Stem cells and genome editing: approaches to tissue regeneration and regenerative medicine

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Abstract

Understanding the basis of regeneration of each tissue and organ, and incorporating this knowledge into clinical treatments for degenerative tissues and organs in patients, are major goals for researchers in regenerative biology. Here we provide an overview of current work, from high-regeneration animal models, to stem cell-based culture models, transplantation technologies, large-animal chimeric models, and programmable nuclease-based genome-editing technologies. Three-dimensional culture generating organoids, which represents intact tissue/organ identity including cell fate and morphology are getting more general approaches in the fields by taking advantage of embryonic stem cells, induced pluripotent stem cells and adult stem cells. The organoid culture system potentially has profound impact on the field of regenerative medicine. We also emphasize that the large animal model, in particular pig model would be a hope to manufacture humanized organs in in vivo empty (vacant) niche, which now potentially allows not only appropriate cell fate identity but nearly the same property as human organs in size. Therefore, integrative and collaborative researches across different fields might be critical to the aims needed in clinical trial.

Introduction

Tissues and organs in the body perform essential bioactivity over the life. A sensory nervous system such as brain and retina play roles in recognition and visual functions. A digestive system, which consists of the gastrointestinal tract plus the accessory organs of digestion (tongue, salivary glands, pancreas, liver, and gallbladder) is critical to maintain a homeostasis, including a nutrient uptake. A circulation system such as heart, kidneys, and vasculature is also important for a fluid circulation, including cells, nutrients, gases and waste products. Loss of those systems by accidents and diseases could lead to life-threatening (or lethal) problems and loss of life quality.

The self-renewal process including self-repair is known to be essential for tissue turn over and tissue restoration upon degeneration and injury. For the replacement or self-restoration of damaged tissues important for homeostasis, a better understanding of the mechanism of regeneration is important. Over the last few decades, researchers have investigated model species that have highly regenerative tissues. To this end, we realize that the ability to return to the embryonic state, alternatively a regenerative ability in mammalian species is not as advanced as it is in fishes, amphibians, and birds although the morphology, the cell types, and the function of the several organs are relatively conserved among vertebrates. Therefore, to improve clinical treatments in regenerative medicine, we need to learn not only about highly regenerative mechanisms but also about those missing in less regenerative mammalian species, such as humans. Generally speaking, in humans, the loss of a body part requires external sources to partially replace and recover the original function. One such approach is transplantation-mediated replacement with full (or parts of) organs from a donor. Since donor organs are in short

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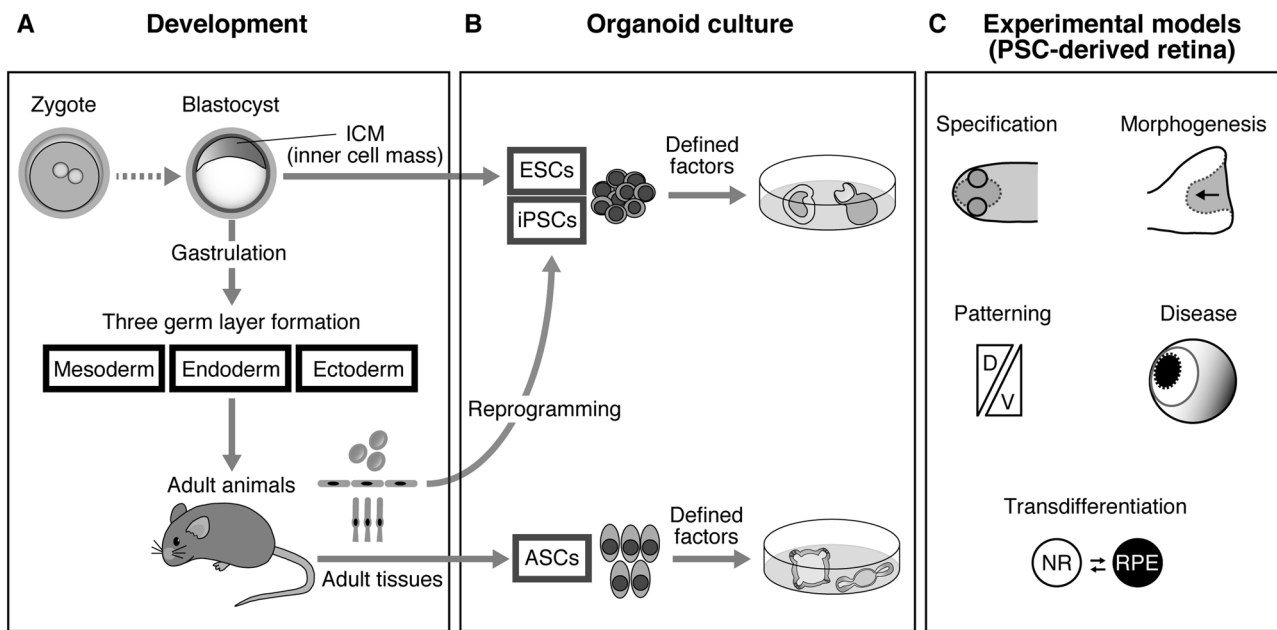
Fig. 1 Stem cell resources and experimental models using in vitro culture. **a** In normal development, a zygote becomes a blastocyst, which contains an inner cell mass (ICM), a body proper. Through a gastrulation, the ICM generates three germ layers: ectoderm, mesoderm, and endoderm. These layers further develop (or differentiate) into each organ until adulthood. Embryonic stem cells (ESCs) are derived from the ICM in the blastocyst. Induced pluripotent stem cells (iPSCs) can be generated from adult cells, such as dermal, blood, and photoreceptor cells, using Yamanaka factors (reprogramming factors) through a reprogramming process. **b** These pluripotent stem cells can be maintained in culture conditions and have an ability to differentiate into the three germ layers in response to specific stimulation such as growth factors. Adult stem cells (ASCs) have a restricted ability to generate specific tissues or organs in vitro with their original identity. **c** As an example, pluripotent stem cell (PSC)-derived eye organoids have been used in several experimental models, such as eye specification, morphogenesis, dorsal (D ventral (V) patterning, transdifferentiation, and disease models. NR, neuroretina; RPE, retinal pigment epithelium

supply, alternative approaches that allow us to produce tissues and organs are needed.

Recent progress in the biotechnologies is providing new approaches to regenerative medicine that mainly fall into five categories: formation of tissues and organs from stem cells or tissues in a dish [1–3], stem-cell derived humanized organs in large animal models, three-dimensional (3D) bioprinting using biocompatible materials [4], ex vivo decellularization [5–7], and Xenotransplantation [8–10]. We do not discuss 3D bioprinting and decellularization technologies in detail in this review.

The use of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) as the source of customized organs has become much more achievable than it was decades ago. For example, starting from a floating culture of ESCs (or iPSCs) in serum-containing media, called embryoid body culture, ESC/iPSCs can differentiate into specific cell types with individual marker expression characteristic of the three germ layers in vivo (Fig. 1). This differentiation ability, or pluripotency, is fundamental and essential for generation of desired tissues and organs.

Adult stem cells (ASCs), stem cells with a specific lineage, also have a remarkable ability to generate tissues with specific architecture when given an appropriate in vitro environment (Fig. 1). Substantial progress in stem cell culture techniques have been achieved recently, due to our better understanding of development in vivo: cell-type specification, embryonic cell-specific roles and crucial genes have been discovered by gene loss- and gain-of-function approaches. Thus, in vitro approaches using primary cells (tissues/organs) and cell lines have seen advances in how we manipulate and culture specific cells, tissues and organs using defined conditions to maintain their fate (or lineage) during growth.

There have been some remarkable developments in the use of human patient-derived cells as well. These stem cells are now considered to be a potentially valuable resource for regenerative approaches, as the problems of immune rejection and quantity of supply would be avoided. However, some roadblocks still remain, such as the fact that patient-derived cells are likely to have intrinsic genetic disorders that should be rescued or modified before their treatment.

Programmable nuclease-based genome editors, or so-called genome editing, are now recognized as a realistic genetic tool for curing diseases that are difficult to treat using currently available technologies. Genome editors are developing rapidly and in a diverse array of contexts. Zinc
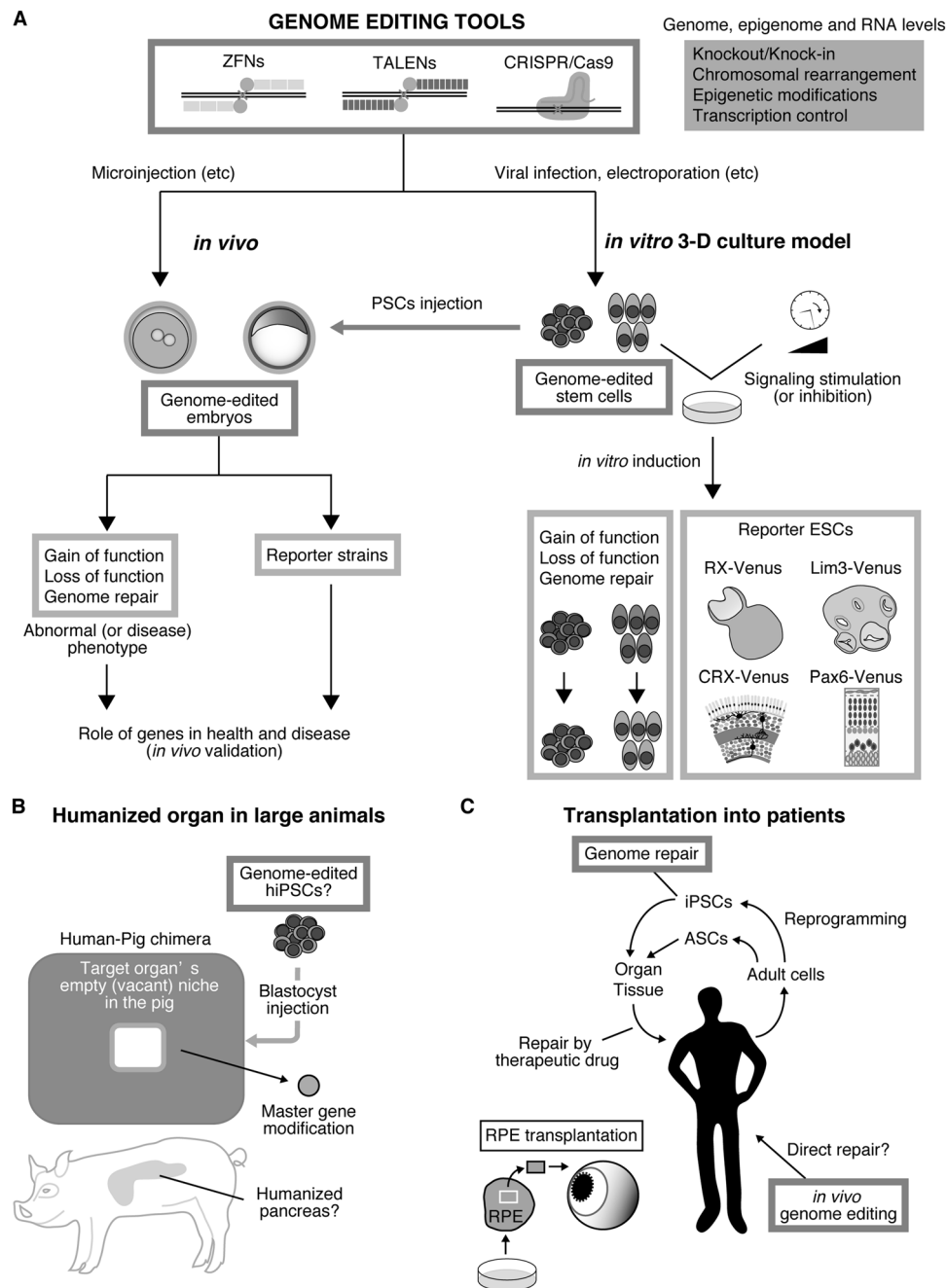
Fig. 2 In vitro and in vivo organ generation, and genome editing. **a** There are three genome-editing tools, ZFNs, TALENs, and CRISPR/Cas9. Taking advantage of genome-editing technologies in several different ways will allow us to perform better experiments, and lead to better ideas for regenerative medicine. Validation of the roles of gene function: gain of function and loss of function. Further modulation of additional genes potentially rescue an abnormal phenotype: functional repair in genome level. By modulating essential factors found in vivo studies in a time- and dose-dependent manner, stem cell-derived tissues and organs can be now generated even in vitro. Genome-edited pluripotent stem cells (PSCs) are also useful for monitoring a specific cell lineage tracing, disease models, and developmental analysis, which might be one of the standard ways for understanding pathogenesis. The resultant knowledge obtained in vitro would help to perform better treatments in future clinical setting. **b** A in vivo humanized organ

model, a large animal pig lacking (or modifying) an essential gene for pancreas development provide an empty (vacant) niche for human pluripotent stem cell-derived pancreas production in vivo. The genome-editing technology would be combined with the organ production technology outside a human body. Then, a better transplantation method for each organ would be necessary once individual humanized organs are mature enough to implant into patients. hiPSC, human iPSC. **c** Human-derived somatic cells can be used for iPSCs generation using reprogramming methods. iPSCs and adult stem cells (ASCs) from patients would have a cause of disease, which now can be repaired by genome-editing tools in clinical use. Even drug treatment might lessen the severity of diseases. Recent efforts are underway to develop in vivo genome editing, for more direct and local repair in the human body. ESCs, embryonic stem cells; RPE, retinal pigment epithelium

finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated proteins 9 (CRISPR/Cas9) are well-known tools [11]. They can create random insertion or deletion of DNA, precise base pair changes, large deletions, inversions or duplications. Alternatively, a knock-in of a therapeutic transgene at a desired location, chromosomal rearrangement, epigenetic modifications, and gene activation (or repression) in transcriptional level is also possible [12–20] (Fig. 2a).

Among them, CRISPR/Cas9 is currently the most popular tool [21, 22], and the winner of Science Magazine's Breakthrough of the 2015 Year award [23]. Since CRISPR loci were first described in 1987 [24], this system has been discovered to be necessary for acquired resistance against phages in bacteria and archeae [25]. It has turned out to be a bacterial immune system, which cleaves an invading foreign DNA in a sequence-specific manner [26]. Several experimental applications have been described [27] and they would be beneficial for biology, biotechnology, and biomedicine [28]. Further potential applications of the CRISPR/Cas9 tool include human gene therapy, viral gene disruption, agriculture, and next-generation therapies [28].

Arbitrarily introduced mutations using genome-editing tools alone showed recapitulation of several developmental abnormalities and diseases, including cancer. In contrast, by using a single-stranded oligonucleotide or double-stranded oligonucleotide as a repair template, these genome-editing tools have great potential for repairing patient-derived genetic mutations. To date, genome editing has worked in most well-studied and genome-deciphered model organisms and mammalian culture cells, including human cells [29–40]. The protocol for each of these model organisms is now being standardized. The combination of genome-editing and stem cell technologies might be quite essential for regenerative medicine.

In this review, we first describe regeneration (using the retina as an example). Then we focus on several in vitro methods for organ generation, and finally we discuss in vivo organ generation in large animal models and the use of genome-editing technologies as potential approaches toward regeneration and regenerative medicine.

Tissue and organ regeneration

Tissue turn over normally requires tissues or cells equivalent to bipotent or multipotent stem cells in order to provide newly generated cells. Regeneration of a complex system where acquisition of stemness from quiescent cells through a dedifferentiation (or reprogramming) process can be seen in the retina [41–44] and heart [45, 46]. The process requires reversion of a differentiated cell to a less differentiated state to allow proliferation or differentiation.

Mammalian species have limited regenerative capacity while some non-mammalian species like fishes and amphibians are known to have remarkable regenerative abilities. In highly regenerative model animals, heart and retina are used to identify genes and fundamental mechanisms for basic understanding of the regeneration; perhaps those mechanisms are useful for mammalian regeneration [47, 48].

Although the liver in mammals has a regeneration ability that is equivalent to other species after injury [49], the heart in mice has a restricted ability to regenerate after damage by neonatal day 7 [50, 51]. Recently Kenneth D. Poss and colleagues provided new insight in heart tissue regeneration, identifying tissue regeneration enhancer elements (TREEs) upon injury suggesting TREEs would be useful to modulate the regenerative potential of vertebrate organs [52]. Introduction of genes important for regeneration, such as Nrg1, using enhancer-based targeting by genome editing, might trigger repair processes even in the mammalian heart [52, 53].

The retina is an important sensory organ in vision. Vision loss by retinal degeneration is a critical issue that reduces the quality of life. There are three resources for the potential regeneration of retina (reviewed in [54–56]). First, retinal pigment epithelium (RPE) located outside the retina could transdifferentiate into the retina only in the amphibian upon removal of retina [57–59]; transdifferentiation is including a regenerative phenomenon where one cell type converts to another [60]. Second, ciliary marginal zone (CMZ) functions located at the rim of the retina are known to provide retinal neurons to the peripheral retina in larval and adult fish [61, 62]. Interestingly, several reports imply the stem-like cells in the mammalian CMZ as well [63–65]. The CMZ is also suggested to have contribution in regeneration of the damaged retina in frogs and fish [61, 66]. However, the CMZ seems to be present in the post-hatched bird but not in the adult and absent in mammals (reviewed in ref. 54). Third, the literature indicates that Müller glial cells are the major glial component of the retina, which is one of the last retinal cell types to be born during development [67, 68]. Müller glia are the only cells to span all retinal layers and have processes that contact neighboring neurons and form part of the outer and inner limiting membranes. Thus, Müller glia are well positioned to monitor retinal homeostasis and contribute to retinal structure and function [67]. Mammalian Müller glia have the capacity to locally de-differentiate following retinal injury to become multipotent retinal progenitors, preferentially supplying photoreceptors [69] (reviewed in ref. 70). In this process, Wnt signaling plays a crucial role [71] and this mechanism might share the similar signaling found in fish [43]. Interestingly,

the level of photoreceptor cell death-induced Müller glia proliferation is different even among mouse strains (12961/SvJ vs C57BL/6), correlating with the gene expression changes of Cyclin D1 and Nestin [72]. It suggests that the gene regulation could control this damage-induced Müller glia proliferation. Although mammalian Müller glia are still insufficient for fully repairing a damaged retina [67], Müller glial cell-dependent spontaneous regeneration is remarkable in some species such as fish [73]. The mechanisms behind the retina regeneration are also getting more understood in the high regenerative species.

Recent work in Medaka fish showed that a single neurogenic factor, Atoh7, directs Müller glia into proliferation [74]. Atoh7 forces quiescent radial glia into neurons through Notch signaling activation, which is also involved in the maintenance of the glial fate [74, 75]. mTor signaling has been shown to support the formation of proliferating Müller glia progenitor cells in the chick [76]. Comparing the methylation profiles of Müller glia and Müller glia progenitor cell provides DNA methylation landscape during cellular reprogramming and regeneration [77].

Therefore, key to a successful self-repair process in the adult mammalian retina upon injury or degenerative disease is controlling Müller glial regenerative function around in the degenerative regions. Surprisingly, a very recent report showed indeed the mechanisms underlying neuronal regeneration in adult mouse retina [78], suggesting that the regeneration of mammalian retina is not impossible. A promising strategy would be to analyze differences between the transcriptomes and epigenomes of fish and mammalian Müller glia (and Müller glia progenitors) [67, 79]. A recent novel technology, in vivo genome editing, might be an important tool for future approaches to promote a self-repair program in mammalian retina [80] (perhaps by combining with the recent finding that stimulates neuronal regeneration in the retina [78]). A combination of approaches will be needed to probe essential signaling pathways and define those signals that trigger conversion of tissue or specific cells to multipotent retinal progenitors.

In vitro tissue and organ generation

Multicellular organisms have been shown to have a self-organizing ability at the cellular level. Plant calluses, for example, are formed by de-differentiated cells that have tissue-forming ability accompanied by organogenesis [81–83]. In animals, dissociated primitive multicellular sponge cells are able to grow and differentiate into a whole sponge [84]. Single-cell suspension culture can reconstitute chick organs [85]. Tumor-derived multi-potent stem cells have tissue-forming potential and these tissues are called embryoid bodies [86]. A single human keratinocyte can generate a stratified squamous epithelium where proliferating and differentiating cells are in a basal layer and upper layer, respectively [87, 88]. The development of growth media has been the basic principle of in vitro tissue culture. Historical aspects of cell, tissue and organoid culture, and their potential applications have been extensively described by Lancaster and Knoblich [89], and Clevers [90].

Organoids are in vitro 3D tissues and organs derived from stem cells or parts of tissues, which have bipotent, multipotent, or pluripotent cells. They also show self-renewal and differentiation capacities in response to a culture environment. Organoid formation is mostly driven by self-organization in a minimal growth factor condition. The organoids typically consist of more than two different cell types and exhibit morphogenetic features closely mimicking an equivalent tissue and organ in vivo. Studies using in vitro systems tend to benefit from an abundant source, production, and easier manipulation.

ESCs can be derived from the inner cell mass of blastocysts, and iPSCs [91, 92] can be reprogramed from dermal fibroblasts [93], blood cells [94, 95], RPE [96], and photoreceptor cells [97, 98] using reprogramming factors (Yamanaka factors) (Fig. 1). Because the inner cell mass generates three germ layers—ectoderm, mesoderm, and endoderm—though gastrulation, its in vitro derivative also shows three-germ-layer differentiation (Fig. 1a, b). iPSCs show a similar extent of differentiation ability to ESCs [99].

These new stem cell technologies facilitate several lines of research related to regenerative medicine. For example, transplantation of ESC- and iPSC-derived retina has been shown to restore vision in rd1 end-stage retinal degeneration mice [100]. A clinical trial is underway, in which human ESC-derived RPE is transplanted into patients with age-related macular degeneration (RPE degeneration leading to photoreceptor loss) [101–103]. An advanced personal medicine technique is being tested, involving autologous transplantation of RPE from iPSCs that were generated from patient skin [104].

Repairing damaged heart from the limited regenerative capacity of the adult mammal is a major challenge in medicine. For this purpose, the use of stem cells would be helpful for understanding and enhancing cardiac regeneration [105, 106]. iPSC-derived multi-layered cardiovascular cell sheets that consist of cardiomyocytes, endothelial cells, and vascular mural cells, showed long-term survival of the cell graft to the heart in vivo [107]. Artificial manipulation of cell composition and geometry brings an ideal framework for scale-up to pre-clinical studies [108]. These results suggest that tissue engineering is a promising approach in regenerative medicine [109–111].

There are a wide variety of applications for stem cell technologies; infectious disease models using *Helicobacter*

pylori and Zika virus [112, 113], a congenital disease model, microcephaly [114], and an acquired disease model, cancer [115]; Toxicology models such as liver organoid testing hepatocyte function act as a gold standard [116–118]. These remarkable advances hold hope for the future of gene therapy and regenerative medicine as more personalized treatments.

Another aspect of stem cell research useful in fundamental research is that it reflects species-specific differences such as organ size, which are hard to analyze via two-dimensional culture systems [112, 114, 119–121]. Unique features of the human brain such as the inner fiber layer, outer subventricular zone, and outer radial glia [122–125], can now be generated in vitro [114]. The period of differentiation in the human brain is longer than that in the mouse [114, 126–128]. These human-specific features likely contribute to human brain-specific identity and functions [129]. Advances in this line of research would be supported by genome-editing technologies as powerful tools for basic and biomedical research [130, 131].

We have been trying to develop an ESC-derived brain and retina organoid system in 3D culture using mouse and human embryonic stem cells, asking what are the minimum or essential factors during several phases of brain development. To date, there are several successful methods for generating cerebral cortex, neuroretina, hypothalamus, and adenohypophysis from mouse embryonic stem cells [126, 128, 132–135]. After we developed mouse ESC methods, further efforts substantially advanced the 3D technologies that enable us to make human brain parts, including retina, in a dish [119, 127, 136, 137]. A key to developing in vitro organogenesis methods is visualizing key marker gene expression in living condition. To do this, researchers typically design a fluorescent reporter system, which is knocked-in at the specific gene loci by genome-editing tools, ZFN, TALEN, and CRISPR/Cas9, resulting in fluorescent cell lines such as Lim3-Venus (pituitary primordium marker), RX-Venus (neuroretina marker), CRX-Venus (photoreceptor progenitor marker), FOXG1-Venus (telencephalic progenitor marker), PAX6-Venus (cells in cortical ventricular zone), Fgf5-mTurquoise2 (epiblast marker), Six3-Tomato (early rostral brain marker), and Irx3-Venus (early caudal brain marker) [119, 127, 135, 138, 139]. This knock-in reporter approach would facilitate performing real-time monitoring including lineage tracing [119], enrichment of specific cell populations, and identification of the reporter-expressing cells when transplanted in vivo in order to rescue hypopituitarism, endocrinological disorders [135]. Using RX-Venus and CRX-Venus human ESCs, a ciliary margin-like stem cell niche was found in optic cups culture [65]. A few other examples showing fluorescent reporter knock-in in pluripotent stem cells have also been described [140–144] (also,

see a recent report, showing a 2A-peptide-based knock-in strategy for human iPSC [145]). The genome editing-assisted reporter knock-in strategy might support the identification of specific cell types responsible for self-repair and regeneration.

Because of its simple but remarkable window into self-organization, the 3D method approach is not only providing benefits for regenerative medicine but also modeling organ formation from a new angle in which the intrinsic aspects of organ development—self-assembly, self-patterning and self-morphogenesis—can be observed [146, 147]. For instance, successful live-imaging and live-manipulation of in vitro eye organogenesis allows us to find essential factors involved in the processes [133]. Similar visualization of in vivo processes is extremely difficult in mammals.

Following is a list of recent findings related to the formation of eye organoids (Fig. 1c). Using a diencephalic tissue culture condition, we showed that optic vesicle specification requires Wnt signaling [148]. Using an atomic force microscope and laser-ablation techniques, we demonstrated that relaxation of retina is a critical step for optic cup morphogenesis [133, 149]. By temporally and locally manipulating the Wnt gradient formation, we found that a dorsal–ventral retinal pattern self-forms [150]. Genome-wide analysis and bioinformatics analysis indicates transcriptional profiling of retina and RPE using a transdifferentiation assay [151]. We also provided the first example of a freezing-mediated tissue-storage model using ZFN modified-reporter knock-in human ESCs [119].

Organoids derived from adult stem cells

The adult body has several different tissue (organ)-specific ASCs, which retain the ability to self-renew and replenish all of the cell types as seen in their intact condition through a damage-repair process [152]. Understanding and manipulation of adult stem cells are quite essential for the future of basic and clinical studies in terms of developmental and regenerative aspects.

New technologies allow us to recapitulate organ formation in vitro from ASCs, as their identities are similar to the organ from which they were derived (Fig. 1b). ASC-derived organoids appear to be genetically stable even when cultured over long periods of time [152]. Like pluripotent stem cells, the genomic stability issue is considerably important when researchers try to use ASCs in in vitro culture. Keeping genomic information intact is essential for organ regeneration and to be a safe cell source for regenerative medicine [3].

There have been numerous reports that several ASC-derived organoids can be generated in vitro (reviewed in ref. 152): liver [153, 154], pancreas [155, 156], stomach

[157, 158], fallopian tube [159], prostate [160, 161], taste buds [162], and salivary gland [163].

Notably, ASCs require a specific culture environment to mimic their original environment supporting growth and stemness [3]. Even more important is the role of ASCs in regeneration upon damage. They are quiescent until needed and there are biopotent (liver or skin etc) and multipotent (intestinel, stomach, mammary stem cells, hair follicle, and so on) cell states, which are dependent on individual cell types (reviewed in ref. 164). Controlling these essential aspects of ASCs in vitro promises the success of translational research [3]. For instance, organoids can be grafted onto injured intestinal regions, and liver organoids differentiate into functional hepatocytes upon transplantation [3].

Thus, the ASC organoid culture system is a useful tool for genetic diseases, host–pathogen interactions and cancer progression models [152]. Furthermore, the combination of ASC-organotypic culture and CRISPR/Cas9-based gene editing would be obviously powerful; genome-editing technologies appear to be useful particularly in genome level manipulation [155, 165]. An initial attempt was performed using intestinal organoids that contained a patient-derived mutation [166] and subsequent work showed a sequential cancer progression by introducing oncogene-related genes [115]. Some of the ASC-derived organoids have been shown to treat the disease portion by transplantation [167–169] (reviewed in refs. 152, 170). Thereby, lineage-restricted stem cells would be expected to be useful for efficient and rapid production of tissues and organs. In this way studying in vitro models that recapitulate human development and pathologies is essential for promoting our understanding of the pathogenesis of diseases, which can lead to the discovery of novel therapeutic paradigms. Genome-editing tools would further widen the potential of ASC technologies.

Vasculature-associated organoid assembles

To support organ growth and homeostasis, the vasculature system performs indispensable roles in vivo. Because organoids show a limited growth potential and maturation level, a main limitation was thought to be the lack of nutrient support from blood vessels. Takebe et al. [171, 172] first demonstrated the creation of a vascularized and functional human liver from human iPSCs by transplantation in an in vivo environment. They succeeded in recapitulating organogenetic interactions between adult tissues, endothelial and mesenchymal cells. This technology highlights liver-specific functions such as protein production and human-specific drug metabolism [171, 172]. This discovery has two important aspects: (1) functional maturation of pluripotent stem-cell-derived tissue requires

vascular support and (2) in vivo derived vasculature can be a powerful source of vascular support by connecting with in vitro-derived vasculature. They further strived to obtain a generalized method for organ bud formation from diverse tissues, such as kidney, pancreas, intestine, heart, lung, and brain by carefully modulating mechanical properties of culture environments [173]. However, whether that vasculature could function physiologically as a stem cell niche for complex brain organoids to support growth and maturation remains unknown. In vivo studies show that direct cell–cell contact with the vascular niche is important for neural stem cell function [174–177]. Therefore, combining several complex in vitro tissues and organs might reproduce better physiological function and provide an advantageous environment for drug screening in regenerative medicine.

Humanized organ generation in vivo

Due to the limitation of availability of donors for organ allotransplantation, exploring other potential resources is essential. In the case of using stem cell technologies, generating organs sufficient for supporting the original function should be considered. It is important that organ size is appropriately fitted for patients. Therefore, to overcome issues with appropriate size, shape and matured functional human-organ requirements upon implantation of organs in the clinical setting is attracting attention. As we mentioned above, even in in vitro conditions, stem cell-derived human organoids reflect species-specific features, such as size and shape. However, complete matured and vascularized human organs are not yet available by in vitro methods. Alternatively, several differences between mice and humans limit the mouse as a model of human disease [178].

Work toward resolving these issues is going to open a new paradigm. It utilizes a chimeric system between closely related species so that cells of a different origin have a less possibility to be rejected in the host environment. Regarding an experimental system of chimerism, there is pioneering work in close species, such as chick–quail, rat–mouse, and sheep–goat [179–182], and more closely related species, Mus musculus-Mus caroli, M. caroli-M. musculus and Bos taurus–Bos indicus [182, 183]. The successful production of interspecies chimaeras is largely dependent on the matching of the species origin of the trophectoderm derivatives and that of the maternal uterus [184–186].

In addition, owing to a dramatic increase in large animal models, mechanisms of pathology and regenerative medicine are becoming more understood and use of those models has become central to new therapeutic strategies [178] and the large animals have been already shown to be engineered (reviewed in refs. 187–189). The domestic pig is one of the most popular animal models for agricultural and biomedical

research. Recently, the pig system has enabled researchers to generate several human organs, overcoming not only the number of organs but also organ size (reviewed in ref. 190) (Fig. 2b). In this large animal model, genetically modified chimaeric pigs are used in order to replace pig organs with humanized organs by combining three powerful techniques: generation of interspecies chimaeras, manipulation of pluripotent stem cells, and genome editing. Based on their initial principle established in rodents [191, 192], Nakauchi and colleagues produced a method that potentially allows human PSC-derived organ generation in the vacant space (or an empty developmental niche; see also the interspecies chimerism between mouse and rat [193]) of a non-rodent large animal, the pig [194]. Belmonte and colleagues indeed showed an interspecies chimerism between human and pig using human iPSC, suggesting the possibility of xeno-generating human tissues and organs in the pig [195] (see also a review paper [186]). Pigs with severe combined immunodeficiency (SCID) that were generated by the TALEN system are also available, perhaps providing a useful model for xenotransplantation [196]. There are a couple of potential examples reviewed in detail by Nakauchi of "genetically engineered organ niches" [186, 197].

Interestingly, work showed an example of combining somatic cell nuclear transfer (SCNT) and direct injection of the CRISPR/Cas9 ribonucleoprotein complex which led the generation of a growth-hormone receptor binding protein-10 (GRB10) ablated pig [198]. As such the CRISPR/Cas9 system-mediated genome-modified pigs have also been reported, raising the possibility of their utility for application in regenerative medicine [199, 200]. Although, so far, larger animals such as dogs, pigs, and primates have been used [178, 201, 202], this work suggests that the pig can serve as a better bridge between rodents and primate models in order to study human diseases [198]. This system might provide an unlimited number of humanized organs. Additional examples of genome editing in pigs are available elsewhere [198, 203–208].

Conclusions

In the near future, a rapid and reliable method may be developed that allows genetically engineered organ niches in large animal models using genome-editing tools. However, ethical issues need to be carefully considered [209]. In particular, human central nervous system (CNS)-derived organs require special attention. It is quite difficult to use large animal models in order to generate humanized CNS. We can not also exclude the possibility of having new hybrid human–pig viruses in this system. Therefore, transplantation technology without the large animal technology is still a realistic way to replace degenerated portions with autologous patient-derived tissues and organs, which are optionally repaired by genome-editing technology. Alternatively, direct repair of patient degenerative portions by genome-editing technology would be a future challenge (Fig. 2c). To accomplish this ambitious aim, a comprehensive understanding of the essential differences between less regenerative species and highly regenerative species would be needed by taking account of evolutionary changes (or aspects) of organ regeneration. It may provide the basic idea to develop alternative strategies to stimulate self-repair in the low regenerative organs. Stimulating regenerative ability by introducing a genome modification, introducing exogenous cells/tissues (enhancing endogenous regenerative ability), or a combination of both could eventually be possible in less regenerative tissues and organs with the assistance of advanced genome editing tools and stem cell technologies.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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