

Modeling mouse and human development using organoid cultures

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Abstract

In vitro 3D cultures are emerging as novel systems with which to study tissue development, organogenesis and stem cell behaviour *ex vivo*. When grown in a 3D environment, embryonic stem cells (ESCs) self-organize into organoids and acquire the right tissue patterning to develop into several endoderm- and ectoderm-derived tissues, mimicking their *in vivo* counterparts. Tissue-resident adult stem cells (AdSCs) also form organoids when grown in 3D and can be propagated in vitro for long periods of time. In this review we discuss recent advances in the generation of pluripotent stem cell- and AdSC-derived organoids, highlighting their potential for enhancing our understanding of human development. We will also explore how this new culture system allows disease modeling and gene repair for a personalized regenerative medicine approach.

Introduction

Modern biology has brought great progress in our understanding of how mammals develop from a single totipotent cell to a complex adult organism. For the past three decades it has been possible to derive embryonic stem cells (ESCs) from the epiblast and expand them continuously *in vitro*. More recently, induced pluripotent stem cells (iPSCs) can be generated from almost any mature cell type in our bodies. These breakthroughs have allowed the differentiation of various pluripotent stem cell (PSC) populations into somatic cell derivatives *in vitro*. Tissue-resident adult stem cells (AdSCs) have also gained much attention recently for their intrinsic abilities to self-renew and differentiate into the cell types present in adult tissues, whilst retaining genomic stability. However, studying tissue patterning and organ morphogenesis *in vitro* has been hindered by the lack of appropriate culture systems that would allow the cell-cell interactions needed for organ formation. The recent development of 3D culture systems has made it possible to partially recapitulate the complexity of mammalian organogenesis *in vitro* (Figure 1 and 2) and has also allowed the generation of transplantable tissues (Assawachananont et al., 2014). Culturing human derivatives (hESCs/hiPSCs/hAdSCs) in 3D has opened up new horizons for the exploration of human development and the development of regenerative medicine approaches.

In 1979, in a seminal paper entitled “Stem Cell Concepts”, Lajtha produced the classical definition of a stem cell as a cell with self-renewal and multilineage potential - a definition to which we will adhere in this review (Lajtha, 1979). This definition covers various PSCs (ESCs/iPSC) as well as some AdSCs and adult tissue progenitors. Culturing these different types of stem cells in 3D under appropriate culture conditions has resulted in the formation of structures that resemble an organ in culture and have been termed “organoids”. In this review we define “organoid” as a three-dimensional structure derived from either PSCs, neonatal tissue stem cells or AdSCs/Adult progenitors, in which cells spontaneously self-organize into properly differentiated functional cell-types and progenitors which resemble their *in vivo* counterpart and recapitulate at least some function of the organ. Thus, this definition encompasses 3D-structures containing mainly epithelial derivatives and, sometimes, also a mesenchymal component. Here, we propose using the terms PSC-derived, neonate-derived or AdSC-derived to indicate the cell/tissue that gives rise to the organoid.

In this review, we will focus on the recent advances in organoid cultures derived from PSCs and AdSCs. We will first explore PSC-derived organoids by focusing on stomach organoids. Then, we will discuss stomach, intestine, liver and pancreas organoids generated from adult or neonatal stem cells. We will then compare the genetic stability of PSCs- and AdSC-derived organoids and discuss their potential uses for understanding human development and modeling human disease. Finally, we will introduce the various emergent genetic engineering tools that hold potential for gene repair in stem cell cultures, with the ultimate aim of personalized regenerative medicine.

Organoids derived from PSCs

In mouse, ESCs can be derived from the inner cell mass (ICM) of 4 day-old blastocysts and cultured on feeder layers (Evans and Kaufman, 1981; Martin, 1981). When injected into blastocysts, ESCs contribute to all germ layers (Bradley et al., 1984), thus indicating pluripotency. Similarly, hESCs have been isolated from human blastocysts prior to implantation (Thomson et al., 1998). Additionally, somatic cells can be reprogrammed to a pluripotent state by cell fusion (Blau et al., 1983), nuclear transfer (Chung et al., 2014; Gurdon, 1962; Tachibana et al., 2013) or by the overexpression of pluripotent factors (Takahashi and Yamanaka, 2006), providing another source of PSCs *in vitro*. All these pluripotent cells have made it possible to generate almost all human and

mouse cell types *in vitro*. Despite this great achievement, *in vitro* differentiation protocols in 2D do not recapitulate organ morphogenesis. In this regard, the conversion of culture systems from 2D to 3D has recently allowed the development of organoids to model tissues of ectoderm [retinal (Eiraku et al., 2011; Kuwahara et al., 2015; Nakano et al., 2012), pituitary (Suga et al., 2011), cerebral organoids (Lancaster et al., 2013; Muguruma et al., 2015), inner ear (Koehler et al., 2013)], endoderm [small intestine (Spence et al., 2011), thyroid (Antonica et al., 2012), liver (Takebe et al., 2013), stomach (McCracken et al., 2014) and lung (Dye et al., 2015)] and mesoderm [cardiac muscle (Stevens et al., 2009)]. Retinal (optic cup), cerebral, pituitary and small intestinal organoid cultures have been excellently reviewed elsewhere (Howell and Wells, 2011; Lancaster and Knoblich, 2014; Sasai, 2013; Sasai et al., 2012; Wells and Spence, 2014) (Figure 1 and Table 1). Therefore, below, we will use human ESC-derived gastric organoid cultures as an example of endodermal organ formation from PSCs *in vitro*.

During mouse embryonic development, the definitive endoderm (DE), which requires Nodal signaling for its specification (Zorn and Wells, 2009), gives rise to the foregut. Around embryonic day E9.5-E10, the stomach arises from the foregut as a very poorly differentiated epithelium that will undergo extensive remodeling during development (Zorn and Wells, 2009). Stomach specification requires transient inhibition of Wnt signaling mediated by sFRP1 and sFRP2 (Kim et al., 2005) and expression of mesenchymal FGF (Nyeng et al., 2007). Terminal cellular fates are induced before the glands are fully formed, between E15.5- E16.5 (Nyeng et al., 2007). Then, the later action of epithelial Shh (Ramalho-Santos et al., 2000) and mesenchymal BMP4 (Fukuda and Yasugi, 2005) will facilitate the further gland maturation, which will terminate during post-natal life (Figure 3A).

The mature stomach is anatomically divided into two distinct areas: the corpus (main body of the stomach), and the pyloric-antrum (close to the intestine). The epithelium is comprised of tubular-shaped invaginations into the lamina propria known as gastric units, which are in turn divided into two parts: the pit and the gland (Lee et al., 1982) (Figure 3A). While the corpus gastric units are responsible for the digestive functions of the stomach (acid and pepsinogen secretion), the pyloric-antrum units mainly produce mucus and enteroendocrine hormones (Karam and Leblond, 1993a; Vries et al., 2010).

By exploiting our knowledge of anterior endoderm patterning and stomach development, gastric tissue has been obtained through the directed differentiation of human ESC or human iPSCs *in vitro* (McCracken et al., 2014). As a first step towards formation of human gastric organoids (hGOs), hESC or hiPSCs were differentiated into DE using Activin, which mimics endogenous Nodal signaling (D'Amour et al., 2005; Kubo et al., 2004), followed by Wnt, FGF4 and Noggin treatment. Noggin, resulted essential to prevent intestinalization and promote a foregut fate (McCracken et al., 2014). Then, after the DE was exposed to RA in the presence of 3D extracellular matrix (Matrigel) to induce antrum specification, these foregut organoids were maintained in an EGF-rich medium to generate gastric organoids containing all cell types present in the stomach antrum (McCracken et al., 2014) (Figure 3B). Because these cultures were specified into antrum instead of corpus, they did not generate acid-producing cells. This is remarkable as it indicates that the system fully recapitulates antrum development and morphogenesis *in vitro*. Of note, mouse PSC-derived corpus cultures that generate functional parietal cells *in vitro* have just been recently achieved (Noguchi et al., 2015). This, again, suggests that culturing hESCs in a 3D environment, combined with our current knowledge on embryonic stomach development, has been instrumental in successfully developing stomach organoids, which will facilitate the study of human stomach development *ex vivo* from embryonic development to postnatal growth.

Organoids derived from adult stem cells

Experiments performed on skin by Barrandon and Green in the late 1980s proved that epidermal stem cells were expandable and could generate vast amounts of epithelium *in vitro*, when supported by lethally irradiated 3T3 cells (Barrandon and Green, 1987). However, expanding stem/progenitor cells from other adult tissues proved to be difficult and for decades, keratinocyte cultures remained the sole example of primary adult stem cell cultures. Recently, the combination of ECM and 3D culture systems with a deeper knowledge of the signalling pathways important for AdSC maintenance and tissue repair have been instrumental for the development of primary AdSCs cultures. Thus, mammary gland (Dontu et al., 2003), bone (Kale et al., 2000), small intestine (Ootani et al., 2009; Sato et al., 2009), stomach (Barker et al., 2010b; Bartfeld et al., 2015; Li et al., 2014; Stange et al., 2013), colon (Jung et al., 2011; Li et al., 2014; Sato et al., 2011), liver (Huch et al., 2013b; Huch et al., 2015), pancreas (Boj et al., 2015; Huch et al., 2013a), lung (Lee et al., 2014), prostate (Gao et al., 2014; Karthaus et al., 2014; Xin et al., 2007), salivary gland (Nanduri et al., 2014) and tongue (Hisha et al., 2013) organoids have all been derived *in vitro* from AdSCs (Figure 2 and Table 1). In this section, we will focus on the development of organoids derived from adult stem/progenitor cells, paying special attention to stomach, intestine, liver and pancreas cultures.

Organoid cultures from high turnover organs: stomach and gut organoids

A recent series of studies has demonstrated that the maintenance and repair of adult tissues with high cellular turnover relies on small populations of actively cycling tissue resident stem cells. A variety of techniques, ranging from 3H-thymidine labelling to Cre-reporter lineage tracing, applied to the stomach and intestine allowed AdSCs to be identified at the very bottom of the invaginations of the mucosa into the submucosa, in the crypt of Lieberkuhn (intestine) and gland bottom (stomach), [reviewed in (Barker et al., 2010a)].

- Stomach Organoids

In the stomach, classical electron microscopy and 3Hthymidine radioautography unveiled the existence of undifferentiated cells in the isthmus of the gastric units, which were proposed as the stem cells of the stomach responsible for the rapid homeostatic turnover of the epithelium (Karam and Leblond, 1993b). However, in 2010, lineage tracing experiments showed that cells expressing the Wnt target gene *Lgr5*, found at the bottom of the antro-pyloric gland, were *bona fide* mouse stomach stem cells and would contribute to the normal homeostasis of the pyloric epithelium during the entire lifespan of a mouse (Barker et al., 2010b). While these results have challenged the previous view that stomach stem cells resided in the isthmus region of the stomach gland (Karam and Leblond, 1993b), they do not conclusively disprove the existence of a -yet to be identified- isthmus stem cell. In this regard, it was shown that upon Interferon- γ stimulation, a rare population of Villin⁺ isthmus cells function as stomach stem cells (Qiao et al., 2007).

The identification of LGR5⁺ pyloric stem cells led to the development of an *in vitro* 3D organoid culture system (Barker et al., 2010b). Pioneering work from Ootani et al. had previously shown that stomach tissue could be maintained for some days in an air-liquid interphase 3D culture system (Ootani et al., 2000). Implementing on this work, the first long-term culture of mouse gastric stem cells from corpus and pylorus was achieved by using Matrigel as 3D basal ECM and a cocktail of growth factors important for stomach stem cell maintenance and/or stomach development (Barker et al., 2010b; Stange et al., 2013) (Figure 3C). The cocktail of growth factors included (1) high WNT signalling by combining WNT3A ligand and RSPO1 (Carmon et al., 2012; de Lau et al., 2011) (2)

the mitogens EGF and FGF10 (Nyeng et al., 2007) and (3) the BMP antagonist, NOGGIN, to prevent the posteriorization and differentiation of the culture. Similar culture conditions combined with a blockade of TGF β signalling have allowed the long-term expansion of human gastric stomach stem cells into 3D organoids that display characteristics of the adult stomach epithelium (Bartfeld et al., 2015) (Figure 3C).

A shortcoming of these cultures was the lack of parietal cell differentiation in the corpus-derived organoids. One potential explanation for this could be the lack of epithelial-mesenchymal interactions, as organoids exclusively form the epithelial component of the stomach tissue. In line with that, some parietal differentiation was observed when neonatal stomach epithelium was cultured in combination with its mesenchymal niche (Li et al., 2014) or when gastric corpus organoids were co-cultured with immortalized stomach mesenchymal cells (Schumacher et al., 2015). One advantage of AdSC- derived gastric organoids over human PSC-derived ones is that they allow the expansion of adult tissue stem cells as well as adult cancer stem cells. Indeed, gastric tumors derived from patient biopsies can be expanded in culture (Bartfeld et al., 2015). Therefore, AdSC-gastric cultures could potentially be used for cancer drug testing and personalized medicine for those patients which biopsies could be expanded and tested in culture.

- *Intestinal organoids*

In the intestine, the signalling pathways involved in the maintenance of the tissue include Wnt, Notch, FGF/EGF and BMP/Nodal [for details refer to (Sato and Clevers, 2013)], with Wnt being key for maintaining and driving the proliferation of the stem cell pool (Korinek et al., 1998; Sato and Clevers, 2013). Small intestinal crypt-base columnar (CBC) cells, marked by Lgr5, Olfm4, CD133 or Tnfrsf19 among others, have been proven to be *bona fide* stem cells by extensive genetic lineage tracing analysis (Barker et al., 2007; Snippert et al., 2009; Stange et al., 2013; van der Flier et al., 2009) (Wong et al., 2012). CBC cells persist during the entire mouse lifespan, and their progeny includes all differentiated cell lineages of the epithelium [for details refer to (Barker et al., 2010a)]. Upon tissue damage, label-retaining LGR5⁺ cells (Buczacki et al., 2013) and DLL1⁺ cells (van Es et al., 2012), both precursors of the secretory lineage, can function as “reserve” stem cells. Along the same lines, non-LGR5⁺ cells marked by BMI1 (Sangiorgi and Capecchi, 2008; Tian et al., 2011) (Powell et al., 2012) or HOPX (Takeda et al., 2011) were shown to behave as intestinal stem cells upon loss of the CBC compartment.

By combining a 3D culture system and the knowledge of the signaling pathways at play in the intestinal stem cell niche, a 3D *in vitro* culture system to grow intestinal epithelial organoids from intestinal epithelium (Ootani et al., 2009) or from a single adult Lgr5⁺ CBC intestinal cell (Sato et al., 2009) was developed. Intestinal organoids grown in Matrigel in a medium supplemented with EGF, NOGGIN and the Wnt agonist RSPONDIN (RSPO) were shown to expand long term in culture while retaining their ability to generate the different intestinal epithelial cells (Ootani et al., 2009; Sato et al., 2009) (Figure 4A). Furthermore, these organoids were expanded for over a year *in vitro*, far beyond the Hayflick limit (Hayflick, 1965), which predicts that primary non-transformed cells only divide 40-60 times – around 2-3 months – before undergoing senescence. Of note, intestinal organoids have also been generated from the above-mentioned non-CBC cells by culturing them in a WNT3A-supplemented medium (van Es et al., 2012). Similarly, mouse- and human-derived colonic stem cells could also be expanded in culture with a minor modification to the culture medium composition (Jung et al., 2011; Sato et al., 2011). For more extensive details, intestinal organoids have been elegantly reviewed elsewhere (Sasai et al., 2012; Sato and Clevers, 2013; Wells and Spence, 2014).

Organoid cultures from organs with slow physiological turnover: liver and pancreas

During embryonic development, the liver and pancreas develop from the same pool of progenitors (Deutsch et al., 2001). The two embryonic tissues express similar sets of transcription factors and are exposed to similar signalling pathways. These include FGF, HGF, WNT, BMP, RA and TGF β , which promote proliferation, migration and survival of the respective progenitor pools [for details refer to (Zaret and Grompe, 2008; Zorn and Wells, 2009)]. This knowledge has been exploited to produce embryonic liver- and pancreas-like cells from ESCs and iPSCs *in vitro*. Despite this groundbreaking work, primary adult liver and pancreas cells have proven difficult to expand in culture for long periods of time. Such cultures could facilitate the study of liver and pancreas function both during homeostasis and disease and increase our understanding of the mechanisms to activate their proliferation during damage response. In the following section we will discuss new advances in obtaining both mouse and human liver and pancreas organoid cultures and their uses for human disease modeling and regenerative medicine.

- Liver organoids

The adult liver is mainly composed of hepatocytes and ductal cells that work in conjunction with endothelial and mesenchymal cells. Both hepatocytes and ductal cells derive from hepatoblasts, the endodermal fetal liver progenitor cell. The characteristics of hepatoblasts, and protocols for their isolation and culture have been recently reviewed elsewhere (Miyajima et al., 2014) and therefore, will not be detailed here. While hepatoblasts generate hepatocytes and ductal cells during development, in normal adult homeostasis, the liver is mostly maintained by the self-replication of existing adult mature hepatocytes and ductal cells. Indeed, extensive lineage tracing approaches indicate that the contribution of non-hepatocytes to the normal homeostasis is negligible, at least in mouse models (Carpentier et al., 2011; Schaub et al., 2014; Tarlow et al., 2014; Yanger et al., 2014). However, *in vitro* and upon transplant, mouse ductal cells (Dorrell et al., 2014; Tarlow et al., 2014) and human EpCAM⁺ ductal cells (Cardinale et al., 2011; Huch et al., 2015; Schmelzer et al., 2007) display characteristics of liver progenitors (i.e., self-renewal and bi-potency) as shown by clonogenic and differentiation assays (Dorrell et al., 2014; Tarlow et al., 2014) (Cardinale et al., 2011; Huch et al., 2015; Schmelzer et al., 2007).

Following damage, the adult liver excels in terms of its regenerative capacity. There is a clear consensus that, upon hepatectomy, adult ductal cells and hepatocytes proliferate to generate ductal cells and hepatocytes, respectively [reviewed in (Michalopoulos, 2014)]. However, the identification of the cells responsible for liver regeneration following toxic damage is an area of extensive investigation. Recent reports using viral-mediated Cre lineage tracing approaches, indicate that hepatocytes are the major player that contributes to the repair of the damaged liver (Schaub et al., 2014; Yanger et al., 2014) whereas other studies found that the ductal population can act as a bi-potent progenitor population that would also contribute to liver regeneration (generating hepatocytes and duct cells) but to a lesser extent (Dorrell et al., 2011; Espanol-Suner et al., 2012; Furuyama et al., 2011; Huch et al., 2013b; Shin et al., 2011) or under extensive damage to the hepatocyte compartment (Lu et al., 2015). Interestingly, this bi-potent ductal population, either from a damaged or an undamaged liver, turned out to be a source for adult liver organoid culture (Dorrell et al., 2014; Huch et al., 2013b; Huch et al., 2015). Here we will focus on the establishment of organoid cultures from cells which, at least *in vitro*, fulfill the stem cell criteria as defined by Lahtja; self-renew and differentiate (as described above) and which were found to have a greater potential in expansion *in vitro* over mature hepatocytes.

Several signaling pathways have been involved in the repair of the liver after damage. Indeed, WNT (Boulter et al., 2012; Monga et al., 2001), HGF (Matsumoto and Nakamura, 1991) and FGF (Takase et al., 2013) are essential for liver regeneration. Despite this knowledge, the expansion of adult primary liver cells *in vitro* had remained a challenge. Pioneering work from Michalopoulos et al. showed that primary liver cells cultured in 3D could be maintained in culture in the presence of EGF, HGF and the corticoid Dexamethasone (Michalopoulos et al., 2001). However, these conditions did not allow the long-term expansion of liver progenitors. A 3D *in vitro* culture system for the long-term expansion of adult liver cells was recently developed by combining Matrigel with HGF, EGF and factors induced during liver damage - FGF (Takase et al., 2013) and RSPO1, produced during liver regeneration following CCl₄ damage - (Huch et al., 2013b)] (Figure 4B). Of note, both damage-induced progenitors and healthy adult mouse liver tissue can be expanded under these culture conditions (Huch et al., 2013b). Typically, the expanded cells self-organize into 3D structures that have a ductal (*Krt19*⁺) single-layered epithelium compartment and a pseudo-stratified compartment that express both ductal (*Krt7*, *Krt19*) and hepatocyte (*Ttr*, *Hnf4a*) markers.

The cellular expansion potential is remarkable as 10⁶ cells can be readily generated in ~5-6 weeks from a single cell. By supplementing the medium with additional factors, it was recently reported that EpCAM⁺ single adult human liver cell can be expanded long-term (>5months) *in vitro* and generate 3D organoid cultures (Huch et al., 2015). Addition of an activator of cAMP signalling and inhibition of TGFβ signalling was crucial to achieve the remarkable longevity of this organoid culture. Of note, only human adult liver tissue, but not mouse, requires inhibition of TGFβ signalling for long-term expansion of organoids. This is a recurrent and very intriguing observation: so far, for all human AdSC-derived organoid cultures developed – colon (Sato et al., 2011), stomach (Bartfeld et al., 2015) and liver (Huch et al., 2015)– the inhibition of the TGFβ pathway is necessary in order to sustain long-term growth. The reason as to why the human cells are sensitive to TGFβ signaling while mouse cells are not is unknown. Further studies will be required to answer this.

In mouse and human liver organoid cultures, blockade of the alternative ductal fate by Notch inhibition (McCright et al., 2002), and Wnt-mediated proliferation in combination with the addition of Dexamethasone and BMP, facilitated hepatocyte differentiation from organoids (Huch et al., 2013b; Huch et al., 2015) (Figure 4B). Differentiated cells displayed characteristics of functional hepatocytes *in vitro* and prolonged the life-span of liver-damaged animals upon transplantation (Huch et al., 2013b). Are we then already ready to start cell therapy transplantations? Probably not yet. It is worth noting that in mouse-to-mouse transplants only 25% of the animals displayed detectable engraftment (Huch et al., 2013b). The reasons for that could vary from the fact that only 33% of the cells to be transplanted acquire a hepatocyte fate, to the lack of an intrinsic ability of the expanded mouse cells to differentiate and proliferate in the host tissue. Unfortunately, the nature of the mouse model used in the human-to-mouse transplantation study precluded the analysis of the human cells functionality *in vivo* (the non-transplanted mice did not display any signs of liver failure). Further optimization of hepatocyte differentiation and transplantation efficiency will be necessary in order to consider therapeutic liver organoid transplants to humans.

One factor to consider when using AdSC-derived liver organoids to study liver development is that they only expand and differentiate into the epithelial derivatives of the liver (hepatocytes and duct cells). However, previous studies have described that reciprocal tissue interactions between the embryonic endoderm and the nearby mesoderm are required for liver organogenesis (Zorn, 2008) and, so far, AdSC-derived liver organoids do not allow the study of such complex interactions. Recently, an elegant attempt at generating a complete developing liver organ with mesodermal and endodermal

components *in vitro* was made by mixing human iPSC derived hepatocytes with mesenchymal stem cells (MSC) and umbilical cord cells (HUVECS) in a 3D culture system. This gave rise to embryonic liver bud organoids formed by proliferating hepatoblasts and other supporting cells that recapitulate aspects of liver embryonic organogenesis *in vitro* (Takebe et al., 2013). When ectopically transplanted into organs such as the mesentery or the brain, these organ buds developed into hepatic tissue exhibiting features of adult liver function. However, further optimization of the protocol is needed to complete a proper hepatic ductal network. Whether such a system can be generated using adult mouse or human liver progenitors in combination with endothelial and/or mesenchymal liver cells is still to be determined.

Finally, an interesting characteristic of AdSC-derived organoids is that they can be expanded long term from one single cell (clonal expansion). This enables the required depth of sequencing to perform studies on the genetic stability of Adult Stem cells/ organoids by using Whole Genome Sequencing (WGS) (Behjati et al., 2014; Huch et al., 2015). Only one synonymous base substitution was identified in AdSC-derived human liver organoids expanded for more than 3 months from one-single cell (Huch et al., 2015), suggesting a great genomic stability. This contrasts with iPSCs or ESCs, which upon long-term expansion in an undifferentiated state (2D-culture), can exhibit aneuploidy and chromosomal alterations [reviewed in (Liang and Zhang, 2013)]. Thus, it seems that AdSC cultured in 3D preserve their genomic integrity. However, more studies will be required to assess whether this is a generalizable attribute of AdSC-derived organoids. Whether this would also apply to ESCs/iPSCs after transferring them to 3D-system for the generation of organoid structures is still to be determined.

- *Pancreas organoids*

Whereas the liver is capable of extensive *de novo* tissue formation after damage, the ability of the pancreas to regenerate is limited [reviewed in (Zaret and Grompe, 2008)] and embryonic and/or adult pancreas cells have proven difficult to maintain in culture. Recently, Anne Grapin-Botton and colleagues elegantly showed that with a 3D Matrigel-based system, mouse embryonic pancreas progenitors could be cultured *in vitro*. The system neatly recapitulates pancreas development and demonstrates exocrine (acinar) and endocrine (insulin⁺) cell differentiation *in vitro*. Interestingly, the authors observed that the use of 3D ECM-based Matrigel promoted the expansion of the embryonic progenitor pool (Greggio et al., 2013), arguing once more in favour of the importance of 3D systems to recapitulate *ex vivo* the interactions that occur in physiological conditions. While this very elegant system allows pancreas development to be studied *ex vivo*, it does not support the long-term expansion of pancreas cells. To address this, 3D adult mouse pancreas cultures were established using similar culture conditions as those used for the liver and small intestinal organoids. Unlike liver cultures, Noggin is required for the culture of pancreas organoids, in agreement with pancreas development (Wandzioch and Zaret, 2009). Thus, adult pancreas organoid cultures can be expanded long-term in 3D Matrigel in the presence of FGF10, Noggin, RSPO1 and EGF (Huch et al., 2013a) (Figure 4C). The resulting organoid structures are entirely composed of ductal cells that express the embryonic progenitor marker *Pdx1* (Ahlgren et al., 1996). So far, differentiation of these cells into endocrine lineages *in vitro* has been unsuccessful. A potential reason could be that, in contrast to their embryonic counterparts, adult ductal cells have lost their bi-potency with regard to endocrine differentiation. However, it has been shown that adult ductal cells can revert to their embryonic state and re-activate the endocrine program (Sancho et al., 2014; Xu et al., 2008). Consistent with this, when the adult duct-derived organoid cells were mixed with embryonic pancreas and immediately transplanted into the kidney capsule, a high percentage of the cells differentiated into fully mature, mono-hormonal cells (insulin, glucagon or somatostatin positive) (Huch et al., 2013a). Other reasons

could also account for the lack of differentiation observed from adult organoids *in vitro*, ranging from deficiencies in the culture components (e.g. growth factors and ECMs), to the lack of stromal or other supporting cells (e.g. endothelial cells). In fact, it has been shown that endothelial cues facilitate embryonic endocrine differentiation (Lammert et al., 2001). Thus, further studies are needed to address this lack of endocrine differentiation *in vitro*.

Organoid cultures: uses and applications

Unveiling developmental processes with human organoid cultures

An interesting feature of AdSC cultures is that they preserve their tissue identity. PSC-derived cultures are generated by the manipulation of medium conditions, which results in the acquisition of different cell fates. In contrast, AdSC organoid cultures cannot be pushed to differentiate into other tissue types by simply changing the external factors. Changing tissue identity in these systems requires genetic manipulation. In this regard, only the loss of the master regulator *Cdx2* can impose a stomach fate in adult intestinal organoid cultures (Simmini et al., 2014). Thus, in homeostatic adult tissue, trans-differentiation between different tissue types seems unlikely to occur spontaneously or by simple environmental changes and tissues seem resistant to lineage changes. This is in clear contrast to what is observed in hPSCs-derived small intestine and stomach cultures, where a change in BMP signalling at the pre-specification stage, results in specification of stomach versus intestinal fate (McCracken et al., 2014). It is reasonable to speculate that the genetic/epigenetic programs that establish tissue identity become fixed at some point in the path from embryonic development to adult organ formation. Human organoid cultures have the potential to allow the identification of these regulatory mechanisms.

Modeling human disease using organoid stem cell cultures derived from patients

The establishment of adult human cultures directly from patients with rare genetic diseases has the potential to identify the mechanisms underlying such diseases and hence participate to the identification of treatments (Table 1). For example, the analysis of small intestinal organoids from a patient with multiple intestinal atresia (a rare genetic disease that causes bowel obstruction) showed that this disease arises from a defect in the apicobasal polarity of the epithelium, which can be reverted by inhibiting Rho kinase (Bigorgne et al., 2014). Similarly, intestinal organoids derived from Cystic Fibrosis patients (a genetic disorder caused by mutations on the CFTR gene, a chloride and thiocyanate ion channel) represent a fantastic platform to assay experimental treatments since CFTR channel functionality is easy to assess *in vitro* (Dekkers et al., 2013).

In addition to diseases of the intestine, liver genetic diseases will also benefit from the possibility of culturing primary liver tissue from patients bearing pathogenic genetic mutations. In support of this concept, direct liver biopsies from patients with two rare liver disorders [Alpha1-Antitrypsin deficiency (A1AT-D) and Alagille syndrome] were used to generate 3D organoids that partially phenocopied the corresponding disease (Huch et al., 2015). A1AT-D is caused by specific mutations of the A1AT gene. However, the development of the disease is highly variable between patients: some present severe symptoms and develop liver cancer while others hardly develop any symptoms (Fairbanks and Tavill, 2008). This difference in the presentation of the pathology could be due to passenger mutations that can now be identified by deriving organoids directly from the diseased tissue.

In addition to their use for the study of human development and human monogenic diseases, human organoids could have a prominent role in enhancing our understanding of human cancer. Indeed, human primary prostate cancer- (Gao et al., 2014), stomach cancer- (Bartfeld et al., 2015), pancreas cancer- (Boj et al., 2015) and colon cancer- (van de Wetering et al., 2015) derived organoids have

been expanded *in vitro* and observed to retain the heterogeneity of genetic alterations present in patient samples. The mechanism by which this heterogeneity is retained will require further investigation.

Genetic engineering in human ESC/iPSC and organoid cultures

The genetic stability of the organoid cultures, combined with their ability to closely phenocopy human diseases *in vitro* and the emergence of novel genetic engineering tools to manipulate the human genome, open up incredible opportunities to obtain molecular insights into human development and disease. Here, we will discuss novel methods of genetic engineering and their application to stem cell cultures for gene correction of human monogenic disorders. Homologous recombination (HR) has been actively employed in mouse ESCs, starting with the monumental work of Mario R. Capecchi, who showed the high level of HR events in mouse ESCs (Thomas and Capecchi, 1987). This method made possible the selective modification of the mouse genome and thus the generation of gene-modified mutant mice, which contributed immensely toward the understanding of mammalian gene function. In addition, HR-mediated gene targeting will potentially enable the editing of our own genetic code to cure monogenic disorders or to eradicate viruses integrated into our genome. Much effort towards establishing this technology in human cells has therefore been deployed. However, unlike mouse ESCs, the frequency of HR events in human PSCs is extremely low. In the following sections, we will detail recent genetic tools developed to overcome HR inefficiency in hPSCs and human organoids.

Zinc finger endonucleases-mediated genome editing in pluripotent stem cells

A hint on how to solve HR inefficiency in hPSCs came from the initial studies describing the mechanism of HR: a double strand break (DSB) triggers HR-mediated DNA repair. In fact, DSBs enhance HR efficiency by 2- to 3-orders of magnitude in mouse 3T3 cells (Rouet et al., 1994). However, generating a DSB at a desired site in the genome presented an insurmountable problem until the introduction of programmable zinc-finger nucleases (ZFNs). ZFNs are composed of two domains: DNA-binding zinc fingers and a FokI restriction endonuclease. DNA-targeting specificity is determined by the composition of the zinc fingers. Using a pair of ZFNs, it became feasible to generate a DSB at a targeted DNA sequence with 18 - 24 bps of recognition site, which greatly enhanced efficient gene targeting in human cells (Porteus and Baltimore, 2003). Subsequently, ZFN technology was rapidly adopted to generate desired mutations in many different cell types including primary human T cells (Urnov et al., 2005), human stem cells (Lombardo et al., 2007), human ESCs and iPSCs (Hockemeyer et al., 2009; Zou et al., 2009).

Starting from the initial success of applying ZFNs to human genome editing, a number of groups reported and demonstrated the versatility of ZFNs for targeted gene correction in human stem cells, by demonstrating functional gene repair for A1AT-D (A1AT) (Yusa et al., 2011), sickle cell anemia (β -globin) (Sebastiano et al., 2011; Zou et al., 2011), and Parkinson's disease (a-synuclein) (Soldner et al., 2011). In particular, Yusa *et al.* achieved several key milestones: the efficient gene correction of human iPSCs, the use of a PiggyBac selection cassette to achieve the clean correction of a point mutation without leaving any foreign DNA sequence, and the subsequent production of liver cells from the modified iPSCs. This work demonstrated a nearly complete cycle for autologous cell-based therapy – human iPSC generation from patient somatic cells, clean gene correction at the clinical level and finally target cell differentiation and production. However, there are still several technical problems to solve before we can use this new technology in a clinical context as a general medical practice. First, designing a functional pair of ZFNs is very challenging and the success rate is

relatively low (20- 25 %). Second, the major steps involved in autologous cell therapy are still under development or need further optimisation. For example, the gene-free or vector-free reprogramming of human somatic cells to iPSCs is under investigation in order to achieve the highest efficiency without inducing any genetic errors. Another significant challenge lies in establishing the protocols to differentiate all the required somatic cell types. Recent advances in genome editing methods (e.g. TALEN and CRISPR/Cas9) and 3D adult stem cell organoid culture techniques provide alternative choices for successful disease gene correction in autologous cell sources.

Novel programmable endonucleases – TALENs and CRISPR/Cas9 – for genome editing and 3D organoid cultures

Two types of programmable endonucleases have become a popular option for genome editing. Both come from prokaryotes: TALEN, the first to be introduced in the field, comes from *Xanthomonas* and CRISPR/Cas9 from *Streptococcus*. A TALEN DNA binding finger recognizes one nucleotide of DNA. Thus, sequentially assembled TALEN fingers are highly modular, simplifying the design step and greatly improving the success rate of gene targeting. CRISPR/Cas9 is composed of a guide RNA (gRNA) template and the Cas9 endonuclease. The specificity of the CRISPR/Cas9 system is determined by the gRNA. Due to the simplicity of gRNA sequence modification, targeting Cas9 to different genes has become a routine practice in labs. These new genome-editing tools, which respective advantages and drawbacks are listed in Table 2, have widened the choice and improved the feasibility of HR-mediated gene targeting. Two groups have elegantly used these new genome editing tools in hPSCs for gene modification, inducible genome editing and multiplexing (Ding et al., 2013; Gonzalez et al., 2014).

3D adult stem cell organoid cultures represent the other innovation that facilitates autologous cell therapy. As detailed above, an appropriate growth factor cocktail with a 3D basement matrix allows the generation and long-term culture of human organoids from diverse tissues. Their remarkable longevity, genetic stability and ability to be implanted *in vivo* make them a suitable platform for gene correction of monogenic disorders. A proof-of-concept was reported in colonic organoids from cystic fibrosis (CF) patients (Dekkers et al., 2013) in which the CRISPR/Cas9 system was used to correct the F508 mutation. When combined with a targeting vector encoding the correct sequence, the DSB generated by CRISPR/Cas9 on the mutant sequence facilitated the HR-mediated integration of the targeting vector, thus reverting the F508 mutation back to wild-type and restoring CFTR chloride channel function (Schwank et al., 2013). This approach – organoid establishment, genome engineering and transplantation (Figure 5), represents a novel short cycle of autologous cell therapy for monogenic diseases that bypasses challenging biotechnological processes such as error-free reprogramming and iPSC differentiation into highly pure and stable target cell types, apart from the inevitable genome engineering step. Taken together, all these innovative tools and technologies represent a huge advance towards the clinical application of organoids to regenerative medicine.

Conclusion

The recent development of 3D stem cell-derived culture systems has allowed the generation and the long-term expansion of adult tissues far beyond the predicted limit. Transferring this technology to the human arena has facilitated the generation of primary tissues from patients, thus opening the possibility of studying the molecular mechanisms involved in certain diseases. The parallel development of powerful genetic engineering tools to manipulate human genomes has opened the avenue for the manipulation of stem cells *in vitro*, which, when combined with 3D culture systems, might facilitate not only gene correction for regenerative medicine purposes but also the delineation of gene function during human development and disease. As we have discussed in this review, these

achievements would not have been possible without a thorough understanding of the signaling pathways important for organ development, organ growth and adult tissue maintenance and repair. Because this is an emerging field, it seems important to clearly define the terms and concepts that should be used moving forward. Hence, in this review, we defined organoid as a 3D-structure which should harbor cells with differentiation potential and organ functionality and suggested using the following nomenclature: PSC-derived, neonate-derived or AdSC-derived organoid cultures, to specify the cell type from which the organoid originates.

The era of 3D organ stem cell culture is just emerging. Thus, many questions remain to be answered. While some questions that wait immediate answer (short-term directions) have already been suggested through the manuscript, more challenging ones (long-term future directions) are exposed below. For example, can these cultures complete our knowledge of what happens during embryonic development, postnatal growth and adult organ formation? We have discussed the example of the human stomach organoids where the development of a full organ can be achieved *in vitro* from ESCs, by manipulating the medium composition, or from the adult stomach stem cells contained in human biopsies. Therefore, it seems plausible to speculate that the combination of the two systems might help bridging the gap between our knowledge of development and that of organ maintenance in the adult. Unveiling those steps of human development and growth has been impossible until now and organoid cultures have the potential to allow the easy identification of the regulatory networks involved in such processes. This should also hold true for other tissues from which organoid cultures can be obtained but await further development.

Another outstanding question relates to the robust genetic stability displayed by some AdSCs-derived organoids. We have discussed that long-term organoid cultures from mouse small intestine (Behjati et al., 2014) and human liver (Huch et al., 2015) have shown that the cells accumulate very few base pair substitutions after months in culture while actively proliferating. Is this some intrinsic property of these cells or can it be generalized to other 3D AdSC cultures? Are the DNA repair pathways similar between different AdSC cultures? And does this hold true for PSC-derived organoid cultures? It is now well established that ESCs and iPSCs expanded in 2D-culture can suffer from genetic instability (Liang and Zhang, 2013). What are the intrinsic differences with AdSC cultures that would allow them to remain genetically stable?

Linked to the previous question, can we generate AdSC from ESC? So far, for intestine and stomach, both PSC-derived and AdSC-derived organoids have been obtained (Barker et al., 2010b; Bartfeld et al., 2015; McCracken et al., 2014; Sato et al., 2009; Spence et al., 2011). Whether adult tissue-specific stem cells could be isolated from PSC-derived organoids and propagated in culture as AdSC-derived organoids has not been demonstrated yet. If this challenge is achieved, these cultures then hold the potential to explain the developmental journey that pluripotent ESCs follow to generate adult tissue stem cells that lose their pluripotency but gain tissue-specific self-renewal and multipotency while keeping their “adult” tissue-characteristics.

Another question is whether organoids could enable the study of mesenchymal-epithelial interactions in a spatio-temporal manner. We have discussed a recent study whereby mixing human iPSC-derived hepatocytes with MSCs and HUVECS generated an organoid structure that developed into hepatic tissue *in vivo* upon transplantation (Takebe et al., 2013). Applying such a system to the study of human stem/progenitor cells (PSCs or AdSCs) could provide crucial information as to how human stem cells interact with their endogenous mesenchymal and vascular niches and address our lack of knowledge on human organ development, maintenance and repair.

Finally, can these systems be used to generate entire transplantable organs *in vitro*? It has been shown that PSCs-derived optic cup organoids developed by self-organization [reviewed in (Sasai et al., 2012)] can be engrafted into a mouse model with advanced retinal degeneration and exhibited

synaptic connection with the host retina (Assawachananont et al., 2014). The challenge in the field will now be to prove whether upon integration these develop into electrophysiologically competent grafts capable of restoring the lost vision. Nevertheless, this represents the first proof-of-concept that organoids generated *in vitro* can be used as a transplantable tissue *in vivo*. It will be interesting to explore whether similar organoid systems, such as the ones described in this review (endoderm-derived) also result in transplantable organs in the future.

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Figure Legends:

Figure 1: Pluripotent stem cell (PSC)- derived organoids.

PSCs (embryonic stem cells or induced PSCs) can be derived into the different germ layers (endoderm, mesoderm and ectoderm) *in vitro* with specific step-wise differentiation protocols. After the initial germ layer specification, cells are transferred into 3 Dimensional systems and generate organoids that faithfully recapitulate *ex-vivo* the developmental steps that occur *in vivo*.

Figure 2: Adult stem cell (AdSC)-derived organoids.

Adult primary tissues derived from endodermal organs harbouring cells with stem cell potential have been cultured *in vitro* into AdSC derived-organoids. Organoids can be derived from both, isolated adult stem/progenitor cells or isolated fragments of tissue from the corresponding organ (e.g., intestinal crypts, liver or pancreas ducts). In these conditions, the cells expand long-term in culture while maintaining their genetic stability and commitment to their tissue of origin. Trans-differentiation has not yet been observed. These cultures can be used to study stem cell biology, as model of adult functional tissue and to study somatic mutational processes. Ma, mammary gland; Lv, Liver; Pc, Pancreas; Tg, Tongue; Lg, Lung; St, Stomach; Pr, Prostate.

Figure 3: Stomach organoids.

A) Stomach development and adult cellular composition. Schematic representation of stomach development. The stomach derives from the foregut which will bulge to make a stomach primordium at ~E13.5. Note that while the stomach cellular identities are formed at the later stages of development (from E16.5), the formation of the mature glandular structure is finalized post-natally. The composition of an adult antro-pyloric gland is shown. Indicated are the molecules known to influence stomach development. SHH, sonic hedgehog; SFRP1 and SFRP2, secreted Frizzled related protein 1 and 2; FGF, Fibroblast Growth Factor. BMP, Bone Morphogenetic Protein. B-C) Gastric organoids derived from PSC (B) or AdSCs (C). The gastric organoids self-organize into 3D-structures that contain a glandular-like domain (gland base, arrow in C) and a central empty lumen (pit region, dotted white circle in C). In both cases (B-C), the empty lumen gets filled with the dead cells as they are pushed from the gland base to the pit region and reach the top of the lumen, in a similar manner as they do *in vivo*, during normal tissue homeostasis. Enteroendocrine cells are scattered all over the organoid structure. **B)** An overview of stomach development *in vitro* from hESCs. Adapted from (McCracken et al., 2014). W, Wnt; F, FGF4; N, NOGGIN; Ra, Retinoic Acid **C)** Overview of

stomach development *in vitro* from AdSCs. The gland is formed by *Lgr5*⁺ stomach stem cells, chief (zymogen producing cells) cells and a Transit Amplifying compartment while the pit region faces the lumen of the organoid structure. Adapted from (Barker et al., 2010a). Ti, Tgfb inhibitor; Nic, Nicotinamide. Ti and Nic are only needed for Human stomach AdSC-cultures, but not mouse. E, Egf; N, NOGGIN; Rspo, Rspodin; g, gastrin; F, Fgf10, W, Wnt.

Figure 4: AdSC-derived small intestine, liver and pancreas organoid cultures

A) Small intestine organoids are obtained from either adult intestinal crypts or adult intestinal stem cells (isolated from the bottom of the crypts by FACS sorting using specific markers). Sorted cells or crypts are cultured in ENRspo medium. Intestinal stem cells (Crypt Base columnar cells, Label retaining cells (LRC), +4 cells as well as Dll1⁺ cells) have all been successfully grown into SI organoids in the presence of ENRspo + addition of Wnt for the first days. Addition of Ti and p38i and Wnt (in blue) is required only for the growth of human gut organoids. E, EGF; N, NOGGIN; Rspo, Rspodin. W, Wnt; Ti (TGFbinhibitor) and p38i (p38 inhibitor). **B)** Liver organoids have been obtained from both mouse and human ductal structures or from liver ductal cells isolated from healthy and damaged-induced livers after culturing these in medium supplemented with EFHNicRspo. Ti and A (in blue) are required for the growth of human liver organoids. Under expansion medium conditions (EFHNicRspo) the cells expand in culture as ductal structures (PCK, pan-cytokeratin, red). When transferred to differentiation medium (bottom panel) the ductal cuboidal epithelium differentiates into a polygonal-like epithelium that expresses hepatocyte markers (albumin, red; Zo1, green). Adapted from Huch et al 2015. E, EGF; F, FGF10; H, HGF; Nic, Nicotinamide; Rspo, Rspodin, Ti (TGFbinhibitor), A (cAMP). **C)** Mouse pancreas organoids derived from either duct structures or isolated ductal cells (shown are 2 markers used) grow into pancreas organoids that expand as ductal cells in culture. E, EGF; N, NOGGIN, F, FGF10; Nic, Nicotinamide; Rspo, Rspodin.

Figure 5: Gene correction in Cystic Fibrosis patient-derived intestinal organoids. CRISPR/Cas9 and targeting vectors are used to replace the F508 mutation with a wild type coding sequence in CF patient-derived gut organoids. First, a patient organoid line is established from a patient biopsy. A single cell suspension containing AdSCs is obtained from the established patient organoids by trypsinization and then transfected with CRISPR/Cas9 and targeting plasmid vectors encoding the correct sequence. The DSB, generated by CRISPR/Cas9 on the mutant sequence, facilitates the HR-mediated uptake of the targeting vector, which eventually reverts the F508 mutation back to normal as well as the functionality of CFTR chloride channel in transfected AdSCs. This gene-corrected AdSC can then be cultured as an organoid line, expanded *in vitro* and potentially transplanted back *in vivo*.

Table 1: Summary of the different tissues and diseases modelled with organoids derived from either pluripotent stem cells (PSC), including embryonic stem cells (ESCs), and stem cells derived from adult tissue (AdSCs) and neonatal/embryonic tissue. ND, not determined yet. FGF, Fibroblast Growth Factor; EGF, Epidermal Growth Factor; Rspo, Rspodin; NOG, NOGGIN; Ti, TGFb inhibitor; FCS, Fetal Calf Serum; RA, Retinoic Acid; Nic, Nicotinamide; HGF, Hepatocyte Growth Factor; FSK, Forskolin; Dexam, Dexamethasone; PMA, Phorbol Myristate Acetate; FBS, Fetal Bovine Serum; DHT,

dihydroxitestosterone; NEAA, Non-essential aminoacids; SAG, Smoothened agonist; BMP, Bone Morphogenetic Protein; BMPR, Bone Morphogenetic Protein receptor and TGFB1, Transforming Growth Factor Beta1.

In all PSC-derived endoderm cultures, Step 1 is after Definitive endoderm induction with ActivinA treatment.

Table 2: Comparison of the different genetic engineering tools.

References

- Ahlgren, U., Jonsson, J., and Edlund, H. (1996). The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. *Development* *122*, 1409-1416.
- Antonica, F., Kasprzyk, D.F., Opitz, R., Iacovino, M., Liao, X.H., Dumitrescu, A.M., Refetoff, S., Peremans, K., Manto, M., Kyba, M., *et al.* (2012). Generation of functional thyroid from embryonic stem cells. *Nature* *491*, 66-71.
- Assawachananont, J., Mandai, M., Okamoto, S., Yamada, C., Eiraku, M., Yonemura, S., Sasai, Y., and Takahashi, M. (2014). Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* *2*, 662-674.
- Barker, N., Bartfeld, S., and Clevers, H. (2010a). Tissue-resident adult stem cell populations of rapidly self-renewing organs. *Cell stem cell* *7*, 656-670.
- Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H.J., van Es, J.H., Sato, T., Stange, D.E., Begthel, H., van den Born, M., *et al.* (2010b). Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell stem cell* *6*, 25-36.
- Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., *et al.* (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* *449*, 1003-1007.
- Barrandon, Y., and Green, H. (1987). Three clonal types of keratinocyte with different capacities for multiplication. *Proceedings of the National Academy of Sciences of the United States of America* *84*, 2302-2306.
- Bartfeld, S., Bayram, T., van de Wetering, M., Huch, M., Begthel, H., Kujala, P., Vries, R., Peters, P.J., and Clevers, H. (2015). In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* *148*, 126-136 e126.
- Behjati, S., Huch, M., van Boxtel, R., Karthaus, W., Wedge, D.C., Tamuri, A.U., Martincorena, I., Petljak, M., Alexandrov, L.B., Gundem, G., *et al.* (2014). Genome sequencing of normal cells reveals developmental lineages and mutational processes. *Nature* *513*, 422-425.
- Bigorgne, A.E., Farin, H.F., Lemoine, R., Mahlaoui, N., Lambert, N., Gil, M., Schulz, A., Philippet, P., Schlessler, P., Abrahamsen, T.G., *et al.* (2014). TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *The Journal of clinical investigation* *124*, 328-337.
- Blau, H.M., Chiu, C.P., and Webster, C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell* *32*, 1171-1180.
- Boj, S.F., Hwang, C.I., Baker, L.A., Chio, II, Engle, D.D., Corbo, V., Jager, M., Ponz-Sarvisé, M., Tiriác, H., Spector, M.S., *et al.* (2015). Organoid models of human and mouse ductal pancreatic cancer. *Cell* *160*, 324-338.
- Boulter, L., Govaere, O., Bird, T.G., Radulescu, S., Ramachandran, P., Pellicoro, A., Ridgway, R.A., Seo, S.S., Spee, B., Van Rooijen, N., *et al.* (2012). Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nature medicine* *18*, 572-579.
- Bradley, A., Evans, M., Kaufman, M.H., and Robertson, E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* *309*, 255-256.
- Buczacki, S.J., Zecchini, H.I., Nicholson, A.M., Russell, R., Vermeulen, L., Kemp, R., and Winton, D.J. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* *495*, 65-69.
- Cardinale, V., Wang, Y., Carpino, G., Cui, C.B., Gatto, M., Rossi, M., Berloco, P.B., Cantafora, A., Wauthier, E., Furth, M.E., *et al.* (2011). Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* *54*, 2159-2172.
- Carmon, K.S., Lin, Q., Gong, X., Thomas, A., and Liu, Q. (2012). LGR5 interacts and cointernalizes with Wnt receptors to modulate Wnt/beta-catenin signaling. *Molecular and cellular biology* *32*, 2054-2064.
- Carpentier, R., Suner, R.E., van Hul, N., Kopp, J.L., Beaudry, J.B., Cordi, S., Antoniou, A., Raynaud, P., Lepreux, S., Jacquemin, P., *et al.* (2011). Embryonic ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells. *Gastroenterology* *141*, 1432-1438, 1438 e1431-1434.
- Chung, Y.G., Eum, J.H., Lee, J.E., Shim, S.H., Sepilian, V., Hong, S.W., Lee, Y., Treff, N.R., Choi, Y.H., Kimbrel, E.A., *et al.* (2014). Human somatic cell nuclear transfer using adult cells. *Cell stem cell* *14*, 777-780.
- D'Amour, K.A., Agulnick, A.D., Eliazer, S., Kelly, O.G., Kroon, E., and Baetge, E.E. (2005). Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nature biotechnology* *23*, 1534-1541.
- de Lau, W., Barker, N., Low, T.Y., Koo, B.K., Li, V.S., Teunissen, H., Kujala, P., Haegebarth, A., Peters,

P.J., van de Wetering, M., *et al.* (2011). Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* *476*, 293-297.

Dekkers, J.F., Wiegerinck, C.L., de Jonge, H.R., Bronsveld, I., Janssens, H.M., de Winter-de Groot, K.M., Brandsma, A.M., de Jong, N.W., Bijvelds, M.J., Scholte, B.J., *et al.* (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nature medicine* *19*, 939-945.

Deutsch, G., Jung, J., Zheng, M., Lora, J., and Zaret, K.S. (2001). A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* *128*, 871-881.

Ding, Q., Lee, Y.K., Schaefer, E.A., Peters, D.T., Veres, A., Kim, K., Kuperwasser, N., Motola, D.L., Meissner, T.B., Hendriks, W.T., *et al.* (2013). A TALEN genome-editing system for generating human stem cell-based disease models. *Cell stem cell* *12*, 238-251.

Dontu, G., Abdallah, W.M., Foley, J.M., Jackson, K.W., Clarke, M.F., Kawamura, M.J., and Wicha, M.S. (2003). In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes & development* *17*, 1253-1270.

Dorrell, C., Erker, L., Schug, J., Kopp, J.L., Canaday, P.S., Fox, A.J., Smirnova, O., Duncan, A.W., Finegold, M.J., Sander, M., *et al.* (2011). Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. *Genes & development* *25*, 1193-1203.

Dorrell, C., Tarlow, B., Wang, Y., Canaday, P.S., Haft, A., Schug, J., Streeter, P.R., Finegold, M.J., Shenje, L.T., Kaestner, K.H., *et al.* (2014). The organoid-initiating cells in mouse pancreas and liver are phenotypically and functionally similar. *Stem Cell Res* *13*, 275-283.

Dye, B.R., Hill, D.R., Ferguson, M.A., Tsai, Y.H., Nagy, M.S., Dyal, R., Wells, J.M., Mayhew, C.N., Nattiv, R., Klein, O.D., *et al.* (2015). In vitro generation of human pluripotent stem cell derived lung organoids. *Elife* *4*.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* *472*, 51-56.

Espanol-Suner, R., Carpentier, R., Van Hul, N., Legry, V., Achouri, Y., Cordi, S., Jacquemin, P., Lemaigre, F., and Leclercq, I.A. (2012). Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. *Gastroenterology* *143*, 1564-1575 e1567.

Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* *292*, 154-156.

Fairbanks, K.D., and Tavill, A.S. (2008). Liver disease in alpha 1-antitrypsin deficiency: a review. *The American journal of gastroenterology* *103*, 2136-2141; quiz 2142.

Fukuda, K., and Yasugi, S. (2005). The molecular mechanisms of stomach development in vertebrates. *Development, growth & differentiation* *47*, 375-382.

Furuyama, K., Kawaguchi, Y., Akiyama, H., Horiguchi, M., Kodama, S., Kuhara, T., Hosokawa, S., Elbahrawy, A., Soeda, T., Koizumi, M., *et al.* (2011). Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nature genetics* *43*, 34-41.

Gao, D., Vela, I., Sboner, A., Iaquinta, P.J., Karthaus, W.R., Gopalan, A., Dowling, C., Wanjala, J.N., Undvall, E.A., Arora, V.K., *et al.* (2014). Organoid cultures derived from patients with advanced prostate cancer. *Cell* *159*, 176-187.

Gonzalez, F., Zhu, Z., Shi, Z.D., Lelli, K., Verma, N., Li, Q.V., and Huangfu, D. (2014). An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells. *Cell stem cell* *15*, 215-226.

Greggio, C., De Franceschi, F., Figueiredo-Larsen, M., Gobaa, S., Ranga, A., Semb, H., Lutolf, M., and Grapin-Botton, A. (2013). Artificial three-dimensional niches deconstruct pancreas development in vitro. *Development* *140*, 4452-4462.

Gurdon, J.B. (1962). Adult frogs derived from the nuclei of single somatic cells. *Developmental biology* *4*, 256-273.

Hayflick, L. (1965). The Limited in Vitro Lifetime of Human Diploid Cell Strains. *Experimental cell research* *37*, 614-636.

Hisha, H., Tanaka, T., Kanno, S., Tokuyama, Y., Komai, Y., Ohe, S., Yanai, H., Omachi, T., and Ueno, H. (2013). Establishment of a novel lingual organoid culture system: generation of organoids having mature keratinized epithelium from adult epithelial stem cells. *Sci Rep* *3*, 3224.

Hockemeyer, D., Soldner, F., Beard, C., Gao, Q., Mitalipova, M., DeKelver, R.C., Katibah, G.E., Amora, R., Boydston, E.A., Zeitler, B., *et al.* (2009). Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nature biotechnology* *27*, 851-857.

Howell, J.C., and Wells, J.M. (2011). Generating intestinal tissue from stem cells: potential for research and therapy. *Regenerative medicine* *6*, 743-755.

Huch, M., Bonfanti, P., Boj, S.F., Sato, T., Loomans, C.J., van de Wetering, M., Sojoodi, M., Li, V.S., Schuijers, J., Gracanin, A., *et al.* (2013a). Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *The EMBO journal* *32*, 2708-2721.

Huch, M., Dorrell, C., Boj, S.F., van Es, J.H., Li, V.S., van de Wetering, M., Sato, T., Hamer, K., Sasaki, N., Finegold, M.J., *et al.* (2013b). In vitro expansion of single Lgr5+ liver stem cells induced by Wnt

driven regeneration. *Nature* 494, 247-250.

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M.M., Ellis, E., van Wenum, M., Fuchs, S.A., de Ligt, J., *et al.* (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 160, 299-312.

Jung, P., Sato, T., Merlos-Suarez, A., Barriga, F.M., Iglesias, M., Rossell, D., Auer, H., Gallardo, M., Blasco, M.A., Sancho, E., *et al.* (2011). Isolation and in vitro expansion of human colonic stem cells. *Nature medicine* 17, 1225-1227.

Kale, S., Biermann, S., Edwards, C., Tarnowski, C., Morris, M., and Long, M.W. (2000). Three-dimensional cellular development is essential for ex vivo formation of human bone. *Nature biotechnology* 18, 954-958.

Karam, S.M., and Leblond, C.P. (1993a). Dynamics of epithelial cells in the corpus of the mouse stomach. *The Anatomical record* 236, 259-279.

Karam, S.M., and Leblond, C.P. (1993b). Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. *The Anatomical record* 236, 259-279.

Karthus, W.R., Iaquina, P.J., Drost, J., Gracanin, A., van Boxtel, R., Wongvipat, J., Dowling, C.M., Gao, D., Begthel, H., Sachs, N., *et al.* (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 159, 163-175.

Kim, B.M., Buchner, G., Miletich, I., Sharpe, P.T., and Shivdasani, R.A. (2005). The stomach mesenchymal transcription factor Barx1 specifies gastric epithelial identity through inhibition of transient Wnt signaling. *Developmental cell* 8, 611-622.

Koehler, K.R., Mikosz, A.M., Molosh, A.I., Patel, D., and Hashino, E. (2013). Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. *Nature* 500, 217-221.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P.J., and Clevers, H. (1998). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nature genetics* 19, 379-383.

Kubo, A., Shinozaki, K., Shannon, J.M., Kouskoff, V., Kennedy, M., Woo, S., Fehling, H.J., and Keller, G. (2004). Development of definitive endoderm from embryonic stem cells in culture. *Development* 131, 1651-1662.

Kuwahara, A., Ozone, C., Nakano, T., Saito, K., Eiraku, M., and Sasai, Y. (2015). Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. *Nature communications* 6, 6286.

Lajtha, L.G. (1979). Stem cell concepts. *Differentiation; research in biological diversity* 14, 23-34.

Lammert, E., Cleaver, O., and Melton, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science* 294, 564-567.

Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345, 1247-1255.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature* 501, 373-379.

Lee, E.R., Trasler, J., Dwivedi, S., and Leblond, C.P. (1982). Division of the mouse gastric mucosa into zymogenic and mucous regions on the basis of gland features. *The American journal of anatomy* 164, 187-207.

Lee, J.H., Bhang, D.H., Beede, A., Huang, T.L., Stripp, B.R., Bloch, K.D., Wagers, A.J., Tseng, Y.H., Ryeom, S., and Kim, C.F. (2014). Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell* 156, 440-455.

Li, X., Nadauld, L., Ootani, A., Corney, D.C., Pai, R.K., Gevaert, O., Cantrell, M.A., Rack, P.G., Neal, J.T., Chan, C.W., *et al.* (2014). Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nature medicine* 20, 769-777.

Liang, G., and Zhang, Y. (2013). Genetic and epigenetic variations in iPSCs: potential causes and implications for application. *Cell stem cell* 13, 149-159.

Lombardo, A., Genovese, P., Beausejour, C.M., Colleoni, S., Lee, Y.L., Kim, K.A., Ando, D., Urnov, F.D., Galli, C., Gregory, P.D., *et al.* (2007). Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nature biotechnology* 25, 1298-1306.

Lu, W.Y., Bird, T.G., Boulter, L., Tsuchiya, A., Cole, A.M., Hay, T., Guest, R.V., Wojtacha, D., Man, T.Y., Mackinnon, A., *et al.* (2015). Hepatic progenitor cells of biliary origin with liver repopulation capacity. *Nature cell biology* 17, 971-983.

Martin, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 78, 7634-7638.

Matsumoto, K., and Nakamura, T. (1991). Hepatocyte growth factor: molecular structure and implications for a central role in liver regeneration. *Journal of gastroenterology and hepatology* 6, 509-519.

McCracken, K.W., Cata, E.M., Crawford, C.M., Sinagoga, K.L., Schumacher, M., Rockich, B.E., Tsai, Y.H., Mayhew, C.N., Spence, J.R., Zavros, Y., *et al.* (2014). Modelling human development and disease in

pluripotent stem-cell-derived gastric organoids. *Nature* *516*, 400-404.

McCright, B., Lozier, J., and Gridley, T. (2002). A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. *Development* *129*, 1075-1082.

Michalopoulos, G.K. (2014). The liver is a peculiar organ when it comes to stem cells. *The American journal of pathology* *184*, 1263-1267.

Michalopoulos, G.K., Bowen, W.C., Mule, K., and Stolz, D.B. (2001). Histological organization in hepatocyte organoid cultures. *The American journal of pathology* *159*, 1877-1887.

Miyajima, A., Tanaka, M., and Itoh, T. (2014). Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. *Cell stem cell* *14*, 561-574.

Monga, S.P., Padiaditakis, P., Mule, K., Stolz, D.B., and Michalopoulos, G.K. (2001). Changes in WNT/beta-catenin pathway during regulated growth in rat liver regeneration. *Hepatology* *33*, 1098-1109.

Muguruma, K., Nishiyama, A., Kawakami, H., Hashimoto, K., and Sasai, Y. (2015). Self-Organization of Polarized Cerebellar Tissue in 3D Culture of Human Pluripotent Stem Cells. *Cell reports*.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., and Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell stem cell* *10*, 771-785.

Nanduri, L.S., Baanstra, M., Faber, H., Rocchi, C., Zwart, E., de Haan, G., van Os, R., and Coppes, R.P. (2014). Purification and ex vivo expansion of fully functional salivary gland stem cells. *Stem Cell Reports* *3*, 957-964.

Noguchi, T.A., Ninomiya, N., Sekine, M., Komazaki, S., Wang, P.C., Asashima, M., and Kurisaki, A. (2015). Generation of stomach tissue from mouse embryonic stem cells. *Nature cell biology* *17*, 984-993.

Nyeng, P., Norgaard, G.A., Kobberup, S., and Jensen, J. (2007). FGF10 signaling controls stomach morphogenesis. *Developmental biology* *303*, 295-310.

Ootani, A., Li, X., Sangiorgi, E., Ho, Q.T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I.L., Capecchi, M.R., *et al.* (2009). Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nature medicine* *15*, 701-706.

Ootani, A., Toda, S., Fujimoto, K., and Sugihara, H. (2000). An air-liquid interface promotes the differentiation of gastric surface mucous cells (GSM06) in culture. *Biochemical and biophysical research communications* *271*, 741-746.

Porteus, M.H., and Baltimore, D. (2003). Chimeric nucleases stimulate gene targeting in human cells. *Science* *300*, 763.

Powell, A.E., Wang, Y., Li, Y., Poulin, E.J., Means, A.L., Washington, M.K., Higginbotham, J.N., Juchheim, A., Prasad, N., Levy, S.E., *et al.* (2012). The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* *149*, 146-158.

Qiao, X.T., Ziel, J.W., McKimpson, W., Madison, B.B., Todisco, A., Merchant, J.L., Samuelson, L.C., and Gumucio, D.L. (2007). Prospective identification of a multilineage progenitor in murine stomach epithelium. *Gastroenterology* *133*, 1989-1998.

Ramalho-Santos, M., Melton, D.A., and McMahon, A.P. (2000). Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development* *127*, 2763-2772.

Rouet, P., Smih, F., and Jasin, M. (1994). Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Molecular and cellular biology* *14*, 8096-8106.

Sancho, R., Gruber, R., Gu, G., and Behrens, A. (2014). Loss of Fbw7 reprograms adult pancreatic ductal cells into alpha, delta, and beta cells. *Cell stem cell* *15*, 139-153.

Sangiorgi, E., and Capecchi, M.R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. *Nature genetics* *40*, 915-920.

Sasai, Y. (2013). Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. *Cell stem cell* *12*, 520-530.

Sasai, Y., Eiraku, M., and Suga, H. (2012). In vitro organogenesis in three dimensions: self-organizing stem cells. *Development* *139*, 4111-4121.

Sato, T., and Clevers, H. (2013). Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* *340*, 1190-1194.

Sato, T., Stange, D.E., Ferrante, M., Vries, R.G., Van Es, J.H., Van den Brink, S., Van Houdt, W.J., Pronk, A., Van Gorp, J., Siersema, P.D., *et al.* (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* *141*, 1762-1772.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., *et al.* (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* *459*, 262-265.

Schaub, J.R., Malato, Y., Gormond, C., and Willenbring, H. (2014). Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. *Cell reports* *8*, 933-939.

Schmelzer, E., Zhang, L., Bruce, A., Wauthier, E., Ludlow, J., Yao, H.L., Moss, N., Melhem, A., McClelland, R., Turner, W., *et al.* (2007). Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* *204*, 1973-1987.

Schumacher, M.A., Aihara, E., Feng, R., Engevik, A., Shroyer, N.F., Ottemann, K.M., Worrell, R.T.,

Montrose, M.H., Shivdasani, R.A., and Zavros, Y. (2015). The use of murine-derived fundic organoids in studies of gastric physiology. *J Physiol* 593, 1809-1827.

Schwank, G., Koo, B.K., Sasselli, V., Dekkers, J.F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C.K., *et al.* (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell stem cell* 13, 653-658.

Sebastiano, V., Maeder, M.L., Angstman, J.F., Haddad, B., Khayter, C., Yeo, D.T., Goodwin, M.J., Hawkins, J.S., Ramirez, C.L., Batista, L.F., *et al.* (2011). In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. *Stem cells* 29, 1717-1726.

Shin, S., Walton, G., Aoki, R., Brondell, K., Schug, J., Fox, A., Smirnova, O., Dorrell, C., Erker, L., Chu, A.S., *et al.* (2011). Foxl1-Cre-marked adult hepatic progenitors have clonogenic and bilineage differentiation potential. *Genes & development* 25, 1185-1192.

Simmini, S., Bialecka, M., Huch, M., Kester, L., van de Wetering, M., Sato, T., Beck, F., van Oudenaarden, A., Clevers, H., and Deschamps, J. (2014). Transformation of intestinal stem cells into gastric stem cells on loss of transcription factor Cdx2. *Nature communications* 5, 5728.

Snippert, H.J., van Es, J.H., van den Born, M., Begthel, H., Stange, D.E., Barker, N., and Clevers, H. (2009). Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine. *Gastroenterology* 136, 2187-2194 e2181.

Soldner, F., Laganier, J., Cheng, A.W., Hockemeyer, D., Gao, Q., Alagappan, R., Khurana, V., Golbe, L.I., Myers, R.H., Lindquist, S., *et al.* (2011). Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. *Cell* 146, 318-331.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M., *et al.* (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 470, 105-109.

Stange, D.E., Koo, B.K., Huch, M., Sibbel, G., Basak, O., Lyubimova, A., Kujala, P., Bartfeld, S., Koster, J., Geahlen, J.H., *et al.* (2013). Differentiated Troy⁺ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* 155, 357-368.

Stevens, K.R., Pabon, L., Muskheli, V., and Murry, C.E. (2009). Scaffold-free human cardiac tissue patch created from embryonic stem cells. *Tissue Eng Part A* 15, 1211-1222.

Suga, H., Kadoshima, T., Minaguchi, M., Ohgushi, M., Soen, M., Nakano, T., Takata, N., Wataya, T., Muguruma, K., Miyoshi, H., *et al.* (2011). Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* 480, 57-62.

Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H.S., Sritanandomchai, H., *et al.* (2013). Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* 153, 1228-1238.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.

Takase, H.M., Itoh, T., Ino, S., Wang, T., Koji, T., Akira, S., Takikawa, Y., and Miyajima, A. (2013). FGF7 is a functional niche signal required for stimulation of adult liver progenitor cells that support liver regeneration. *Genes & development* 27, 169-181.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N., *et al.* (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499, 481-484.

Takeda, N., Jain, R., LeBoeuf, M.R., Wang, Q., Lu, M.M., and Epstein, J.A. (2011). Interconversion between intestinal stem cell populations in distinct niches. *Science* 334, 1420-1424.

Tarlow, B.D., Finegold, M.J., and Grompe, M. (2014). Clonal tracing of Sox9⁺ liver progenitors in mouse oval cell injury. *Hepatology* 60, 278-289.

Thomas, K.R., and Capecchi, M.R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 51, 503-512.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145-1147.

Tian, H., Biehs, B., Warming, S., Leong, K.G., Rangell, L., Klein, O.D., and de Sauvage, F.J. (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* 478, 255-259.

Urnov, F.D., Miller, J.C., Lee, Y.L., Beausejour, C.M., Rock, J.M., Augustus, S., Jamieson, A.C., Porteus, M.H., Gregory, P.D., and Holmes, M.C. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature* 435, 646-651.

van de Wetering, M., Francies, H.E., Francis, J.M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L., *et al.* (2015). Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 161, 933-945.

van der Flier, L.G., Haegerbarth, A., Stange, D.E., van de Wetering, M., and Clevers, H. (2009). OLFM4 is a robust marker for stem cells in human intestine and marks a subset of colorectal cancer cells. *Gastroenterology* 137, 15-17.

van Es, J.H., Sato, T., van de Wetering, M., Lyubimova, A., Nee, A.N., Gregorieff, A., Sasaki, N., Zeinstra, L., van den Born, M., Korving, J., *et al.* (2012). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nature cell biology* *14*, 1099-1104.

Vries, R.G., Huch, M., and Clevers, H. (2010). Stem cells and cancer of the stomach and intestine. *Molecular oncology* *4*, 373-384.

Wandzioch, E., and Zaret, K.S. (2009). Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. *Science* *324*, 1707-1710.

Wells, J.M., and Spence, J.R. (2014). How to make an intestine. *Development* *141*, 752-760.

Wong, V.W., Stange, D.E., Page, M.E., Buczacki, S., Wabik, A., Itami, S., van de Wetering, M., Poulosom, R., Wright, N.A., Trotter, M.W., *et al.* (2012). Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nature cell biology* *14*, 401-408.

Xin, L., Lukacs, R.U., Lawson, D.A., Cheng, D., and Witte, O.N. (2007). Self-renewal and multilineage differentiation in vitro from murine prostate stem cells. *Stem cells* *25*, 2760-2769.

Xu, X., D'Hoker, J., Stange, G., Bonne, S., De Leu, N., Xiao, X., Van de Casteele, M., Mellitzer, G., Ling, Z., Pipeleers, D., *et al.* (2008). Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* *132*, 197-207.

Yanger, K., Knigin, D., Zong, Y., Maggs, L., Gu, G., Akiyama, H., Pikarsky, E., and Stanger, B.Z. (2014). Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. *Cell stem cell* *15*, 340-349.

Yusa, K., Rashid, S.T., Strick-Marchand, H., Varela, I., Liu, P.Q., Paschon, D.E., Miranda, E., Ordonez, A., Hannan, N.R., Rouhani, F.J., *et al.* (2011). Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature* *478*, 391-394.

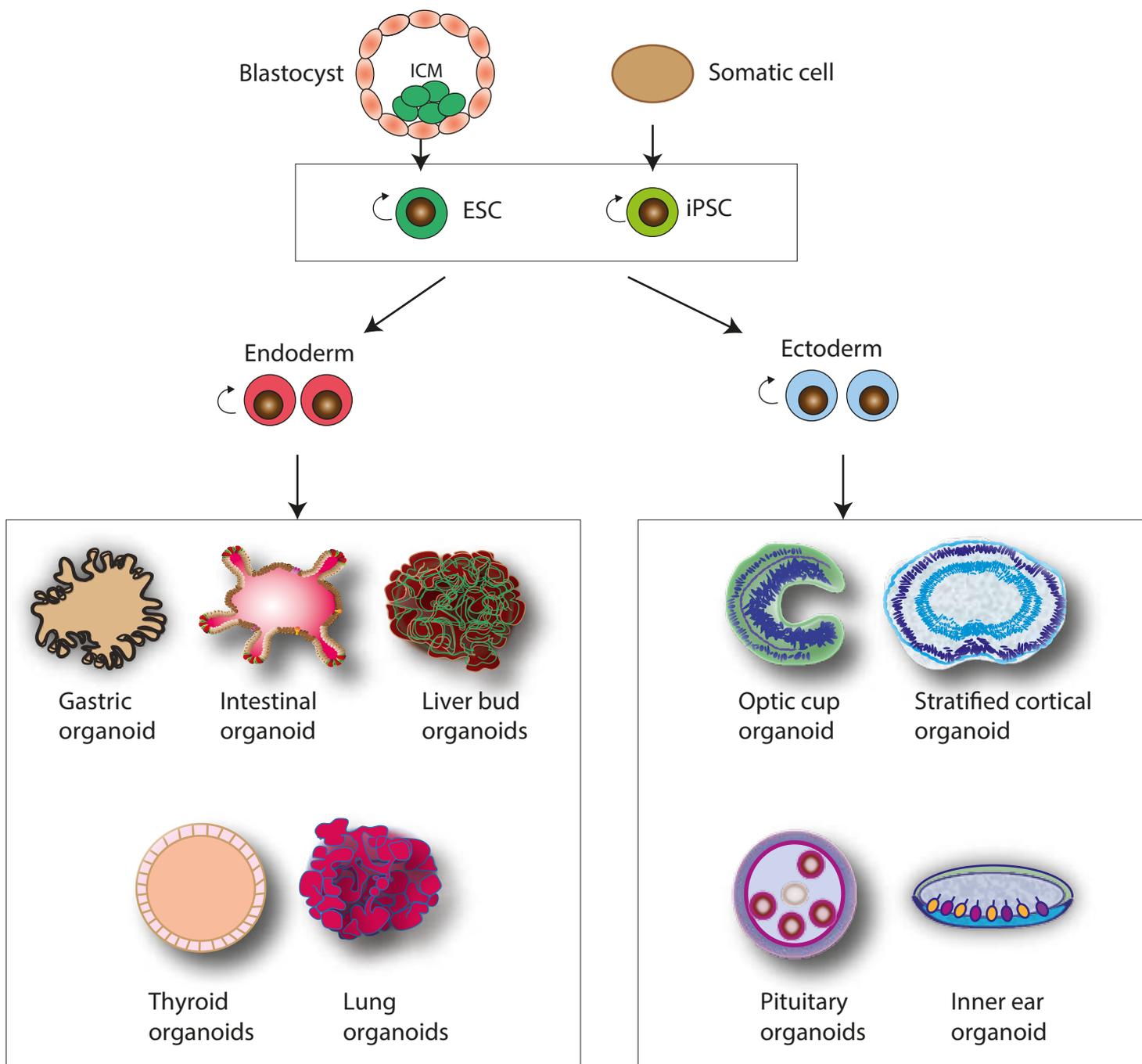
Zaret, K.S., and Grompe, M. (2008). Generation and regeneration of cells of the liver and pancreas. *Science* *322*, 1490-1494.

Zorn, A.M. (2008). Liver development. In *StemBook* (Cambridge (MA)).

Zorn, A.M., and Wells, J.M. (2009). Vertebrate endoderm development and organ formation. *Annual review of cell and developmental biology* *25*, 221-251.

Zou, J., Maeder, M.L., Mali, P., Pruett-Miller, S.M., Thibodeau-Beganny, S., Chou, B.K., Chen, G., Ye, Z., Park, I.H., Daley, G.Q., *et al.* (2009). Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell stem cell* *5*, 97-110.

Zou, J., Mali, P., Huang, X., Dowey, S.N., and Cheng, L. (2011). Site-specific gene correction of a point mutation in human iPS cells derived from an adult patient with sickle cell disease. *Blood* *118*, 4599-4608.

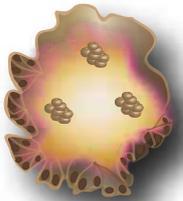


Scheme summarizing the different organoids generated from Pluripotent stem cells. Pluripotent stem cells, either ESC or iPSC have been derived into the different germ layers (endoderm, mesoderm and ectoderm) in vitro after following specific step-wise protocols. Following this first germ layer specification, organoid cultures and organoid buds have been generated in vitro after transferring the specified cells into 3Dimensional systems. These faithfully recapitulate ex-vivo the developmental steps that occur in vivo.

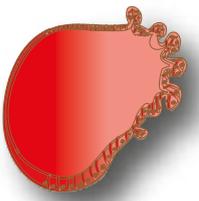
Mammary Gland organoid



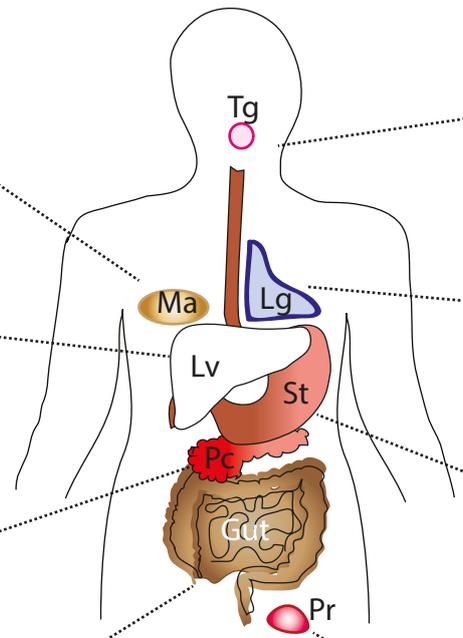
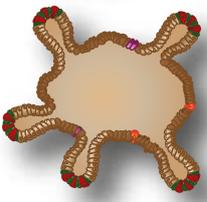
Liver organoid



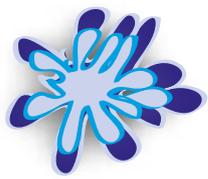
Pancreas organoid



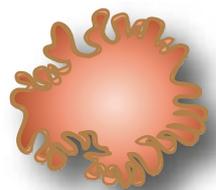
Gut organoid



Tongue organoid



Lung organoid

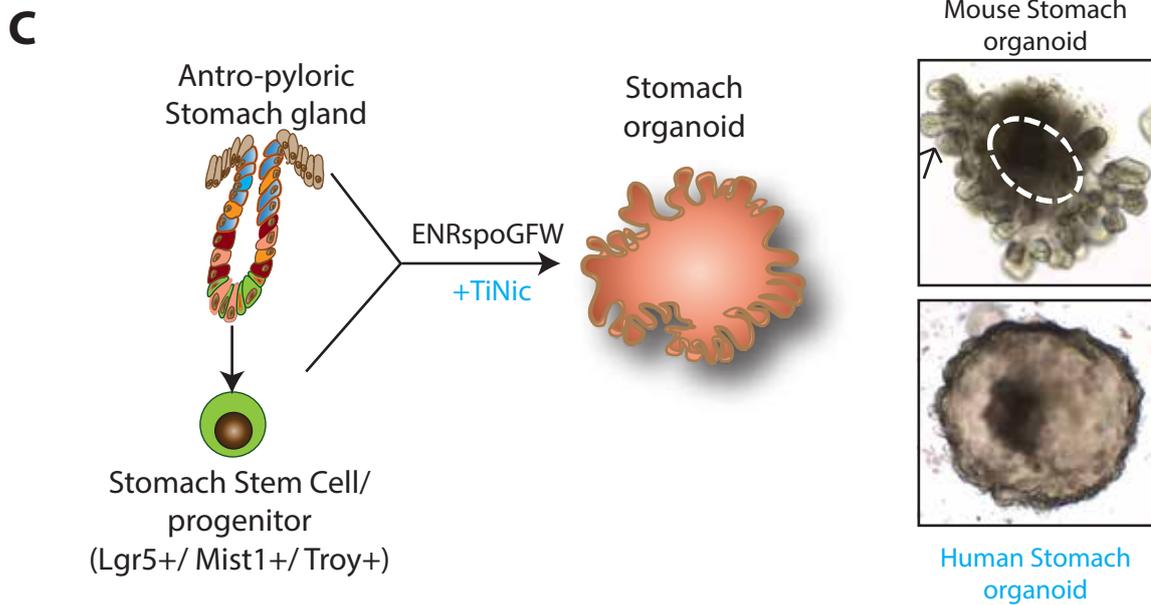
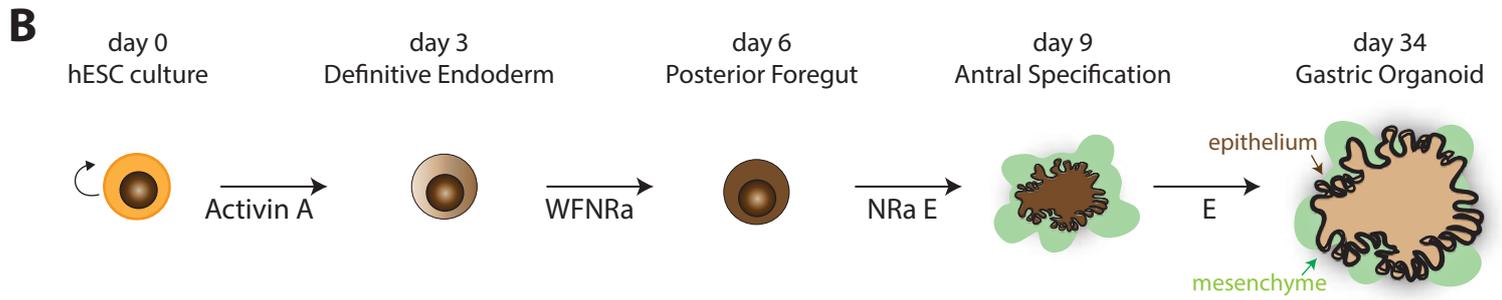
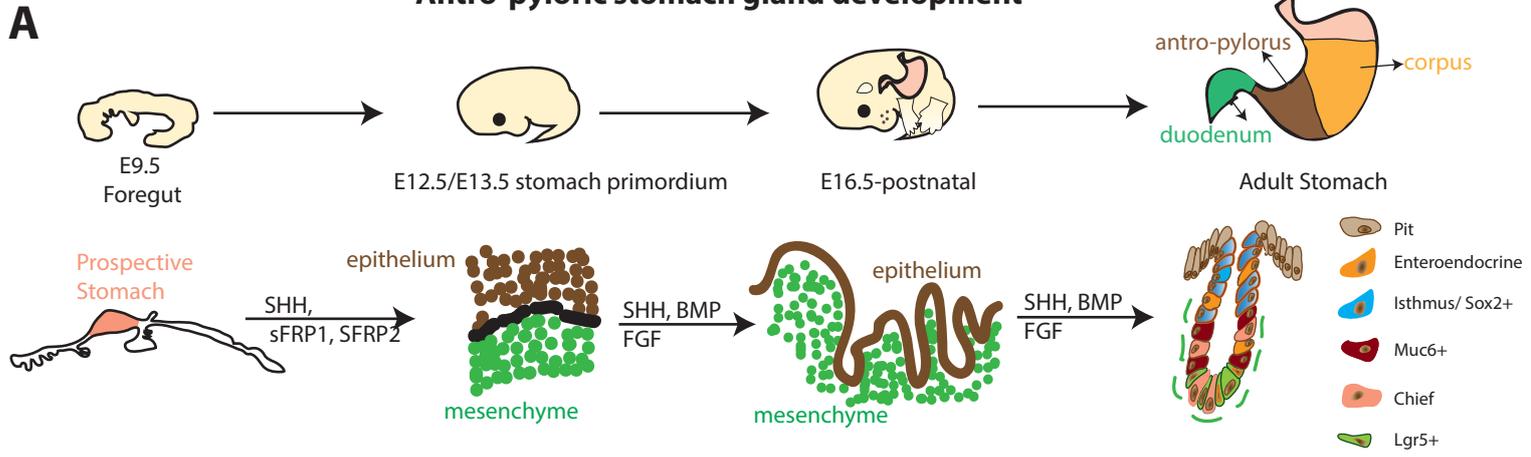


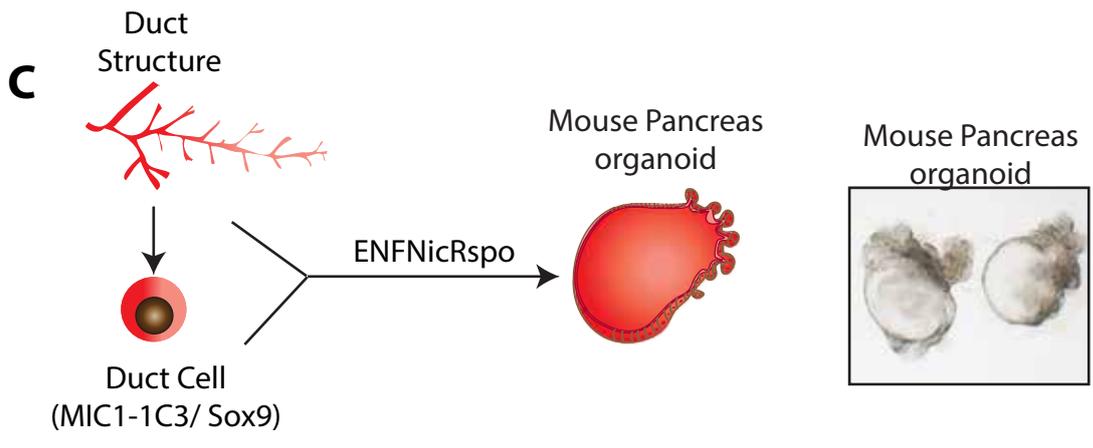
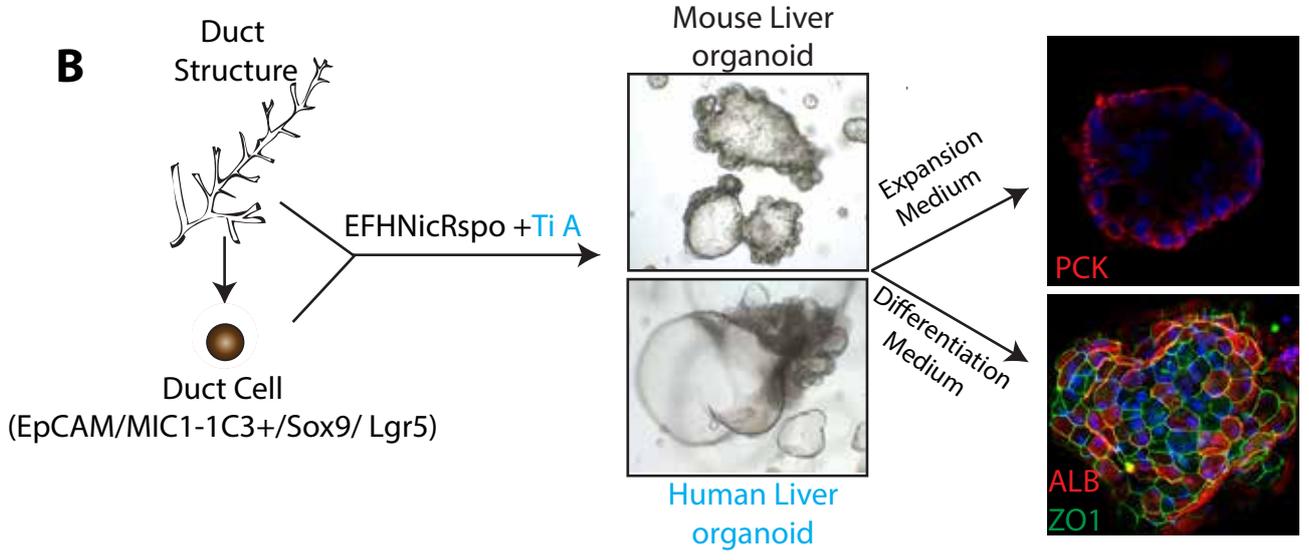
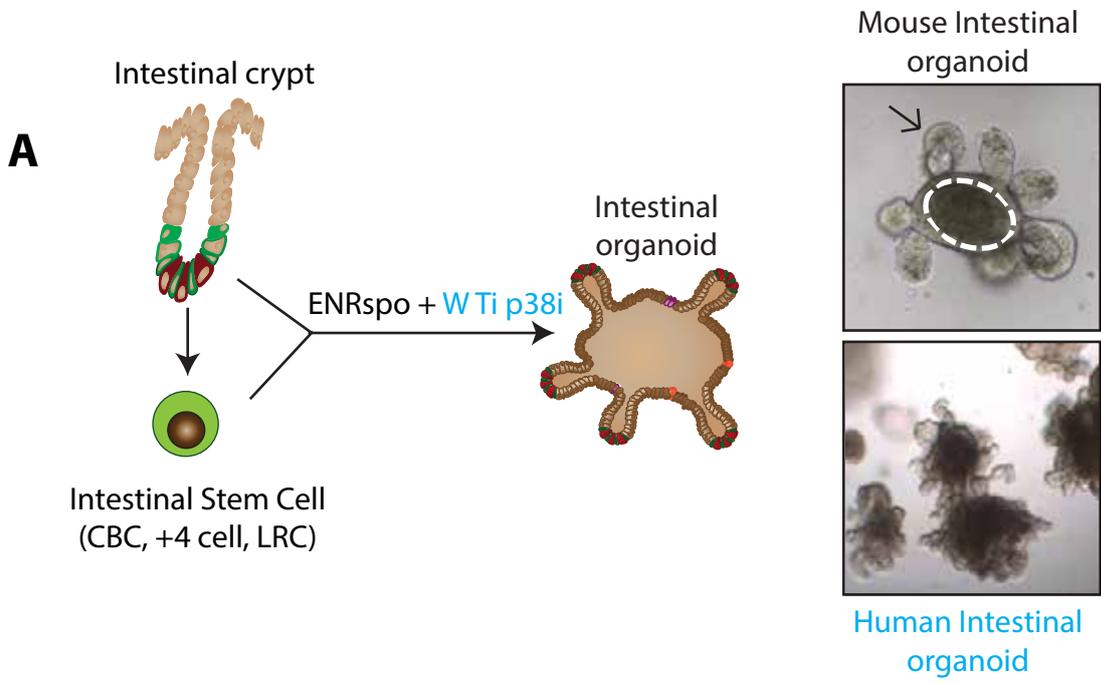
Stomach organoid



Prostate organoid

Antro-pyloric stomach gland development





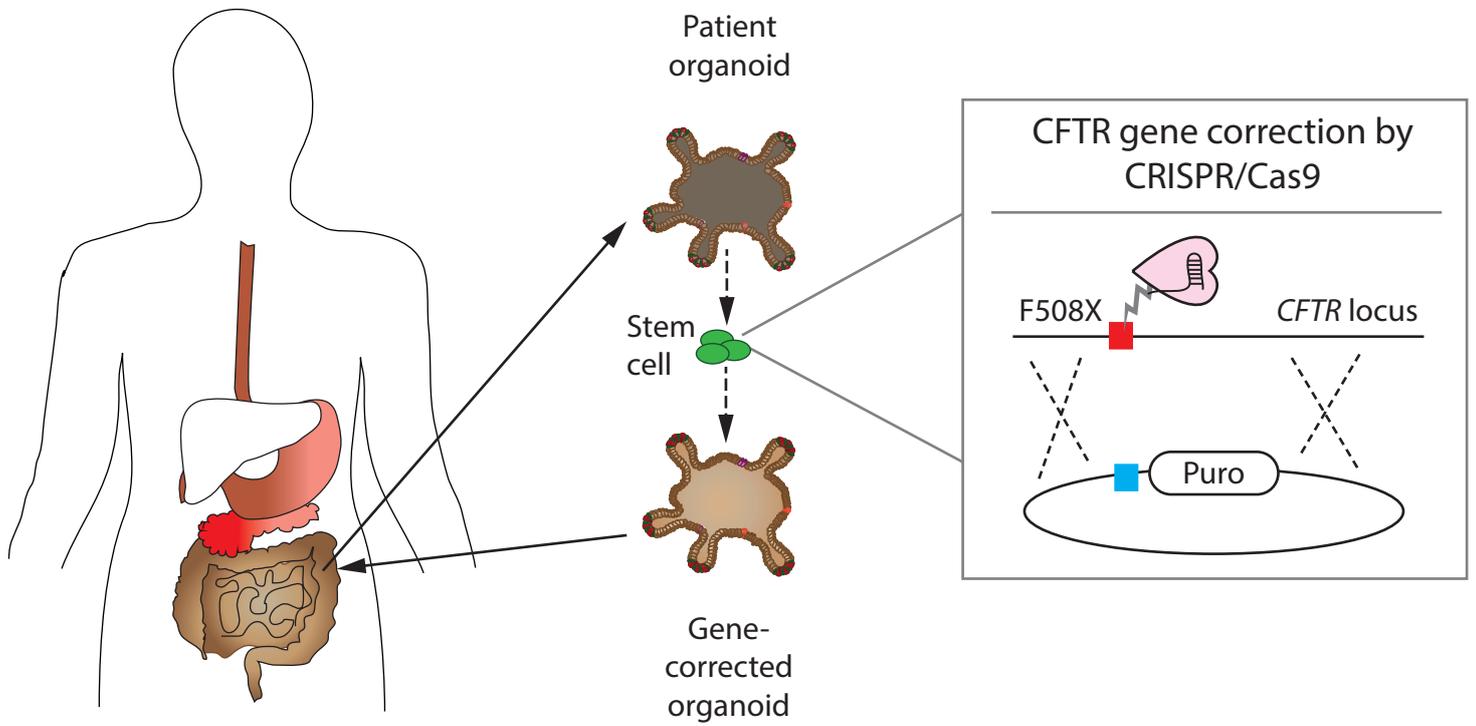


Figure 5: Gene correction in Cystic Fibrosis patient-derived intestinal organoids. CRISPR/Cas9 and targeting vectors are used to replace F508 mutation to wild type coding sequence in CF patient-derived gut organoids. First a patient organoid line is established from a patient biopsy. Single cell suspension containing AdSCs is obtained from the established patient organoids by trypsinization and then transfected with CRISPR/Cas9 and targeting plasmid vectors encoding the correct sequence. DSB, generated by CRISPR/Cas9 on the mutant sequence, facilitated the HR-mediated uptake of targeting vector, which eventually reverts the F508 mutation back to normal as well as the functionality of CFTR chloride channel in a transfected AdSC. This gene-corrected AdSC can be cultured to be an organoid line for expansion in vitro.