrected using CRISPR/Cas9 homologous recombination (Schwank et al., 2013). Of note, while autologous cell therapy transplantation is of great promise of the organoid field, its efficacy, safety and immunogenicity are still pending evaluation. Although cell-derived organoids retain their genetic stability over time (Huch et al., 2015; Blokzijl et al., 2016), their immunogenicity is still an enigma. In fact, for organoid cell therapy to be cost-effective, it will be crucial to determine whether, after being expanded in culture, organoid cells still retain the HLA type of the original patient, which would result in the abolition of any immunosuppressive treatment post-transplantation and be a clear benefit for the patient and the healthcare systems. For details on the translational applications of organoids, see Drost and Clevers (2017) in this issue.

The use of disease-specific organoids will facilitate the analysis of molecular mechanisms behind the disease, the identification of potentially novel biomarkers and the development of patient-specific platforms for drug testing or toxicology studies, thus bringing the promise of personalised medicine to reality. In fact, in a recent study cystic fibrosis patient-derived organoid cultures were used to successfully predict the response to a specific drug treatment (Dekkers et al., 2016). Pancreatic cancer organoids have proven useful in identifying novel genes associated with pancreatic cancer progression (Boj et al., 2015), while colon cancer organoids have permitted the detection of gene-drug associations following high-throughput drug screens (van de Wetering et al., 2015). Similarly, cerebral organoids derived from human iPSCs have demonstrated that premature neuronal differentiation and defects in the orientation of cell division drive congenital microcephaly disorders (Lancaster et al., 2013). Furthermore, brain organoids have provided the first direct human evidence for a causal connection between the Zika virus and microcephaly (Li et al., 2016). Moreover, these models are of great importance for identifying and verifying potential compounds for preventing microcephaly, such as inhibitors for the potential Zika receptor AXL. For details on Zika infection models, see Qian et al. (2017) in this issue.

Overall, we can start to envisage a future scenario in which patient-derived organoid cultures could help to distinguish more effective drugs and determine the correct therapeutic window for a particular patient. Experiments that compare several organoids derived from the same iPSC line could be informative as to how mutations in specific genes differentially affect tissue specification in various organs in a patient carrying a particular congenital allele. Establishing organoids directly from the diseased tissue could even allow clinicians to perform computerised drug screening tests while the patient is still in the hospital bed, mirroring antibiogram tests, which are commonly used in the clinic to determine antibiotic susceptibilities.

Organoids as models to study human development

Organoid cultures also have great potential for the study of human development. Until recently, analysing human organogenesis and development was almost impossible, mainly due to the difficulty in accessing human embryonic material. As organoid cultures can faithfully recapitulate many of the developmental steps that occur *in vivo*, they allow the visualisation and analysis of human developmental processes in real time. Also, organoid cultures can potentially reveal the similarities and differences between humans and other animals during development. This is particularly important for the analysis of human brain development and its perturbation in human-specific congenital diseases. So far, studies using human organoids have already told us that inhibiting BMP signalling is crucial for specifying human posterior foregut endoderm and for stomach formation (McCracken et al., 2014). Analysis of lung organoids has taught us that FGF signalling specifies human anterior foregut and SHH specifies lung fate (Dye et al., 2015), consistent with the role of these pathways in mouse lung growth (Morrisey and Hogan, 2010). Similarly, by differentiating PSCs into stomach organoids, we have learned that Wnt/β-catenin signalling is essential to drive the production of both human and mouse stomach acid-producing cells (Noguchi et al., 2015; McCracken et al., 2017). These pioneering studies are already revolutionising the way we study human development and show that organoid cultures have the potential to advance the field of human development in an unprecedented manner. For an in-depth discussion on the use of PSC-derived organoids to study human development, see McCauley and Wells (2017) in this issue.

Future directions of the organoid field

Organoid cultures represent a novel means for studying human development and human disease. They resemble the original human tissue, contain the correct cell types and perform certain tissue functions. As they faithfully recapitulate aspects of tissue composition, architecture and function *ex vivo*, they open up great possibilities for identifying novel therapeutic strategies in personalised human disease models. Combining organoid cultures with recent developments in live imaging (Pampaloni et al., 2015) will allow us, for the first time, to visualise early events in human development in real time. It will be possible to track cells and to study, for example, how the human cortical plate develops from the very early neural progenitors to the final mature neural cells. It will also be possible to assess the impact of exchanging a single growth factor or of ECM modifications on cellular behaviour. Also, as new synthetic ECM components are developed, organoids will provide a platform for determining how physical forces and cell shapes influence tissue differentiation or organ shape.

In summary, organoid cultures combined with novel developments in live imaging, genetic engineering and biomaterials represent a tour de force that will influence, in the very near future, how we study human development and how we treat human disease. In our view, the combined emergence of these new technologies raises strong hopes for the development of novel therapies and fundamental improvements in the drug discovery process.

Engineering development to turn organoid hype into organ hope

Matthias P. Lutolf and Alfonso Martinez-Arias

Introduction

Nearly six years ago the late Yoshiki Sasai and his team announced their striking discovery that mouse ESCs could be coaxed into making an eye cup in a dish (Eiraku et al., 2011). The group had already reported the emergence of cortical structures from cultures of ESCs (Eiraku et al., 2008; Watanabe et al., 2005), but the emergence of something as seemingly complex and with such crucial functionality as an eye caused a sensation. Further research reported a similar event from cultures of human ESCs (Nakano et al., 2012) with the surprising observation that, in each case, the size of the end product was appropriate for the species. These results were contemporaneous with reports of intestinal organoids by Hans Clevers and his team (Sato et al., 2009), and were soon followed by organoids for multiple tissues from PSCs and adult stem cell cultures. This menagerie was capped by the report of structures resembling regions of the human brain, so called ‘human minibrains’ (Lancaster et al., 2013). The press capitalised on the fact that the human brain is a

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quintessential element of human nature and the possibility that it could be created in a dish was, unsurprisingly, a newsworthy item. One astounding claim, for example, was that scientists at Ohio State University had 'developed a nearly complete human brain in a dish' (Cadwell, 2015). Clearly, such statements are a gross overstatement of the reality of this fledgling field.

As the range of organs emerging from adult tissue and PSCs widens, the hype continues and, in its midst, perhaps it is a good moment to ask what it is that organoids should be used for. What is the purpose of this supposed new field of research? Only if the aims are stated clearly will it be possible to lay down a path to achieve them. We see four main reasons that make efforts in organoid biology a desirable aim: drug testing, disease modelling, regenerative medicine, and as model systems for basic research – in particular for human biology. However, in the path to achieve these goals it is important to be critical and not to be content with only reporting observations from sporadic events in a dish. It is equally important not to create false expectations, and also to address two crucial issues that pervade most of the current organoid systems: a lack of reproducibility and, coupled to this, our lack of understanding of the processes that guide their development.

Reproducibility: the major bottleneck of current organoid systems

The general conclusion from recent organoid studies is that stem cells have a remarkable ability to reproduce in culture what they do in the organism. This promises a gateway to create the functional cell types that adherent or liquid cultures cannot produce but, at present, the experimenter has little or no input into what the cells do when they assemble into organoids. In our opinion, this lack of control over the process is likely to underpin the variability in systems and experiments that, with few exceptions, does not allow them to yield their full potential. Reproducibility, by which we mean that under the same experimental conditions every sample yields very similar phenotypic traits including organoid size, shape, cellular composition and 3D architecture, is essential in order to understand the mechanisms that underlie organoid development in normal and pathological situations, and to use them as targets for manipulation or drug testing. It is important to remember that the reason why studies with model organisms have been so informative is because of the reproducibility of their wild-type counterparts, which enables the systematic detection of any deviation from normal development. Furthermore, in the case of drug testing and regenerative medicine, it is crucial that, in addition to being reproducible, organoid systems are scalable and safe. Thus, achieving robust and reproducible organoid cultures is a most important immediate goal, for only then will it be possible to use them in basic and applied research, as well as a tool to gain a thorough understanding of the processes underlying their generation.

Engineering: a path forward for controllable organoid systems

We believe that interactions with engineers will be central for achieving robust and reproducible organoid cultures, particularly in the case of PSCs where the challenge is most obvious at the moment. Recent developments with intestinal and brain organoids are encouraging, showing that well-defined biomaterials and advanced bioreactor technology can be used to improve organoid cultures (Gjorevski et al., 2016; Qian et al., 2016). Further progress is keenly anticipated: engineers have developed powerful tools that will allow the culture of organoids in 3D contexts that provide more defined environments. Thus, instead of growing organoids without any spatial constraints in ill-defined 3D matrices, well-defined biomaterials and microtechnology may be applied to guide *in vitro* development through geometric and/or mechanical inputs that mimic the embryo environment. Likewise, instead of flooding stem cells with high and uniform concentrations of key morphogens, it should be possible to utilise engineering technology, for instance microfluidics and photochemistry, to deliver morphogens in a highly controlled manner – both spatiotemporally and in terms of dosage. Such controlled, systematic approaches will provide insight into the positional information that may be necessary to overcome the stochasticity in symmetry breaking in current organoid systems. Finally, bioreactor technology or engineered blood vessel systems may be employed to address the major problem of nutrient availability in growing organoids and thereby allow proper long-term growth of complex systems.

Back to basics: organoid biology is developmental biology

An engineering-based approach to organoid culture can and will help with reproducibility, scalability and safety but, whatever the aim might be, there is no escape from the fact that basic research is the fuel of applied progress. Much of organoid research is focused on the final product and does not seem to appreciate that the path to that endpoint matters. But the fact is that the early stages of *in vitro* organogenesis are the basis for later events, and poor foundations will lead to poor final products. Up until now, the field has been able to show what stem cells can do, but now the time is ripe to ask and gain knowledge of how they do it. In essence, organoid biology is developmental biology by another name. Yoshiki Sasai knew the importance of this view point and applied it to his work (De Robertis, 2014; Sasai et al., 2012). Developmental biology is about the molecular and cellular processes that build tissues and organs and, over the last 30 years, has made huge gains in unlocking the networks and strategies that mediate these events. In our view, organoid biology presents an opportunity to test this knowledge and, in the process, learn aspects of organ assembly that we have missed through more classical approaches. After all, most of what we know has been derived from genetic analysis, often via reverse engineering from the malfunctioning that results from removing individual components of the system. Richard Feynman famously said 'that which I cannot build I do not understand', and organoids provide an opportunity to test whether the developmental rules that we have derived from previous studies are indeed the blueprints for the organism. This is as exciting a challenge as that of generating the organoid itself and, together with an engineering-based approach, one that in our view will significantly progress the field. In many ways, developmental biology is to organoids what physics is to many disciplines of engineering: the foundation pillar and guiding element.

A significant prize that will result from these interactions and approaches will be a better understanding of human developmental biology. Progress in understanding human development is rightly challenged by ethical issues that prevent studies after day 13, the onset of gastrulation (Daley et al., 2016; Hyun et al., 2016). Differentiation of human ESCs in culture and, in particular human organoids, can bypass some of these issues and is starting to yield insights into our own biology that cannot be obtained in any other way. However, for this to work and to turn promise into reality, we need to be ambitious and go about the process without unfounded hype. Hype damages because it creates unrealistic expectations and, in the process, delays and discredits science when those expectations are found not to have been fulfilled. Hype also damages basic research because it places the emphasis on unrealistic goals that will be demanded by funding bodies and the public, without appreciating that only by carefully laying the groundwork will it be possible to deliver a meaningful product at the end.

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Organoids have revealed what developmental biologists have suspected for years: that cells have amazing self-organising abilities, the regulation of which is only just beginning to emerge. It is now time to harness control of this phenomenon for our own benefit. Rigour, self-criticism and, sometimes, a slow pace will be essential in this process and are prices worth paying when the stakes are as high and as exciting as they are here.

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Competing interests

J.A.K. is co-author on a patent application for use of cerebral organoid technology in disease modelling and toxicology testing.

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