Interspecies Organogenesis for Human Transplantation

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Abstract

Blastocyst complementation combined with gene editing is an emerging approach in the field of regenerative medicine that could potentially solve the worldwide problem of organ shortages for transplantation. In theory, blastocyst complementation can generate fully functional human organs or tissues, grown within genetically engineered livestock animals. Targeted deletion of a specific gene(s) using gene editing to cause deficiencies in organ development can open a niche for human stem cells to occupy, thus generating human tissues. Within this review, we will focus on the pancreas, liver, heart, kidney, lung, and skeletal muscle, as well as cells of the immune and nervous systems. Within each of these organ systems, we identify and discuss (i) the common causes of organ failure; (ii) the current state of regenerative therapies; and (iii) the candidate genes to knockout and enable specific exogenous organ development via the use of blastocyst complementation. We also highlight some of the current barriers limiting the success of blastocyst complementation.

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Introduction

In the 2017 year-end annual report, the United States Department of Health and Human Services Organ Procurement and Transplantation Network reported that nearly 115,759 patients needed lifesaving organs, but only 34,770 transplants were performed. For many end-stage diseases, organ transplantation is the final resort for survival after all other treatments have been rendered ineffective. The severe shortage in the availability of organs and the increase in their demand have forced the research community to develop novel sources of organs and cells for transplantation in humans. Various in vitro techniques have been described for the differentiation and maturation of numerous cell types in both two-dimensional cultures and three-dimensional "organoid" cultures. While many of these protocols have resulted in cell lines that can mimic various properties of their in vivo counterpart, they cannot yet recapitulate the full spectrum of spatiotemporal signals required to develop fully functional tissues and organs for transplantation.

One approach that may have immense potential in addressing the shortage of organs for transplantation is 'blastocyst complementation' (Fig. 1). In this approach, embryos from one organism are genetically engineered so that they lack a functional gene(s) necessary for the development of the tissue of interest. The organogenesis-disabled embryos are then microinjected with healthy pluripotent stem cells (PSCs) from a second organism, and are then transferred into a maternal surrogate. Through normal mammalian development, the microinjected PSCs occupy the niche left by the gene knockout, and the progeny of these cells develop into a functional organ. Beginning in the early 1990s, with a resurgence in the last decade, blastocyst complementation has surpassed many milestones with the goal of generating human organs within non-human hosts (Table 1). Currently, the pig is the model of choice for chimeric host organism due to anatomical and physiological similarities with humans[7–9]. Gene editing technologies such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) can be used to inactivate (knockout) specific genes in mammalian cells at high efficiency[10]. Specifically, in the pig, genomic editing was achieved using both TALEN[11] and CRISPR/Cas9[12] approaches.

It is highly conceivable that blastocyst complementation can be used for the generation of human pancreas, liver, heart, kidney, lung, skeletal muscle, as well as cells of the immune and nervous systems. Within this review, we will focus on the development of these tissue systems and identify candidate genes for use in blastocyst complementation (Table 2). Knockout of candidate genes should, in theory, create a vacant niche in the organ/tissue system of interest for cells derived from the microinjected PSCs to occupy and proliferate, leading to fully functional tissues. Furthermore, candidate genes should be spatially restricted to limit the potential for off-target chimerism.

Pancreas

The human pancreas performs essential exocrine and endocrine functions. Pancreatic acinar cells produce enzymes critical for food digestion, and hormones from the endocrine cells of the pancreatic islets control blood glucose homeostasis. Medical conditions, such as diabetes and pancreatitis, that result in pancreatic dysfunction are extremely debilitating for patients and place an increasingly untenable economic burden on healthcare services and society. However, current treatment options for pancreatic diseases are limited. Currently, the pancreatic disorder that urgently requires new treatment options is type 1 diabetes mellitus (T1DM). T1DM is an autoimmune disorder that results in the destruction of the insulin-secreting β-cells affecting more than 30 million Americans[13]. Despite improvements in life-sustaining exogenous insulin therapies currently available, such as insulin pumps and continuous glucose monitoring systems, treatment via this route is non-physiologic resulting in secondary complications[14,15].

Allogeneic human pancreatic islet transplantation has demonstrated value as a therapeutic modality for T1DM that can restore insulin independence by providing a physiologic insulin source resulting in near-normoglycemia[16,17]. Due to the limited supply of suitable donor organs, however, alternative sources of pancreases or islets are needed to extend these therapies to the vast majority of patients with T1DM. Although there has been great interest in the development of virtually unlimited sources of islets, such as porcine islets or β-cells derived from human pluripotent stem cells, each of these methods still has major technical hurdles to overcome before they are ready for widespread clinical use[18].

Alternatively, blastocyst complementation may provide a route to a virtually unlimited supply of human pancreas and make pancreatic islet transplantation widely available to patients with T1DM. In fact, blastocyst complementation has successfully generated a functional pancreas in mice, rats, and pigs through blastocyst complementation targeting of the *Pdx1* gene[2–5,19]. Functional PDX1 activity is critical for the development of the pancreas, beta cell maturation, homeostasis between neogenesis and apoptosis, duodenal differentiation, and biliary development. Hence, *Pdx1* represents an obvious target for gene knockout and blastocyst complementation[20]. Through TALEN or CRISPR/Cas9 genome editing, *Pdx1* mutations have previously been introduced into donor mouse or rat embryos[4,5]. Similarly, porcine fibroblasts genetically engineered to express the transcription factor HES1 from the PDX1 promoter repressed pancreatic development in pigs following somatic cell nuclear transfer[3]. The pancreatic disabled embryos from these studies were injected with induced pluripotent stem cells (iPSCs) from the same species (mouse → mouse, rat → rat, pig → pig) or from different species (rat → mouse, mouse → rat)[2–5]. The resulting chimeric offspring presented with pancreata comprised of donor cells, which were sensitive and responsive to glucose. Furthermore, glucose tolerance was restored
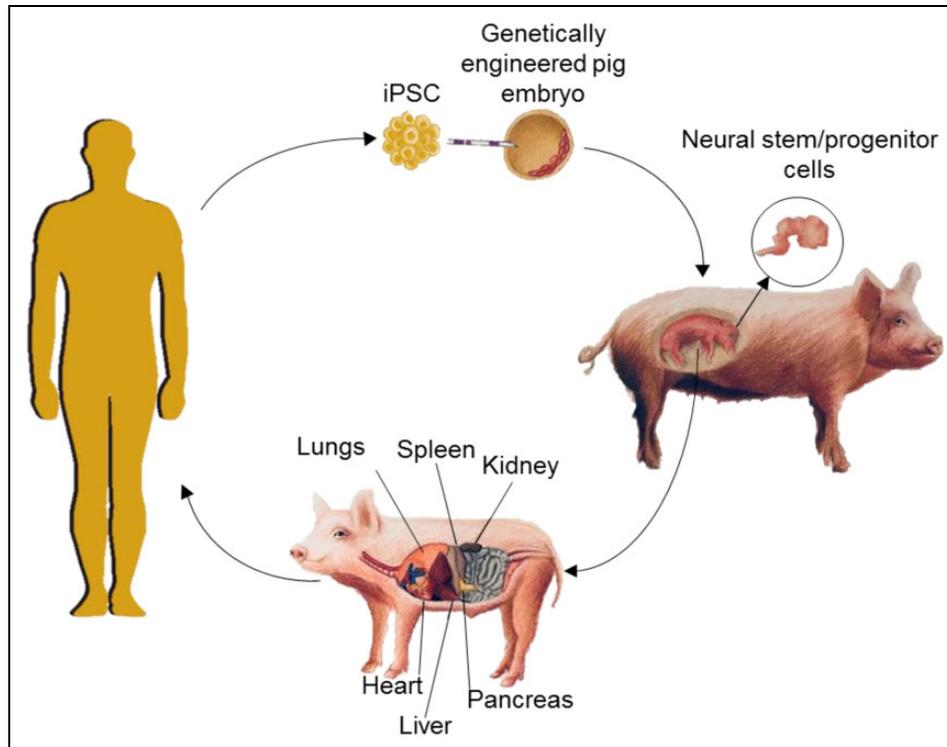
Fig 1. Cartoon schematic of blastocyst complementation. Human pluripotent stem cells grown in vitro are microinjected into genetically engineered porcine blastocysts which are then transferred to surrogate sows. The chimeric blastocysts will develop to a fetal stage in which human neuronal stem/progenitor cells can be harvested or to live-born animals where mature human organs can be harvested and processed for transplantation into patients.

in diabetic mice following transplantation of islet cells derived from mouse → rat chimeras[4].

Other transcription factor candidates involved in fetal development of the pancreas may provide alternative host niches for either generation of the entire pancreas or more targeted pancreas tissue replacement using blastocyst complementation. Additional candidate genes for knockout include *Nkx2.2*, *Pax4*, *Neurod1*, and *OC-1*. Inactivation of genes involved in neogenesis such as *Pdx1* and *Nkx2.2* resulted in a substantial and complete loss of alpha and beta islet cells. *Pax4*, known to be associated with the maturation of beta cells, may also provide a holistic niche for human stem cells to propagate. *Neurod1* inactivation results in reduced beta cell populations and the inability to produce mature islets. This gene has also shown involvement in both pancreatic cell expansion and glucagon expression. In terms of addressing the exocrine component of the pancreas, OC-1 is expressed in early and late pancreatogenesis and is significantly involved in the development of the pancreatic duct[21].

Liver

The liver is the largest organ in the human body, and has the unique potential to regenerate its entire mass even after major resection[22,23]. It also performs a wide variety of complex and critical metabolic, synthetic, immunologic, and detoxification functions. In 2016, more than 40,000 individuals died as a result of chronic liver disease and cirrhosis[24]. Alcohol consumption, obesity, type 2 diabetes, metabolic syndrome, and infection with hepatitis viruses are major causes of acute and chronic liver diseases, as are many inborn errors of metabolism[25]. Even though hepatocyte transplantation could potentially correct a variety of these disorders, whole liver transplantation remains the most viable and appealing option.

Due to the severe shortage of livers for transplantation, efforts are being made to restore the function of damaged livers by alternative means, including hepatocyte transplantation[26,27]. A number of techniques have been developed to optimize the growth and propagation of primary and stem cell-derived hepatocytes in vitro, and to maintain their phenotypic characteristics[28–30]. Investigations aimed at culturing hepatocytes as 3D spheroids have demonstrated improved function when compared with monolayer cultures[31,32], which can be further augmented using arrays[33], bioreactors[34], microencapsulation[35–37], and decellularization/recellularization of biologic scaffolds[38,39]. Successful hepatocyte transplantation, however, requires reliable sources of hepatic cells (both intrahepatic and extrahepatic). High-level propagation of primary hepatocytes that ensures

Table 1. Milestones of Blastocyst Complementation.

Organ/Tissue System	Gene Target	Milestone	Ref
Immune system	*C-kit*	First publication describing blastocyst complementation method	Chen[1]
Pancreas	*Pdx1*	Intraspecies and interspecies complementation to generate functional mouse pancreas and functional rat pancreas in mouse donor	Kobayashi et al.[2]
Pancreas	*Pdx1*	Intraspecies complementation to generate functional pig pancreas	Matsunari et al.[3]
Pancreas	*Pdx1*	Interspecies complementation to generate functional mouse pancreas in rat donor	Yamaguchi et al.[4]
Pancreas, heart, eye	*Pdx1, Nkx2.5, Pax6*	Use of CRISPR/Cas9 to inactivate genes in mouse zygotes for blastocyst complementation	Wu et al.[5]
General	-	Identification of pluripotent state for human stem cells which formed chimeric pig fetus	Wu et al.[5]
Vasculature	*Flk-1*	Providing an avenue to reduce potential organ rejection due to xenogeneic derived vasculature	Hamanaka et al.[6]

Table 2. Candidate Genes for Blastocyst Complementation.

Organ/Tissue System	Major candidate genes	Other candidate genes
Pancreas	*Pdx1*[a]	*Nkx2.2, Pax4, NeuroD1, OC-1*
Liver	*Hhex*	*Tbx3, OC-1, OC-2*
Heart	*Tbx5, Nkx2.5*[a]	*Gata4, Mst1, Id-1, Id-3*
Kidney	*Bmp7, Sall1*[a], *Six1*	*Wnt9, Wnt4, Pax2, Pax8, Six4*
Lung	*Nkx2.1, HoxA5/B5/C5*	*Sox9, Id-2*
Skeletal Muscle	*Pax3, Pax7*	*SGCD*[a], *MyoD, MyoG, Myf5, MRF4*
Immune System/ vasculature	*Il2rγ/C-kit*[a]/ *Rag2* [a], *Flk-1*[a]	
Dopaminergic progenitors	*Lmx1a, Nurr1*	*Otx2, Pitx3*
Oligodendroglia progenitors	*Olig2, Olig1*	*Nkx2.2, Sox10, Pdgfra*

[a]Genes targeted in blastocyst complementation studies

formation of the liver bud, which is then arrested beyond E9.5, suggesting that *Hhex* is an ideal candidate for blastocyst complementation[41,45,48].

Studies using mutant mice have also shown that hepatoblast differentiation into biliary epithelial cells, which develop into the biliary tree, can be prevented through *Tbx3* inhibition of notch signaling[49,50]. Another set of transcription factors, OC-1 and OC-2, promote the expression of α2-macroglobulin and follistatin, inhibitors of the TGF-β/ activin pathway that is critical for the formation of the biliary tree[51]. During liver development, OC-1 and OC-2 also promote the migration of hepatoblasts in the septum transversum by stimulating the degradation of the basal lamina surrounding the liver bud[52]. Unbalanced expression of these transcription factors could lead to impaired migration and enhanced cell clustering.

Heart

Diseases of the heart are the leading cause of death in the United States, responsible for over 600,000 in 2015[53]. Dilated cardiomyopathy (DCM) and coronary artery disease (CAD) are the primary indications for heart transplant, accounting together for over 94% of the heart transplants performed in the US in 2015[54]. Heart transplantation is the definitive cure for DCM and coronary artery bypass graft and percutaneous coronary intervention are revascularization procedures commonly used to treat CAD[55]. Significant advances in immunology, immunosuppression, as well as peri- and post-operative management have dramatically reduced morbidity and mortality related to heart transplantation. Despite these improvements, heart transplantation is still associated with several limitations. Primary among

viability and maintenance of phenotypic characteristics of in vitro expanded hepatocytes, as well as efficient hepatic differentiation protocols, are major roadblocks to achieving clinical success.

The challenges to generating large quantities of hepatocytes can be directly addressed, in part, by creating human-animal liver chimeras via blastocyst complementation. In the mouse embryo, the liver is first detected as an outgrowth bud of proliferating endodermal cells in the ventral foregut on day 8 of gestation[40]. Beyond the induction stage, various transcription factors are needed for endoderm patterning and organ development. Among these, the hematopoietic-specific expression of HHEX plays a pivotal role in liver development[41], where it functions both as a transcriptional repressor and activator[42,43]. It is highly conserved in human, mouse, *Xenopus*, zebrafish and other multicellular organisms[44]. Disruption of the *Hhex* gene has been shown to result in embryonic lethality in mice[41,45–47]. Analysis of *Hhex*-knockout mice demonstrated the initial

these limitations is the need for donor hearts, which has steadily increased since 2007 and has risen 51% overall since 2004[54].

Blastocyst complementation could potentially be used to generate exogenous human hearts, but it is essential to understand the complex process of cardiogenesis, which involves several key genes, including *Tbx5*, *Nkk2.5*, *Gata4*, and *Mst1*. The T-box-containing transcription factor TBX5 has a critical role in cardiogenesis and septation. Overexpression of a dominant-negative form of the *Tbx5* gene in mice results in a severely abnormal or absent heart[56]. Both TBX5 and NKX2.5 interact to promote differentiation of cardiomyocytes[57]. GATA4 is a zinc finger transcription factor and is one of the earliest transcription factors expressed by developing cardiac cells. While *Gata4* is not essential for specifying a cardiac lineage, it is a key regulator of embryo folding. *Gata4*-null mice have generalized severe defects in ventral body patterning that leads to a failure to form a linear heart tube resulting in embryonic lethality between E7.0 and E10.5[58,59]. Mouse studies have demonstrated GATA4 physically interacts with NKX2.5 to synergistically increase atrial natriuretic factor expression[60]. GATA4 also interacts with TBX5, and mutations in *Gata4* can disrupt this binding, causing congenital heart defects in humans[61,62].

Overexpression of pro-apoptotic factor MST1 in mice results in cardiac-specific apoptosis and consequently adult-onset cardiomyopathy in mice. Complemented *Mst1* mutant blastocysts with healthy embryonic stem cells (ESCs) resulted in chimeric mosaicism of the heart with cells derived from both host and donor. The hearts of the chimeric mice functioned normally, with cell densities consistent with those of wild-type mice[63]. Other key components of cardiogenesis are the Inhibitor of DNA binding proteins 1 and 3 (ID1 and ID3) that are expressed during mid-gestation and are responsible for the formation of the epicardium, endocardium, and cardiac valves. Ablation of *Id1* and/or *Id3* results in cardiac defects and lethality in mice. Injection of healthy murine ESCs into *Id1* and *Id3* knockout blastocysts was able to rescue heart functioning to normal functioning levels and correct for otherwise altered gene expression profiles[64].

Finally, the homeobox-containing gene *Nkx2.5* plays an essential role in cardiac development and may be a viable target for blastocyst complementation[65,66]. It is expressed early in heart development and regulates the expression of multiple downstream genes involved in cardiogenesis[67]. Mice with homozygous *Nkx2.5* mutations have abnormal cardiac looping, stunted growth, and die between E9.5 and E11.5[67,68]. Complementing *Nkx2.5* mutant mouse blastocysts with rat iPSCs has been shown to rescue cardiac development and embryo growth at E10.5, but it has not yet been demonstrated to produce a live rescued chimera[5]. Given the many interactions between the genes involved during cardiogenesis, it may be necessary to identify multiple genes for successful organ generation via the approach of blastocyst complementation.

Kidney

According to the National Kidney Foundation, 30 million adults in the United States have chronic kidney disease (CKD)[69]. CKD arises from abnormalities in kidney structure and function that produce a gradual decline in glomerular filtration rate over months to years. In the United States, diabetes mellitus and hypertension are the leading causes of CKD[69]. Other etiologies include polycystic kidney disease, congenital abnormalities of the urinary tract, autoimmune diseases like systemic lupus erythematosus, and primary glomerulonephritis such as IgA nephropathy[70]. Patients with CKD have reduced life expectancy and increased risk of cardiovascular disease[70]. Progression of CKD may result in end-stage kidney failure requiring renal replacement therapy. Dialysis is life-saving but does not produce the same longevity as people with normal functioning kidneys[71]. Besides the high financial burden associated with chronic dialysis, the 5-year survival rate of patients on dialysis is only 36%[72]. Kidney transplantation offers the most effective treatment to restore kidney function but is limited by a severe organ shortage. One potential solution is to generate kidneys via the approach of blastocyst complementation.

Kidney development is a complex process involving reciprocal signaling between the branching ureteric bud, which gives rise to the collecting system, and the adjacent metanephric mesenchyme, which gives rise to the nephrons. Many growth factors, receptors, and transcription factors that are essential for normal kidney development have been identified. Thus, there are many candidate genes for kidney organogenesis via blastocyst complementation. For example, bone morphogenic protein-7 (BMP7) is a potential candidate gene because of its essential role during embryogenesis. BMP7 is expressed in the metanephric mesenchyme and ureteric buds[73], and knockout mice that are deficient in BMP7 show severe kidney abnormalities. Absence of BMP7 affects the expression of molecular markers of nephrogenesis, such as PAX2 and WNT4, between embryonic days 12.5 and 14.5[74]. Both PAX2 and PAX8 are required for branching morphogenesis and nephron formation[75]. *WNT* genes are critically important in embryonic kidney development as they control ureteric bud formation, nephrogenesis, and stem cell renewal. *WNT9*-deficient mice die within 24 h of birth due to kidney agenesis, and *WNT4*-deficient mice die due to a failure of epithelial development[76]. Inactivation of SIX1 in porcine fetuses resulted in failure in the development of the branching ureteric bud, and dual inactivation SIX1/SIX4 arrested kidney development at metanephros formation[77].

Another essential gene that may be a candidate for blastocyst complementation is *Sall1*. SALL1-deficient mice die during the perinatal period from kidney agenesis[78]. The expression of SALL1 in the metanephric mesenchyme surrounding the ureteric bud further suggested that it plays an essential role in metanephros development. Blastocyst

complementation has been successfully used to generate PSC-derived mouse kidneys in *Sall1* mutant mice[78]. In this procedure, both mouse ESCs and mouse iPSCs were capable of forming a kidney within *Sall1*-deficient mice. Flow cytometry and PCR confirmed the cell lineages, and histological analysis indicated that the injected PSCs in *SALL1*-deficient chimeric mice replaced the kidney epithelial cell lineages of the metanephros. Similarly, complementation of *Sall1*-deficient rats with mouse ESCs resulted in kidneys comprised entirely of mouse cells expressing markers indicative of functional tissue[79].

Lung

Lung disease is a major cause of pulmonary morbidity and mortality. When considering the major lung diseases where lung transplantation is a viable option, 81% are either chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) with or without pulmonary fibrosis, and bronchiectasis with or without cystic fibrosis[80]. The most common disease of the lung, COPD, is characterized by a general restriction of airflow to the lung tissue and leads to frequent coughing, sputum production, and shortness of breath[81]. Conversely, ILD represents many different diseases and complications, most of which are related primarily to malformations and fibrosis of the connective tissue surrounding alveoli within the lung. Restriction of air passage leads to mild to extreme shortness of breath and a painful dry cough[82]. Excessive fibrosis and scarring can then lead to pulmonary fibrosis, a more serious condition where lung transplantation becomes necessary[83].

Between 1990 and 2013, there were 45,452 lung transplantations reported globally to treat progressive lung disease. This large cohort had a median survival of 5.7 years after transplant and a survival rate of 31% at 10 years posttransplant. The need for an alternative source of lung tissue is clear, as there were 4218 lung transplants reported to the International Society for Lung and Heart Transplantation in 2015, with many more left on donor waiting lists[84].

Generation of lung tissue has not yet been attempted by blastocyst complementation, in part because there is no clear gene candidate for inactivation. For this reason, development of a lung may require a combination of different gene and transcription factor knockdowns. Currently, the candidate gene with the most potential as a regulator of overall lung development knock-down is *Nkx2.1*, given that it is the first recognizable transcription factor present during the onset of the early lung buds and *Nkx2.1*-null mice lack lung formation[85]. At E9.0 in mice, and 28 days gestation in humans, NKX2.1 is expressed along with LEF1 and AXIN2 on the ventral side of the anterior foregut endoderm and is driven upstream by WNT/β-catenin signaling[86]. Shortly after initial NKX2.1 detection, expression increases and a primitive trachea extends followed by formation of the two lung buds into the mesenchyme. The proximal-distal axis is initially regulated by SOX2 expression at the proximal end

and SOX9/ID2 expression at the distal end and is responsible for the migrating split at E12.5 in the mouse. The proximal SOX2 progenitor cells differentiate into secretory and ciliated lineage cells[87], whereas the distal SOX9/ID2 progenitor cells differentiate into type 1&2 terminal alveolar cells[88]. HOX5 is an external regulator of WNT signaling in the patterning of the lung related to BMP4 feedback and NKX2.1. Triple paralog HOXA5/HOXB5/HOXC5 mutant embryos lose WNT2/2b signaling completely in the mesenchyme and have severe defects in proximal-distal patterning and overall branching[89]. Clearly, similar to all complex organs of the body, there are many different genes that play major roles in the development of the lung. Currently, *Nkx2.1* in combination with all three *Hox5* paralogs represent the best candidate genes for lung organogenesis through blastocyst complementation, as these are the early genes in initial lung bud formation.

Skeletal Muscle

Muscular dystrophies are one of the more prevalent diseases that affects skeletal muscles. The most common form of this affliction is Duchenne muscular dystrophy (DMD), in which symptoms manifest in the form of muscle weakness and can lead to insufficient respiratory muscle function and cardiomyopathy[90]. The disorder itself is a recessive genetic condition associated with the dystrophin gene. Two-thirds of cases are inherited from the parent as an X-linked recessive trait, while the rest are the result of mutations within the gene itself[91]. Muscles that lack functional dystrophin are more prone to injury and have been found to undergo cycles of necrosis and regeneration[90]. There is currently no cure for the disease, but gene therapies have created treatments that are helping to eliminate symptoms and recover some degrees of function[92].

Muscle satellite cells are stem cells that can proliferate to produce myoblasts and regenerate muscle fibers. Muscle satellite cells are quiescent in adults, but, following injury, they are activated to express MYOD and begin the production of myogenic precursors cells for the repair and regeneration of muscle fibers within the body[93]. The potential of satellite cells to aid in the regeneration of muscle systems has led to increased studies for the role of transplantation as a treatment modality. Myoblasts can be isolated from adult muscles and expanded ex vivo[94]. Through this process, the myoblasts maintain their ability to further differentiate into muscle fibers and form connections between tissues, which presents unique opportunities for transplantation therapies. Myoblast transplantation has been successfully performed in both mouse models and patients suffering from DMD[93]. Diseased muscles fuse with the newly introduced myoblasts, leading to improvements in their function as the new cells integrate into the system and differentiate into healthy fibers[94]. However, satellite cells freshly isolated from adult muscles are best suited for regeneration due to their ability to engraft within the native muscle cells and self-renew[93].

Myogenic regulatory factors (MRFs) are key in myogenic specification and characterizing the expression of muscles cells. There are four MRFs that drive myogenesis within muscle stem cells: MYF5, MRF4, MYOD, and MYOG[95]. Knocking out single MRFs or combinations of MRFs can lead to a total loss of body muscles as reported in mouse models. Two of the most important genes for muscle development are *Pax3* and *Pax7* as upstream genes of MRFs. Knocking out the *Pax3* and *Pax7* genes completely halts myogenic differentiation[95].

Through blastocyst complementation, it is possible to induce the growth of human skeletal muscles that are fully functioning and provide satellite cells that produce healthy myoblasts. While they can be used as various therapies for a number of disorders, their greatest potential may be in the treatment of DMD[93]. Blastocyst complementation has been the focus of research to generate healthy skeletal muscle within animal models of muscular dystrophy. Microinjection of healthy mouse ESCs into blastocysts derived from the dystrophin mutation *mdx* mouse model of DMD resulted in chimeric mice, presenting with up to 30% mosaicism in skeletal muscle, and significantly increased levels of muscular dystrophin[96]. Similarly, microinjection of healthy mouse ESCs into blastocysts derived from the sarcoglycan-δ (SGCD) knockout mouse model of Limb-girdle muscular dystrophy-2F resulted in chimeric mice that were observed with a normal sarcoglycan complex within the skeletal and cardiac muscles[97]. Mosaicism of levels lower than 60% in the SGCD complemented mice, however, were unable to ameliorate the pathology seen in SGCD knockout mice[97]. While neither of these studies identified complementation of myoblasts, the degree of chimerism within the skeletal muscle of the *mdx* and SGCD mutant mice may provide targets for future complementation studies.

Immune Cells/Vasculature

Diseases of the immune system include severe combined immunodeficiencies (SCIDs), which are genetic diseases usually present at birth with non-functional T-helper cells combined with the absence of T-lymphocyte and B-lymphocyte function. X-linked SCID accounts for about 19% of all SCIDs and is due to a mutation in the IL-2Rγ gene[98]. Adenosine deaminase deficiency (ADA) makes up 10% of all SCIDs and causes accumulation of toxic dATP, which inhibits dNTP synthesis and resulting in the death of T-lymphocytes[98]. RAG-SCID (Omenn syndrome, OS-SCID) is caused by mutations of the recombinase activating genes (RAG1 or RAG2) that encode for enzymes involved in the first stage of V(D)J recombination and is critical to the immunoglobulin heavy-chain joining of B- or T-cell receptors accounts for roughly 15% of all SCIDS[98].

Enzyme replacement therapy and hematopoietic stem cell (HSC) transplantation are the most common therapies for SCIDs. Although the success rate may reach 90% in matched HSC transplantation and 60–80% in haploidentical parent transplants, graft-versus-host disease remains a major concern in the context of incomplete HLA matching between donor and host. Blastocyst complementation could provide a source of HLA matched HSCs for use in the treatment of SCIDs.

A variety of SCID mice have already been developed that have aided in the study of the human immune system and disease, and can help to identify potential niches for blastocyst complementation[99]. The transgenic IL-2Rγ-deficient mouse presents with almost undetectable levels of circulating lymphocytes and NK cells. The c-kit point mutations lead to pleiotropic developmental defects, especially hematopoietic defects[100,101]. Chen and colleagues complemented RAG-2-deficient blastocysts with mouse ESCs to generate somatic chimeric animals with normal mature B and T cells, all of which were derived from the injected ES cells[1].

Various techniques have been utilized to generate immunological chimeras. One method, currently employed by the Jackson Laboratory, is injection of human CD34+ hematopoietic stem cells into myeloablated adult non-obese diabetic SCID mice[102]. Within 12 weeks, the human CD34+ chimeric mice present with multi-lineage human hematopoiesis[102]. A second technique to generate chimeras is to introduce hematopoietic stem cells *in utero*. This method was published by Sasaki and colleagues, where Cynomolgus macaque ESCs were differentiated, in vitro, into a mesodermal lineage and injected into the primordial liver of sheep during the first trimester of development[103]. Low levels of macaque hematopoietic stem cells were observed in the peripheral blood and bone marrow up to 17 months following engraftment. While these two methods resulted in complementation of hematopoietic cells, a full recapitulation of the immune system was not observed.

Blastocyst complementation may be applicable to generate the full complement of immune cells. To test this hypothesis, Jansson and colleagues injected GFP-labeled mouse ESCs into W(41)/W(41) c-kit mutant mouse blastocysts, which typically present with reduced number and functionality of HSCs[104]. Fetal livers from the chimeric mice were isolated at embryonic day 14.5 chimeric mice, and GFP+ hematopoietic cells were observed at levels greater than 90%. Importantly, the dissociated cells from the chimeric GFP+ fetal liver were then transplanted into lethally irradiated mice, which resulted in near-complete engraftment in the bone marrow and peripheral blood. These results demonstrate an available niche for HSCs that can then be used therapeutically via blastocyst complementation.

A major obstacle in organ generation via blastocyst complementation is that the vasculature of the organ of interest is derived from cells from the host animal. In an effort to make a rejection-free transplantable organ through blastocyst complementation, it is necessary for the vasculature and the organ to be derived from human cells. The Nakauchi lab at the University of Tokyo has attempted to address this issue through complementation of *Flk-1* mutant embryos[6]. *Flk-1* is required for the formation of vascular and hematopoietic

tissues. It was observed that complementation with mouse PSCs resulted in adult mice with donor-derived blood vessels and hematopoietic stem cells. While complementation of *Flk-1* mutant embryos with rat PSCs did not result in live-born chimeras, these results provide an avenue for future complementation experiments where the risk of graft rejection is minimized.

Dopaminergic Neuron Progenitors

Transplantation of a population of dopaminergic neural progenitor cells as a therapy for Parkinson's Disease (PD) has arguably advanced the furthest of any neurological cell replacement therapy, to date. The clinical diagnosis of PD occurs well into disease progression as patients will report symptoms of essential tremor, bradykinesia, and rigidity, which is a result of the loss of dopaminergic neurons within the substantia nigra [105]. While the prevalence of PD can be stratified among different geographical locations/ethnicities, it is generally estimated that almost 2 individuals per every 1000 in the population has symptoms which can be diagnosed as PD, with increased risk associated with aging[106].

The success of cellular transplantation for PD is due to the identification of a population of dopaminergic neural progenitors within the fetal ventral mesencephalon (fVM), which can innervate the striatum and release dopamine to near physiological levels. Clinically, transplantation of fVM was attempted in the 1980s and 1990s in Europe and the USA. Patients were observed to benefit from transplantation with a reduction in off-medication Unified Parkinson's Disease Rating Scale, an increase in radiolabeled 18F-DOPA uptake, and a reduction in anti-PD medication up to 15 years after transplantation[107,108]. Post-mortem analysis of the brain of one PD patient transplanted with fVM tissue revealed survival and integration of the graft nearly a quarter-century following transplantation[109]. In some patients, one unforeseen outcome was the development of debilitating graft-induced-dyskinesias—a poorly understood phenomenon[110–112]. Due to the limited availability and logistical hurdles associated with human fetal tissue, several laboratories have focused on in vitro differentiation[113,114] and transplantation of dopaminergic progenitors using ESCs[114–116] and iPSCs[117–119]. The in vitro differentiation protocol of pluripotent stem cells, however, is not a replacement for in vivo development.

Generating authentic nigral dopamine neurons by blastocyst complementation can provide a source of human VM tissue isolated from the fetal chimera, which has been subjected to all the inductive cues of development. In-depth analyses of the development of the substantia nigra have provided a wealth of knowledge on the transcription factors involved and the timing of gene expression in rodents and humans[120–122]. Identifying a specific gene (or genes) to knock out that will create a specific niche for complementation is difficult. In general, the development of the substantia nigra can be broken down into three stages: regionalization, specification, and maturation. In transgenic mice and chick embryos, knocking out specific genes during the regionalization, specification, or maturation stages has led to varying degrees of cell loss within the substantia nigra. Knockout of *Otx2* during the regionalization stage results in significant malformation of the midbrain[123]. Knockout of *Nurr1* or *Lmx1a* during the specification and maturation stage resulted in a reduction of nigral dopaminergic neurons and also resulted in a decrease in ventral tegmental dopaminergic neurons[124–126]. In the maturation stage, live-born aphakia (*Pitx3* mutant) mice present with a nigral specific reduction in the number of dopamine neurons[127,128].

Oligodendrocyte Progenitors

Oligodendrocytes are the myelinating cells in the central nervous system, aiding in neuronal communication through proper conduction of action potentials. Multiple diseases are associated with oligodendrocyte dysfunction, but four of the most prevalent are multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), and spinal cord injury (SCI). MS is characterized by the loss of oligodendrocytes resulting in motor deficits, tremors, and visual problems. Currently, there is no cure for MS, and it is a neurodegenerative disease that gradually worsens over time. ALS is characterized primarily by a loss of upper and lower motor neurons, but oligodendrocyte dysfunction largely influences the disease progression through failure to remyelinate and support metabolic function. ALS currently has no cure and results ultimately in death, most commonly from an inability to contract the diaphragm. TBI is the most common cause of morbidity in young people in the United States, with 2,800,000 emergency room visits related to TBI per year[129]. According to the World Health Organization, 250,000–500,000 new SCI cases are reported each year worldwide, resulting in compromised mobility, sensation, and paralysis. Currently, treatments for both TBI and SCI are largely palliative, with no FDA-approved therapies to reverse the damage sustained by the injury.

There are multiple clinical trials attempting to transplant human oligodendrocyte precursor cells (OPCs) into the lesioned CNS. Q Therapeutics has developed a human glial-restricted progenitor cell line with the capability of forming myelinating oligodendrocytes in animal models of MS, ALS, and SCI. Similarly, Asterias Biotherapeutics is attempting to use human ESC-derived OPCs to treat SCI in a phase1/2 clinical trial. Other groups such as Neuralstem, Stem Cells Inc., and FortunaFix have either used, or propose to use, other progenitor cell types such as neural stem cells that are designed to generate OPCs in combination with other neural cell types. All of these groups have published results demonstrating safety and efficacy in rodent models of disease[130–133]. These studies suggest that OPCs may be an effective therapeutic target for transplantation.

Oligodendrocytes develop initially from the ganglionic eminences of the developing forebrain[134]. These cells begin

migrating and differentiating largely after neuronal development from a subset of glial-restricted progenitor cells[134–136]. For blastocyst complementation, it is important to choose a genetic knockout that is early in OPC development to stall the endogenous population of glial precursor cells before they fill the OPC niches. Conversely, a gene too early in OPC development will likely affect too many developmental pathways, thus potentially leading to undesired cell types in addition to OPCs. Accordingly, the transcription factors *Olig1*, *Olig2*, and *Sox10* are likely the best candidates to target. *Olig2* has been implicated in a number of other cell types, including motor neurons and cholinergic neurons, but still is a relatively specific transcription factor[137,138]. OLIG1 is a later-expressed transcription factor in OPC development, likely initiating the expression of myelination proteins[139]. Additionally, SOX10 is a transcription factor downstream of OLIG2 and has been shown to interact with OLIG1 to initiate myelination[139,140]. Although none of these targets are completely specific to OPCs, they are early enough to ensure that a vacated niche is present. Other target genes include myelin basic protein, oligodendrocyte marker *Pdgfr*α, or *Nkx2.2*, although these targets are less promising.

Barriers and Challenges to Complementation

Aside from the ongoing ethical debate surrounding blastocyst complementation, many technical barriers currently need to be addressed. Off-target chimerism with human cells present in the brain or reproductive system of the host animal is one major concern[141]. Conversely, mixed chimerism in the target tissue where animal cells are present within the human organ has also raised concerns over the viability of the transplanted organ, as well as immunologic considerations. All of these issues have been discussed previously[142–144]. Other technical barriers that need to be addressed include the feasibility of generating genetically engineered embryos, human donor cell characteristics, and the low efficiency of interspecies chimerism.

One major technical problem is the ability to obtain large quantities of genetically engineered large animal blastocysts. In the mouse, where knockout models are abundant, it is possible to obtain fertilized blastocysts carrying the desired genotype from transgenic mouse breeders. As validated and characterized transgenic livestock are in limited supply, it is currently necessary to harvest wild-type oocytes, which can be used to generate parthenogenetic embryos through chemical or electrical activation[145,146]. The uncleaved embryos are then genetically engineered through TALEN or CRISPR injections to inactivate the desired gene[11,147]. This process results in limited knockout blastocysts, as success rate at each step is compounded. To promote survival of the embryos and increase the rate of blastocyst development, anti-apoptotic reagents have been added to the cultures, but there still is no consensus on optimal conditions[148–150].

Another technical problem is the chimeric potential of the donor cells. Results from interspecies chimera studies, to date, have not identified the characteristics of a cell line that will best incorporate with the developing embryo. Recent reports suggest that developmental synchronization between host embryo and donor cells appears to be required, with naïve-iPSCs and intermediate-iPSCs contributing to chimeras[5,151–153]. Methods to enhance the chimeric potential of donor cells have been attempted through inhibiting apoptosis until the developmental stage matches that of the host[151,154].

Low efficiency of successful interspecies complementation is another critical issue that must be addressed. Theunissen and colleagues reported that 0.2% of human-mouse chimeric embryos implanted into a maternal surrogate developed into a fetus with roughly 1 out of 10,000 cells of human origin[155]. Similarly, less than 0.1% of human-pig chimeric embryos developed into a fetus with roughly 1 out of 100,000 cells of human origin[5]. In a study on intraspecies complementation, 5% of complemented blastocyst resulted in fetuses demonstrating complementation within the pancreas[3].

Conclusions

The potential of new gene editing technologies, most notably genome modifications using CRISPR and TALENs, is evident from the recent exponential surge in research activity. Use of these technologies in combination with blastocyst complementation could greatly enhance our ability to produce human organs and cells in a relatively short period of time. Furthermore, generation of human-animal organ chimeras together with their associated vasculature could overcome the problems of organ shortage for transplantation, in addition to those associated with immune rejection. However, it will be imperative, for a variety of reasons, that we understand the conditions of stem cell growth in a developmental environment after their injection into a blastocyst, and development of the chimeric tissue. To achieve clinical success in human transplantation using complemented organs produced in animals, we must address critical issues such as species-specific differences in the mechanisms of fetal development, size of developed chimeras for transplantation, the possibility of transmission of zoonotic diseases through human-animal chimeras. The combination of gene editing and blastocyst complementation has created an entirely new and exciting field of medicine—one in which the role of precision medicine becomes a key to long and/or improved quality of life.

Ethical Approval

This study was approved by our institutional review board.

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References

1. Chen J. RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. Proc Natl Acad Sci USA. 1993;90:4528–4532.
2. Kobayashi T, Yamaguchi T, Hamanaka S, Kato-Itoh M, Yamazaki Y, Ibata M, Sato H, Lee YS, Usui J, Knisely AS, Hirabayashi M, Nakauchi H. Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. Cell. 2010;142(5):787–799.
3. Matsunari H, Nagashima H, Watanabe M, Umeyama K, Nakano K, Nagaya M, Kobayashi T, Yamaguchi T, Sumazaki R, Herzenberg LA, Nakauchi H. Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. Pro Natl Acad Sci USA. 2013;110(12):4557–4562.
4. Yamaguchi T, Sato H, Kato-Itoh M, Goto T, Hara H, Sanbo M, Mizuno N, Kobayashi T, Yanagida A, Umino A, Ota Y, Hamanaka S, Masaki H, Rashid ST, Hirabayashi M, Nakauchi H. Interspecies organogenesis generates autologous functional islets. Nature. 2017;542(7640):191–196.
5. Wu J, Platero-Luengo A, Sakurai M, Sugawara A, Gil MA, Yamauchi T, Suzuki K, Bogliotti YS, Cuello C, Morales Valencia M, et al. Interspecies chimerism with mammalian pluripotent stem cells. Cell. 2017;168(3):473–486.
6. Hamanaka S, Umino A, Sato H, Hayama T, Yanagida A, Mizuno N, Kobayashi T, Kasai M, Suchy FP, Yamazaki S, Masaki H, et al. Generation of vascular endothelial cells and hematopoietic cells by blastocyst complementation. Stem Cell Reports. 2018;11(4):988–997.
7. Cooper DKC, Ekser B, Ramsoondar J, Phelps C, Ayares D. The role of genetically engineered pigs in xenotransplantation research. J Pathol. 2016;238(2):288–299.
8. Nagashima H, Matsunari H. Growing human organs in pigs-A dream or reality? Theriogenology. 2016;86(1):422–426.
9. Hara H, Campanile N, Tai HC, Long C, Ekser B, Yeh P, Welchons D, Ezzelarab M, Ayares D, Cooper DKC. An in vitro model of pig liver xenotransplantation–pig complement is associated with reduced lysis of wild-type and genetically modified pig cells. Xenotransplantation. 2010;17(5):370–378.
10. Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405.
11. Carlson DF, Tan W, Lillico SG, Stverakova D, Proudfoot C, Christian M, Voytas DF, Long CR, Whitelaw CBA, Fahrenkrug SC. Efficient TALEN-mediated gene knockout in livestock. Proc Natl Acad Sci U S A. 2012;109(43):17382–17387.
12. Zhang W, Wang G, Wang Y, Jin Y, Zhao L, Xiong Q, Zhang L, Mou L, Li R, Yang H, Dai Y. Generation of complement protein C3 deficient pigs by CRISPR/Cas9-mediated gene targeting. Sci Rep. 2017;7(1):5009.
13. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta (GA): Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2017;20.
14. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. Diabetes. 2006;55(5):1463–1469.
15. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes. 2008;57(12):3169–3176.
16. Bellin MD, Barton FB, Heitman A, Harmon JV, Kandaswamy R, Balamurugan AN, Sutherland DER, Alejandro R, Hering BJ. Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. Am J Transplant. 2012;12(6):1576–1583.
17. Niclauss N, Meier R, Bédat B, Berishvili E, Berney T. Beta-cell replacement: pancreas and islet cell transplantation. Endocr Dev. 2016;31:146–162.
18. Moore SJ, Gala-Lopez BL, Pepper AR, Pawlick RL, Shapiro AJ. Bioengineered stem cells as an alternative for islet cell transplantation. World J Transplant. 2015;5(1):1–10.
19. Kobayashi T, Kato-Itoh M, Nakauchi H. Targeted organ generation using Mixl1-inducible mouse pluripotent stem cells in blastocyst complementation. Stem Cells Dev. 2014;24(2):182–189.
20. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development. 1996;122(3):983–995.
21. Bansal VS. Genes involved in pancreatic islet cell rejuvenation. Indian J Med Res. 2013;137:695–703.
22. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology. 2004;39(6):1477–1487.
23. Michalopoulos GK, DeFrances MC. Liver regeneration. Science. 1997;276(5309):60–66.

24. Xu J, Murphy SL, Kochanek KD, Bastian B, Arias E. Deaths: Final Data for 2016. Natl Vital Stat Rep. 2018;67(5):1–76.

25. Aravalli RN, Belcher JD, Steer CJ. Liver-targeted gene therapy: approaches and challenges. Liver Transpl. 2015;21(6):718–737.

26. Fitzpatrick E, Mitry RR, Dhawan A. Human hepatocyte transplantation: State of the art. J Internal Med. 2009;266(4):339–357.

27. Zhang W, Tucker-Kellogg L, Narmada BC, Venkatraman L, Chang S, Lu Y, Tan N, White JK, Jia R, Bhowmick SS, Shen S, Dewey CF Jr, Yu H. Cell-delivery therapeutics for liver regeneration. Adv Drug Deliv Rev. 2010;62(7–8):814–826.

28. Hasebe Y, Okumura N, Koh T, Kazama H, Watanabe G, Seki T, Ariga T. Formation of rat hepatocyte spheroids on agarose. Hepatol Res. 2005;32(2):89–95.

29. Choi HJ, Choi D. Successful mouse hepatocyte culture with sandwich collagen gel formation. J Korean Surg Soc. 2013;84(4):202–208.

30. Koide N, Sakaguchi K, Koide Y, Asano K, Kawaguchi M, Matsushima H, Takenami T, Shinji T, Mori M, Tsuji T. Formation of multicellular spheroids composed of adult rat hepatocytes in dishes with positively charged surfaces and under other nonadherent environments. Exp Cell Res. 1990;186(2):227–235.

31. Herzog N, Hansen M, Miethbauer S, Schmidtke K-U, Anderer U, Lupp A, Sperling S, Seehofer D, Damm G, Scheibner K, Kupper JH. Primary-like human hepatocytes genetically engineered to obtain proliferation competence display hepatic differentiation characteristics in monolayer and organotypical spheroid cultures. Cell Biol Int. 2016;40(3):341–353.

32. Soltys KA, Soto-Gutiérrez A, Nagaya M, Baskin KM, Deutsch M, Ito R, Shneider BL, Squires R, Vockley J, Guha C, Roy-Chowdhury J, Strom SC, Platt JL, Fox IJ. Barriers to the successful treatment of liver disease by hepatocyte transplantation. J Hepatol. 2010;53(4):769–774.

33. Okuyama T, Yamazoe H, Mochizuki N, Khademhosseini A, Suzuki H, Fukuda J. Preparation of arrays of cell spheroids and spheroid-monolayer cocultures within a microfluidic device. J Biosci Bioeng. 2010;110(5):572–576.

34. Cipriano M, Freyer N, Knöspel F, Oliveira NG, Barcia R, Cruz PE, Cruz H, Castro M, Santos JM, Zeilinger K, Miranda JP. Self-assembled 3D spheroids and hollow-fibre bioreactors improve MSC-derived hepatocyte-like cell maturation in vitro. Arch Toxicol. 2017;91(4):1815–1832.

35. Mitry RR, Jitraruch S, Iansante V, Dhawan A. Alginate encapsulation of human hepatocytes and assessment of microbeads. Methods Mol Biol. 2017;1506:273–281.

36. Li L, Zhang Y, Pan X. Preparation and characterization of alginate-chitosan microcapsule for hepatocyte culture. Methods Mol Biol. 2017;1479:199–206.

37. Meier RPH, Montanari E, Morel P, Pimenta J, Schuurman H-J, Wandrey C, Gerber-Lemaire S, Mahou R, Bühler LH. Microencapsulation of hepatocytes and mesenchymal stem cells for therapeutic applications. Methods Mol Biol. 2017;1506:259–271.

38. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. 2011;13:27–53.

39. Faulk DM, Wildemann JD, Badylak SF. Decellularization and cell seeding of whole liver biologic scaffolds composed of extracellular matrix. J Clin Exp Hepatol. 2015;5(1):69–80.

40. Matsumoto K, Yoshitomi H, Rossant J, Zaret KS. Liver organogenesis promoted by endothelial cells prior to vascular function. Science. 2001;294(5542):559–563.

41. Keng VW, Yagi H, Ikawa M, Nagano T, Myint Z, Yamada K, Tanaka T, Sato A, Muramatsu I, Okabe M, Sato M, Noguchi T. Homeobox gene Hex is essential for onset of mouse embryonic liver development and differentiation of the monocyte lineage. Biochem Biophys Res Commun. 2000;276(3):1155–1161.

42. Soufi A, Jayaraman P-S. PRH/Hex: an oligomeric transcription factor and multifunctional regulator of cell fate. Biochem J. 2008;412(3):399–413.

43. Tanaka T, Inazu T, Yamada K, Myint Z, Keng VW, Inoue Y, Taniguchi N, Noguchi T. cDNA cloning and expression of rat homeobox gene, Hex, and functional characterization of the protein. Biochem J. 1999;339 (Pt 1):111–117.

44. Morgutti M, Demori E, Pecile V, Amoroso A, Rustighi A, Manfioletti G. Genomic organization and chromosome mapping of the human homeobox gene HHEX. Cytogenet Cell Genet. 2001;94(1–2):30–32.

45. Bort R, Signore M, Tremblay K, Martinez Barbera JP, Zaret KS. Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Dev Biol. 2006;290(1):44–56.

46. Martinez Barbera JP, Clements M, Thomas P, Rodriguez T, Meloy D, Kioussis D, Beddington RS. The homeobox gene Hex is required in definitive endodermal tissues for normal forebrain, liver and thyroid formation. Development. 2000;127(11):2433–2445.

47. Paz H, Lynch MR, Bogue CW, Gasson JC. The homeobox gene Hhex regulates the earliest stages of definitive hematopoiesis. Blood. 2010;116(8):1254–1262.

48. Kubo A, Kim YH, Irion S, Kasuda S, Takeuchi M, Ohashi K, Iwano M, Dohi Y, Saito Y, Snodgrass R, Keller G. The homeobox gene Hex regulates hepatocyte differentiation from embryonic stem cell-derived endoderm. Hepatology. 2010;51(2):633–641.

49. Suzuki A, Sekiya S, Büscher D, Izpisúa Belmonte JC, Taniguchi H. Tbx3 controls the fate of hepatic progenitor cells in liver development by suppressing p19ARF expression. Development. 2008;135(9):1589–1595.

50. Lüdtke TH-W, Christoffels VM, Petry M, Kispert A. Tbx3 promotes liver bud expansion during mouse development by suppression of cholangiocyte differentiation. Hepatology. 2009;49(3):969–978.

51. Clotman F, Jacquemin P, Plumb-Rudewiez N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. Genes Dev. 2005;19(16):1849–1854.

52. Margagliotti S, Clotman F, Pierreux CE, Beaudry J-B, Jacquemin P, Rousseau GG, Lemaigre FP. The Onecut transcription factors HNF-6/OC-1 and OC-2 regulate early liver expansion by controlling hepatoblast migration. Dev Biol. 2007;311(2): 579–589.

53. National. Center for Health Statistics (US). Health, United States, 2016: With Chartbook on Long-term Trends in Health. Hyattsville (MD): National Center for Health Statistics; 2017.

54. Colvin M.OPTN/SRTR 2015 annual data report: heart. Am J Transplant. 2017;17:286–356.

55. Serruys PW. Comparison of coronary-artery bypass surgery and stenting for the treatment of multivessel disease. N Engl J Med. 2001;344:1117–1124.

56. Horb ME. Tbx5 is essential for heart development. Development. 1999;126:1739–1751.

57. Hiroi Y. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. Nat Genet. 2001;28: 276–280.

58. Kuo CT. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev. 1997; 11:1048–1060.

59. Molkentin JD. Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. Genes Dev. 1997;11:1061–1072.

60. Lee Y. The cardiac tissue-restricted homeobox protein Csx/Nkx2.5 physically associates with the zinc finger protein GATA4 and cooperatively activates atrial natriuretic factor gene expression. Mol Cell Biol. 1998;18:3120–3129.

61. Garg V. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003; 424:443–447.

62. Maitra M. Interaction of Gata4 and Gata6 with Tbx5 is critical for normal cardiac development. Dev Biol. 2009;326: 368–377.

63. Zhao Q, Beck AJ, Vitale JM, Schneider JS, Chang C, Gao S, del Re D, Bhaumik M, Yehia G, Sadoshima J, Fraidenraich D. Injection of wild type embryonic stem cells into Mst1 transgenic blastocysts prevents adult-onset cardiomyopathy. Stem Cell Rev. 2011;7(2):326–330.

64. Fraidenraich D, Stillwell E, Romero E, Wilkes D, Manova K, Basson CT, Benezra R. Rescue of cardiac defects in Id knockout embryos by injection of embryonic stem cells. Science. 2004;306(5694):247–252.

65. Komuro I. Csx: a murine homeobox-containing gene specifically expressed in the developing heart. Proc Natl Acad Sci USA. 1993;90:8145–8149.

66. Lints TJ. Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development. 1993;119:419–431.

67. Tanaka M. The cardiac homeobox gene Csx/Nkx2.5 lies genetically upstream of multiple genes essential for heart development. Development. 1999;126:1269–1280.

68. Lyons I. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. Genes Dev. 1995;9:1654–1666.

69. About chronic kidney disease. https://www.kidney.org/atoz/content/about-chronic-kidney-disease. 2017. [Accessed 2018 July 3].

70. Levey AS. Chronic kidney disease. Lancet. 2012;379: 165–180.

71. Stevens PE. Chronic kidney disease and life expectancy. Nephrol Dial Transplant. 2012;27:3014–3015.

72. Powe NR.Choosing a treatment for kidney failure. The National Institute of Diabetes and Digestive and Kidney Diseases Health Information Center. https://www.niddk.nih.gov/health-information/kidney-disease/kidney-failure/choosing-treatment. 2016. [Accessed 2018 July 3].

73. Tsujimura T. Roles and regulation of bone morphogenetic protein-7 in kidney development and diseases. World J Stem Cells. 2016;8:288–296.

74. Luo G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev. 1995;9:2808–2820.

75. Narlis M. Pax2 and Pax8 regulate branching morphogenesis and nephron differentiation in the developing kidney. J Am Soc Nephrol. 2007;18:1121–1129.

76. Merkel CE. Molecular regulation of kidney development: is the answer blowing in the Wnt? Pediatr Nephrol. 2007;22: 1825–1838.

77. Wang J, Liu M, Zhao L, Li Y, Zhang M, Jin Y, Xiong Q, Liu X, Zhang L, Jiang H, Chen Q, et al. Disabling of nephrogenesis in porcine embryos via CRISPR/Cas9-mediated SIX1 and SIX4 gene targeting. Xenotransplantation. 2019;26(3):e12484.

78. Usui J, Kobayashi T, Yamaguchi T, Knisely AS, Nishinakamura R, Nakauchi H. Generation of kidney from pluripotent stem cells via blastocyst complementation. Am J Pathol. 2012; 180(6):2417–2426.

79. Goto T, Hara H, Sanbo M, Masaki H, Sato H, Yamaguchi T, Hochi S, Kobayashi T, Nakauchi H, Hirabayashi M. Generation of pluripotent stem cell-derived mouse kidneys in Sall1 -targeted anephric rats. Nat Comm. 2019;10(1):451.

80. Yusen RD. The registry of the international society for heart and lung transplantation: thirty-second official adult lung and heart-lung transplantation report—2015; focus theme: early graft failure. J Heart Lung Transplant. 2015;34:1264–1277.

81. Barnes PJ. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165–1185.

82. Bourke SJ. Interstitial lung disease: progress and problems. Postgr Med J. 2006;82:494–499.

83. Gross TJ. Idiopathic pulmonary fibrosis. N Engl J Med. 2001; 345:517–525.

84. Chambers DC. The registry of the international society for heart and lung transplantation: thirty-fourth adult lung and heart-lung transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant. 2017;36:1047–1059.

85. Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev. 1996;10(1):60–69.

86. Goss AM. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. Dev Cell. 2009;17:290–298.

87. Que J. Multiple roles for Sox2 in the developing and adult mouse trachea. Development. 2009;136:1899–1907.

88. Rockich BE. Sox9 plays multiple roles in the lung epithelium during branching morphogenesis. Proc Natl Acad Sci USA. 2013;110: E4456–E4464.

89. Hrycaj SM. Hox5 genes regulate the Wnt2/2b-Bmp4-signaling axis during lung development. Cell Rep. 2015;12: 903–912.

90. Harper SQ.Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. Nat Med. 2002;8:253–261.

91. Mann CJ. Aberrant repair and fibrosis development in skeletal muscle. Skelet Muscle. 2011;1:21.

92. Schiaffino S. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. Skelet Muscle. 2011;1:4.

93. Gonçalves MA. Genetic complementation of human muscle cells via directed stem cell fusion. Mol Ther. 2008;16: 741–748.

94. Motohashi N. Isolation, culture, and transplantation of muscle satellite cells. J Vis Exp. 2014;(86):e50846.

95. Bismuth K. Genetic regulation of skeletal muscle development. Exp Cell Res. 2010;316:3081–3086.

96. Stillwell E, Vitale J, Zhao Q, Beck A, Schneider J, Khadim F, Elson G, Altaf A, Yehia G, Dong JH, Liu J, et al. Blastocyst injection of wild type embryonic stem cells induces global corrections in mdx mice. PloS One. 2009;4(3):e4759.

97. Vitale JM, Schneider JS, Beck AJ, Zhao Q, Chang C, Gordan R, Michaels J, Bhaumik M, Fraidenraich D. Dystrophin-compromised sarcoglycan-δ-knockout diaphragm requires full wild-type embryonic stem cell reconstitution for correction. J Cell Sci. 2012;125(Pt 7):1807–1813.

98. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, Baker M, Ballow M, Bartoshesky LE, Bonilla FA, Brokopp C, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014;312(7):729–738.

99. Owen J. Kuby Immunology. New York (NY): W.H. Freeman and Company; 2013.

100. DiSanto JP, Müller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc Natl Acad Sci USA. 1995;92:377–381.

101. Bernstein A, Chabot B, Dubreuil P, Reith A, Nocka K, Majumder S, Ray P, Besmer P. The mouse W/c-kit locus. Ciba Foundation Symp. 1990;148:158–166; discussion 166-172.

102. McDermott SP, Eppert K, Lechman ER, Doedens M, Dick JE. Comparison of human cord blood engraftment between immunocompromised mouse strains. Blood. 2010;116(2): 193–200.

103. Sasaki K, Nagao Y, Kitano Y, Hasegawa H, Shibata H, Takatoku M, Hayashi S, Ozawa K, Hanazono Y. Hematopoietic microchimerism in sheep after in utero transplantation of cultured cynomolgus embryonic stem cells. Transplantation. 2005;79(1):32.

104. Jansson L, Larsson J. W41/W41 blastocyst complementation: a system for genetic modeling of hematopoiesis. Blood. 2010; 115(1):47–50.

105. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008;79(4):368–376.

106. Pringsheim T, Jette N, Frolkis A, Steeves TDL. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014;29(13):1583–1590.

107. Lindvall O.Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. Arch Neurol. 1989;46:615–631.

108. Piccini P, Brooks DJ, Björklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehncrona S, Widner H, Lindvall O. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. Nature Neurosci. 1999; 2(12):1137.

109. Li W, Englund E, Widner H, Mattsson B, Westen D van Lätt J, Rehncrona S, Brundin P, Björklund A, Lindvall O, Li JY. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. Proc Natl Acad Sci U S A. 2016;113(23): 6544–6549.

110. Barker RA, Drouin-Ouellet J, Parmar M. Cell-based therapies for Parkinson disease—past insights and future potential. Nat Rev Neurol. 2015;11(9):492–503.

111. Steece-Collier K, Rademacher DJ, Soderstrom K. Anatomy of graft-induced dyskinesias: circuit remodeling in the parkinsonian striatum. Basal Ganglia. 2012;2(1):15–30.

112. Politis M, Oertel WH, Wu K, Quinn NP, Pogarell O, Brooks DJ, Bjorklund A, Lindvall O, Piccini P. Graft-induced dyskinesias in Parkinson's disease: high striatal serotonin/dopamine transporter ratio. Mov Disord. 2011;26(11):1997–2003.

113. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009;27(3):275–280.

114. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, Yang L, et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature. 2011;480(7378):547–551.

115. Grealish S, Diguet E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, Van Camp N, Perrier AL, Hantraye P, Björklund A, Parmar M. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell. 2014;15(5):653–665.

116. Kirkeby A, Nolbrant S, Tiklova K, Heuer A, Kee N, Cardoso T, Ottosson DR, Lelos MJ, Rifes P, Dunnett SB, Grealish S, Perlmann T, Parmar M. Predictive markers guide differentiation to improve graft outcome in clinical translation of hESC-

based therapy for Parkinson's disease. Cell Stem Cell. 2017; 20(1):135–148.

117. Doi D, Samata B, Katsukawa M, Kikuchi T, Morizane A, Ono Y, Sekiguchi K, Nakagawa M, Parmar M, Takahashi J. Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Reports. 2014;2(3):337–350.

118. Hallett PJ, Deleidi M, Astradsson A, Smith GA, Cooper O, Osborn TM, Sundberg M, Moore MA, Perez-Torres E, Brownell AL, Schumacher JM, Spealman RD, Isacson O. Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. Cell Stem Cell. 2015;16(3):269–274.

119. Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, Mizuma H, Takara S, Takahashi R, Inoue H, Morita S, et al. Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature. 2017; 548(7669):592.

120. Arenas E, Denham M, Villaescusa JC. How to make a midbrain dopaminergic neuron. Development. 2015;142(11): 1918–1936.

121. Kee N, Volakakis N, Kirkeby A, Dahl L, Storvall H, Nolbrant S, Lahti L, Björklund ÅK, Gillberg L, Joodmardi E, Sandberg R, Parmar M, Perlmann T. Single-cell analysis reveals a close relationship between differentiating dopamine and subthalamic nucleus neuronal lineages. Cell Stem Cell. 2017;20(1): 29–40.

122. La Manno G, Gyllborg D, Codeluppi S, Nishimura K, Salto C, Zeisel A, Borm LE, Stott SRW, Toledo EM, Villaescusa JC, Lönnerberg P, et al. Molecular diversity of midbrain development in mouse, human, and stem cells. Cell. 2016;167(2): 566–580.e19.

123. Fossat N, Chatelain G, Brun G, Lamonerie T. Temporal and spatial delineation of mouse Otx2 functions by conditional self-knockout. EMBO Rep. 2006;7(8):824–830.

124. Kadkhodaei B, Alvarsson A, Schintu N, Ramsköld D, Volakakis N, Joodmardi E, Yoshitake T, Kehr J, Decressac M, Björklund A, Sandberg R, et al. Transcription factor Nurr1 maintains fiber integrity and nuclear-encoded mitochondrial gene expression in dopamine neurons. Proc Natl Acad Sci U S A. 2013;110(6):2360–2365.

125. Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, Perlmann T, Ericson J. Identification of intrinsic determinants of midbrain dopamine neurons. Cell. 2006; 124(2):393–405.

126. Deng Q, Andersson E, Hedlund E, Alekseenko Z, Coppola E, Panman L, Millonig JH, Brunet J-F, Ericson J, Perlmann T. Specific and integrated roles of Lmx1a, Lmx1b and Phox2a in ventral midbrain development. Development. 2011;138(16): 3399–3408.

127. Liégeois NJ, Horner JW, DePinho RA. Lens complementation system for the genetic analysis of growth, differentiation, and apoptosis in vivo. Proc Natl Acad Sci U S A. 1996;93(3): 1303–1307.

128. Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP. Pitx3 is required for development of substantia nigra

dopaminergic neurons. Proc Natl Acad Sci U S A. 2003; 100(7):4245–4250.

129. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic brain injury-related emergency department visits, hospitalizations, and deaths - United States, 2007 and 2013. MMWR Surveill Summ. 2017;66(9):1–16.

130. Haidet-Phillips AM, Doreswamy A, Gross SK, Tang X, Campanelli JT, Maragakis NJ. Human glial progenitor engraftment and gene expression is independent of the ALS environment. Exp Neurol. 2015;264:188–199.

131. Kim H, Walczak P, Muja N, Campanelli JT, Bulte JWM. ICV-transplanted human glial precursor cells are short-lived yet exert immunomodulatory effects in mice with EAE. Glia. 2012;60(7):1117–1129.

132. Lepore AC, O'Donnell J, Kim AS, Williams T, Tuteja A, Rao MS, Kelley LL, Campanelli JT, Maragakis NJ. Human glial-restricted progenitor transplantation into cervical spinal cord of the SOD1 mouse model of ALS. PloS One. 2011;6(10): e25968.

133. Shin JC, Kim KN, Yoo J, Kim I-S, Yun S, Lee H, Jung K, Hwang K, Kim M, Lee I-S, Shin JE, et al. Clinical trial of human fetal brain-derived neural stem/progenitor cell transplantation in patients with traumatic cervical spinal cord injury. Neural Plas. 2015;2015:1–22.

134. Kessaris N FM. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci. 2006;9:173–179.

135. Jakovcevski I. Sequence of oligodendrocyte development in the human fetal telencephalon. Glia. 2005;49:480–491.

136. Jakovcevski I. Oligodendrocyte development and the onset of myelination in the human fetal brain. Front Neuroanat. 2009;3:5.

137. Erceg S, Ronaghi M, Oria M, Roselló MG, Aragó MAP, Lopez MG, Radojevic I, Moreno-Manzano V, Rodríguez-Jiménez FJ, Bhattacharya SS, Cordoba J, et al. Transplanted oligodendrocytes and motoneuron progenitors generated from human embryonic stem cells promote locomotor recovery after spinal cord transection. Stem Cells. 2010;28(9):1541–1549.

138. Furusho M. Involvement of the Olig2 transcription factor in cholinergic neuron development of the basal forebrain. Dev Biol. 2006;293:348–357.

139. Li H. Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes. J Neurosci. 2007;27:14375–14382.

140. Küspert M. Olig2 regulates Sox10 expression in oligodendrocyte precursors through an evolutionary conserved distal enhancer. Nucleic Acids Res. 2011;39:1280–1293.

141. Crane AT, Voth JP, Shen FX, Low WC. Human-animal neurological chimeras: humanized animals or human cells in an animal? Stem Cells. 2019;37(4):444–452.

142. Freedman BS. Hopes and difficulties for blastocyst complementation. Nephron. 2018;138(1):42–47.

143. Suchy F, Nakauchi H. Interspecies chimeras. Curr Opin Genet Dev. 2018;52:36–41.

144. Wu J, Greely HT, Jaenisch R, Nakauchi H, Rossant J, Belmonte JCI. Stem cells and interspecies chimaeras. Nature. 2016;540(7631):51–59.

145. Cheng W-M, Sun X-L, An L, Zhu S-E, Li X-H, Li Y, Tian J-H. Effect of different parthenogenetic activation methods on the developmental competence of in vitro matured porcine oocytes. Anim Biotechnol. 2007;18(2):131–141.

146. Liu S, Cui K, Li HL, Sun JM, Lu XR, Shen KY, Liu QY, Shi DS. Comparison of chemical, electrical, and combined activation methods for in vitro matured porcine oocytes. In Vitro Cell Dev Biol Anim. 2015;51(2):103–112.

147. Tan W, Carlson DF, Lancto CA, Garbe JR, Webster DA, Hackett PB, Fahrenkrug SC. Efficient nonmeiotic allele introgression in livestock using custom endonucleases. Proc Natl Acad Sci U S A. 2013;110(41):16526–16531.

148. Kim J-S, Song B-S, Lee K-S, Kim D-H, Kim S-U, Choo Y-K, Chang K-T, Koo D-B. Tauroursodeoxycholic acid enhances the pre-implantation embryo development by reducing apoptosis in pigs. Reprod Domest Anim. 2012;47(5):791–798.

149. Lu F, Li Z, Ruan Z, Liu X, Du S, Ruan Q, Deng Y, Jiang J, Shi D. Effects of tauro ursodeoxycholic acid on development of bovine embryos from in vitro fertilization. Reprod Fertil Dev. 2014;27:153.

150. Zhang JY, Diao YF, Oqani RK, Han RX, Jin DI. Effect of endoplasmic reticulum stress on porcine oocyte maturation and parthenogenetic embryonic development in vitro. Biol Reprod. 2012;86(4):128.

151. Cohen MA, Markoulaki S, Jaenisch R. Matched developmental timing of donor cells with the host is crucial for chimera formation. Stem Cell Reports. 2018;10(5):1445–1452.

152. Fang R, Liu K, Zhao Y, Li H, Zhu D, Du Y, Xiang C, Li X, Liu H, Miao Z, Zhang X, Shi Y, Yang W, Xu J, Deng H. Generation of naive induced pluripotent stem cells from rhesus monkey fibroblasts. Cell Stem Cell. 2014;15(4):488–496.

153. Mascetti VL, Pedersen RA. Human-Mouse chimerism validates human stem cell pluripotency. Cell Stem Cell. 2016; 18(1):67–72.

154. Masaki H, Kato-Itoh M, Takahashi Y, Umino A, Sato H, Ito K, Yanagida A, Nishimura T, Yamaguchi T, Hirabayashi M, Era T, Loh KM, Wu SM, Weissman IL, Nakauchi H. Inhibition of apoptosis overcomes stage-related compatibility barriers to chimera formation in mouse embryos. Cell Stem Cell. 2016;19(5):587–592.

155. Theunissen TW, Friedli M, He Y, Planet E, O'Neil RC, Markoulaki S, Pontis J, Wang H, Iouranova A, Imbeault M, Duc J, et al. Molecular criteria for defining the naive human pluripotent state. Cell Stem Cell. 2016;19(4):502–515.