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Mouse vs man: Organoid models of brain development & disease

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HIGHLIGHTS

● We show how brain organoids can be used to study development and disease.
● We compare advantages and disadvantages of mouse and human organoids.
● We argue that mice and mouse organoids remain useful for research in this field.

ABSTRACT

Brain organoids have rapidly become established as promising tools for studying both the normal embryonic development of the brain and the mechanistic roots of neurodevelopmental disorders. Most recent studies are based on brain organoids derived from human pluripotent stem cells (PSCs), as these are likely to be the best way to understand normal human development and disease. However, brain organoids grown from mouse cells still have a role to play. We discuss recent work showing how mice and mouse organoids can be employed to complement studies using human organoids. Mouse stem cell-derived organoids are useful for the development of improved protocols to generate organoids, including brain region-specific organoids. Importantly, the wealth of existing in vivo data on mouse brain development together with detailed descriptions of mutant phenotypes provide invaluable points of comparison to validate organoids as tools to study the genetics of brain development. Further, organoids have significant potential to replace or reduce the numbers of animals used in studies of normal brain development.

1. Introduction – modelling brain development

Understanding how the brain normally develops during embryogenesis represents a major challenge. It is important because many neurodevelopmental disorders most likely arise as a consequence of early development going wrong in some way, so we can only fully understand the root causes of such disorders if we first understand how the brain develops normally. Ultimately, understanding how the brain develops will allow us to devise rational strategies for alleviating neurodevelopmental disorders. It is very difficult to study early human brain development directly because of the ethical and practical constraints on research with human embryos, so scientists have relied on alternatives instead, such as animal models. In the last few decades, the mouse has been probably the most commonly used model to understand brain development, mainly due to the relative ease by which mice can be genetically manipulated; for example by the addition or inactivation of specific genes in their genome. Mouse models have allowed us to learn a great deal about normal brain development, to identify and study the function of many of the genes required for this complex process and has greatly improved our understanding of the developmental origins of neurodevelopmental disorders (Del Pino et al., 2018). While there are clear similarities between the developing brains of humans and mice, as we learn more about human development, it has become increasingly clear that there are also important differences between these species; notably, cell cycle times of neural progenitors are much longer in humans than in the mouse, and humans show a greater complexity of progenitor types (Fig. 1) (reviewed by Florio and Huttner, 2014; Mason and Price, 2016). Further, differences in the overall structure of mouse and human brains can make it difficult to use mice to model certain neurodevelopmental disorders. For example,
Organoids have been reported to show these interspecies differences. In humans there is a greater abundance of oRG progenitors including IPCs and oRGs, before differentiation to neurons. Radial glial cells (RGCs) proliferate and create subpopulations of progenitor cells in human organoids show increased cell cycle times compared to mouse, and human organoids contain many more outer radial glia (oRG) progenitors than do mouse organoids, as found in vivo (Kadoshima et al., 2013; Lancaster et al., 2013). The potential applications and limitations of brain organoids as tools to understand development and disease have been extensively reviewed recently (including Amin and Pasca, 2018; Brown et al., 2018; Giandomenico and Lancaster, 2017; Qian et al., 2019). As organoids have opened up the possibility of studying human embryonic brain development directly, this raises the question of whether there is still a place for studies using organoids grown from mouse cells. Here, we argue that cerebral organoids derived from mouse cells still have useful roles to play.

A large number of studies using cerebral organoids have been published in the last few years, the overwhelming majority of which employed human PSCs. The two most likely reasons that human stem cell-derived brain organoids have largely supplanted studies based on mouse cells are (i) as outlined above, the best way to study human-specific development is to use human cells and tissues and (ii) the availability of induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). iPSCs can be derived from any individual, including those affected by a neurodevelopmental disorder, to form patient-specific cell lines. This makes them ideal tools to investigate mechanisms underlying genetic disorders, as they have exactly the same genetic makeup as the patient (Lancaster and Knoblich, 2014). Further, CRISPR/Cas9 gene targeting allows the straightforward correction of patient-specific mutations, to demonstrate that specific phenotypes are indeed due to specific mutations. The relative merits of mouse and human stem cell-based methods specifically to model schizophrenia and autism were recently reviewed by St Clair and Johnstone (2018).

In studies where the primary focus is on the details of specific mechanisms underlying a particular neurodevelopmental disorder, brain organoids derived from human PSCs will usually be more appropriate. However, mutant mice lacking the equivalent gene have no such defects (Corbo et al., 2002), at least in part because the mouse cortex is normally smooth, in contrast to the gyrencephalic human cortex. Given these limitations to the mouse as a model for understanding human brain development, scientists have continued to seek alternative models, to complement or even replace studies using mice. Currently, brain organoids represent the most promising such alternative model (see Table 1).

2. Brain organoids

Brain organoids are complex three-dimensional structures grown from pluripotent stem cells (PSCs) that mimic the cell-type composition and tissue organization of the embryonic brain (Lancaster and Knoblich, 2014). Organoids can readily be grown from either mouse or human PSCs and the latter allow ready access to early stages of human brain development for the first time. Interestingly, key differences between mouse and human cerebral cortex development are reproduced in organoids – progenitor cells in human organoids show increased cell cycle times compared to mouse, and human organoids contain many more outer radial glia (oRG) progenitors than do mouse organoids, as found in vivo (Kadoshima et al., 2013; Lancaster et al., 2013). The potential applications and limitations of brain organoids as tools to understand development and disease have been extensively reviewed recently (including Amin and Pasca, 2018; Brown et al., 2018; Giandomenico and Lancaster, 2017; Qian et al., 2019). As organoids have opened up the possibility of studying human embryonic brain development directly, this raises the question of whether there is still a place for studies using organoids grown from mouse cells. Here, we argue that cerebral organoids derived from mouse cells still have useful roles to play.

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than mouse brain organoids. Similarly, in studies aimed at understanding aspects of normal brain development that are unique to human (i.e. not found in mouse), such as folding of the cerebral cortex, mouse organoids are not likely to be appropriate as they do not exhibit human-specific aspects of development. This was illustrated neatly in a study exploring the function of the PTEN gene, which encodes an important component of growth factor signalling pathways. Inactivating PTEN in human-PSC derived brain organoids caused an increase in proliferation of neural progenitors, leading to an expansion in organoid size and induced folding in the organoids. In contrast, when PTEN was deleted in mouse brain organoids no folding of the organoid tissue occurred (Li et al., 2017).

Our focus here is specifically on brain organoids, but it should be noted that organoids corresponding to a great variety of other tissues can also be grown (reviewed by Rossi et al., 2018). Mouse as well as human PSCs continue to be widely used in organoid-based studies on at least some of these other tissues. In part, this could reflect the fact that the brain is more complex than most other tissues, and that many tissues are relatively similar in mouse and human - the differences between mouse and human brain are much greater than those between mouse and human intestine, so mouse intestinal organoids may reasonably be expected to model human intestine more closely than would be the case for brain. For example, a recent study exploring the mechanism by which symmetry is broken during the formation of intestinal organoids employed mouse ES cells (Serra et al., 2019). Similarly, in an elegant series of experiments from the Martinez Arias lab, mouse ES cells were used to generate gastruloids – organoid-type structures which accurately reproduce key aspects of gastrulating mouse embryos (Beccari et al., 2018; Turner et al., 2017; van den Brink et al., 2014).

3. Applications for mouse cell-derived brain organoids

We envisage at least four areas where mouse cell-derived brain organoids are likely to continue to be useful to scientists for the foreseeable future.

3.1. Making better brain organoids

As described above, brain organoids have very rapidly become established as popular models to explore mechanisms of brain development and understand neurodevelopmental disorders. Nonetheless, the technology is at an early stage, and current protocols used to grow brain organoids are not optimal. In particular, there are limitations on the size to which organoids grow, many organoids contain heterogeneous mixtures of differentiated cell types and the overall efficiency and consistency of differentiation can vary considerably between batches of organoids (Qian et al., 2019). Therefore, there is a continuing need for new and improved protocols that will consistently produce high quality organoids that closely resemble embryonic brain. Early protocols for growing brain organoids were mainly pioneered in the lab of the late Yoshiki Sasai, initially using mouse embryonic stem (ES) cells (Eiraku et al., 2008; Nasu et al., 2012; Watanabe et al., 2005). Protocols for growing brain organoids from human stem cells were subsequently developed (Kadoshima et al., 2013; Lancaster et al., 2013; Paşca et al., 2015; Qian et al., 2016; Quadrato et al., 2017). The strategy of first optimising protocols using mouse stem cells, then adapting the protocols to work with human cells can be very effective. It is attractive because organoids retain their species-specific developmental timescales, so mouse brain organoids can be grown in a much shorter time period than human ones. Typical timescales to produce mouse brain organoids are around 10–14 days, whereas human brain organoids can take months to grow (Eiraku et al., 2008; Lancaster et al., 2013; Nasu et al., 2012; Paşca et al., 2015). Further, many of the reagents required to culture and maintain human iPSCs are expensive compared to those required for mouse ES cells. Optimising protocols can be challenging – often a number of factors, such as signalling molecules, need to be added, each of which must be present at the correct concentration, at only the correct time and for the correct duration. Optimising such a large number of variables using human cells is both time-consuming and expensive, so it can be both time and cost-efficient to derive protocols using mouse ES cells in the first instance. It is, of course, likely that the precise timings and duration of exposure to signalling molecules and other factors required for organoid development will vary between mouse and human (O’Leary et al., 2007; Paridaen and Huttner, 2014; Rash and Grove, 2006; Tan and Shi, 2013; Taverna et al., 2014), however a robust protocol developed in mouse is likely to be a good starting point for devising a human protocol. Overall, we consider that developing improved differentiation protocols is likely to be one of the major areas in which mouse organoids continue to be of value.

This approach has also been successfully applied to growing organoids corresponding to other complex tissues, such as the kidney. Taguchi and Nishinakamura (2017) developed a sophisticated protocol for the production of kidney organoids, by recapitulating key steps of normal embryonic kidney development. They first developed the protocol using mouse ES cells, then successfully adapted it for use with human iPSCs.

3.2. Protocols to generate region-specific brain organoids

Most existing protocols for growing brain organoids from human PSC cells fall into one of two main types. In the first type, exemplified by the protocol developed by Lancaster et al. (2013) cells are not guided towards any one particular brain region, so organoids commonly contain cells that resemble those found in multiple different parts of the brain. As organoids lack the antero-posterior and dorso-ventral axes found in the embryonic brain in vivo, these regions do not become organised within organoids into the normal anatomical arrangement found in the embryo (Lancaster et al., 2013). Many brain functions require the formation of specific connections between regions of the developing brain, but these connections cannot form normally in the organoids, because the various subregions of the brain are not arranged in the correct spatial patterns. The second type of protocol for differentiating organoids attempts to circumvent this issue by using specific combinations of factors to generate organoids that correspond to just one brain region, such as cortex, ventral telencephalon or thalamus (Birey et al., 2017; Qian et al., 2016; Shiraishi et al., 2017). These region-specific organoids can then be combined in culture, to form assemblies of two different regions, which subsequently interact with each other in the same way as the cognate brain regions do. For example, GABA-ergic interneurons formed in ventral telencephalic organoids migrate into co-cultured cortical organoids and produce functional synapses (Birey et al., 2017). Similarly, reciprocal connections can be formed between thalamus and cortical organoids, analogous to those found in the embryonic brain (Xiang et al., 2019).

Mouse stem cell-derived organoids are of continuing value in developing such brain region-specific organoids, in the same way as described above. Indeed, the protocol employed by Xiang et al. (2019) to grow thalamus-like organoids from human iPSCs was closely based on one originally designed using mouse ES cells (Shiraishi et al., 2017).

3.3. Developing and optimising new technologies

Just as mouse ES cell-derived brain organoids are of value in optimising protocols to make better organoids, they can be used as tools to optimise other types of experimental protocols. For example, they were used to help validate a refined version of CRISPR/Cas9 technology which employs two gRNAs in combination to greatly increase the efficiency of biallelic inactivation of several genes implicated in brain development in mouse ES cells (Acosta et al., 2018).
3.4. Studying normal mouse brain development

It is also important to note that brain organoids offer significant potential to reduce the number of animals used in studies of brain development by replacing the use of mice. One of the key attractions to using organoids rather than intact embryos to study brain development is their accessibility – this allows investigators to visualise and track the behaviours of individual cells within organoids in a way that would not be possible in intact embryos, especially at the earliest stages when embryos are particularly inaccessible. Examples of this include the tracking of interkinetic nuclear migration of GFP-marked radial in mouse ES cell-derived organoids (Nasu et al., 2012) and quantitation of the rate of neurogenesis in brain organoids grown from primary cortical progenitor cells isolated from mouse embryos expressing a Tbr2-GFP reporter transgene (Kosodo et al., 2017). Mouse brain organoids are therefore useful as tools to study basic cell biological processes that are required for normal brain development.

Analysis of chimaeric mouse embryos is a powerful tool for exploring gene functions and mechanisms (West, 1999). Making chimaeraeic organoids is a straightforward case of mixing mutant and control ES cells together in appropriate ratios, then letting organoids develop. This method was used recently with mouse ES cell-derived organoids to show that cell-surface heparan sulphate proteoglycans (HSPGs) are essential for setting up gradients of the signalling molecule sonic hedgehog (Shh) which has multiple roles in the development of the nervous system (Guo and Roelink, 2019).

Mouse brain organoids have also been employed to great effect in a study of the mechanisms by which antero-posterior pattern is initially set up in the early neuroectoderm (Takata et al., 2017). This is important, because understanding how the early regionalisation of the nervous system is achieved in vivo will help guide future attempts to make high-quality organoids corresponding to specific brain regions.

3.5. Comparing phenotypes in mutant organoids and embryos.

One particular advantage of mouse organoids that should not be overlooked is the wealth of existing published in vivo data on mouse brain development, including detailed descriptions of the phenotypic consequences of mutations in genes that regulate brain development. This means that we can compare the effects of mutating such genes in organoids with the well described phenotypes found in mutant mice, giving us confidence that the regulatory mechanisms acting within brain organoids are indeed the same as those acting in embryos, and therefore that organoids are truly valid models of brain development. This will be increasingly important in studies exploring gene functions in human PSC-derived organoids, where the high degree of variability between organoids can make it difficult to be confident whether an apparent phenotype is truly caused by a mutation, or whether it is a result of inter-experiment variability.

As yet, there are few examples in the literature of studies comparing the effects of the same mutation in mouse organoids to those in the equivalent mutant mouse. One such study used mouse organoids to investigate the transcription factor Foxg1 (Mall et al., 2017). Foxg1 is a high-level regulator of multiple aspects of forebrain development. Homozygous mutant mice completely lack ventral telencephalon, have dramatically smaller cerebral cortices and die at birth (Martynoga et al., 2005; Xuan et al., 1995). Mutations in human FOXG1 cause FOXG1 syndrome, a severe neurodevelopmental disorder (Vegas et al., 2018). Brain organoids grown from mouse ES cells in which both copies of Foxg1 had been inactivated showed reduced differentiation of cortical progenitor cells, which is consistent with part of the mouse phenotype (Mall et al., 2017), however as findings were based mainly on qRT-PCR data and no immunohistochemically stained sections were included, it’s difficult to be certain from this study exactly how closely the organoid phenotype matches the mouse one.

4. Some limitations of organoids can be overcome using mice

Although organoids closely resemble normal tissue, it is important to remember that there are key differences. One of the major limitations of current cerebral organoids (both human and mouse) is that they lack vasculature and blood circulation. This imposes severe constraints on the maximum size that organoids can grow to, and the extent to which they can develop normally (Qian et al., 2019). Mice have been used in attempts to circumvent this limitation. Successful xenografting of human brain organoid tissue into mouse brain in vivo has been reported by several groups. In this scenario, transplanted organoid tissue becomes vascularised, allowing it to survive and mature for longer than is possible in vitro (Daviaud et al., 2018; Mansour et al., 2018). It has also been suggested that this approach could create a more realistic model for drug discovery research as it includes a blood brain barrier (Waldau, 2019).

Cerebral organoids have been used to explore the mechanisms by which infection with Zika virus can lead to microcephaly. Several studies showed that Zika virus can infect neural progenitor cells in human cerebral organoids, causing them to cease proliferating and therefore potentially accounting for the microcephaly seen in babies born to Zika-infected mothers (Dang et al., 2016; Gabriel et al., 2017; Qian et al., 2016; Qian et al., 2017; Shao et al., 2016; Yoon et al., 2017). These papers clearly demonstrate the potential of human organoids to investigate disease states, however, the situation in vivo is more complex. The virus must first cross the placenta, then the blood brain barrier, neither of which is present in organoids. Therefore, it is important to complement the organoid experiments with in vivo studies, using mice, which more closely resemble the normal situation of Zika infection (Dowall et al., 2016; Lazear et al., 2016; Li et al., 2016; Tripathi et al., 2017). This highlights that in vivo models such as mice remain an important tool to complement some types of human organoid studies.

Finally, an important future avenue for organoid research will involve assembling circuits that resemble those found in the normal human brain and nervous system. As we cannot yet produce organoids that perfectly recreate all parts of the brain, an intermediate step in this work would be to assemble circuits between organoids and explanted tissue – mouse embryos are the obvious source for obtaining such tissue, given the practical and ethical constraints on the use of human embryonic tissue. This principle was recently demonstrated in an exciting study in which human cortical organoids were coupled together with explants mouse spinal cord and muscle. The organoids formed functional innervation with the explanted tissue, such that electrical stimulation of the organoid caused muscle contractions (Giandomenico et al., 2019).

5. Conclusion

Brain organoids present an exceptionally promising new tool to help understand brain development and disease. While much research in this area has employed human cells, we believe that organoids grown from mouse stem cells still have important roles to play, in particular to help improve protocols and make better organoids. Mouse and human brain organoids should be thought of as complementary, rather than competing tools. Similarly, mouse models are likely to remain important as tools to complement organoid-based studies for many years to come.

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