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PRIMER

Translational applications of adult stem cell-derived organoids

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ABSTRACT

Adult stem cells from a variety of organs can be expanded long-term *in vitro* as three-dimensional organotypic structures termed organoids. These adult stem cell-derived organoids retain their organ identity and remain genetically stable over long periods of time. The ability to grow organoids from patient-derived healthy and diseased tissue allows for the study of organ development, tissue homeostasis and disease. In this Review, we discuss the generation of adult stem cell-derived organoid cultures and their applications in *in vitro* disease modeling, personalized cancer therapy and regenerative medicine.

KEY WORDS: Adult stem cells, Organoids, Disease modelling, Cancer

Introduction

The human body contains multiple different tissue-specific adult stem cell populations. These adult stem cells have the ability to self-renew and generate all the different cell types present in that organ, thereby playing a key role in tissue homeostasis and/or repair. In recent years, it has become possible to culture adult stem cells *in vitro* and, by growing them under certain conditions, coax them into producing miniaturized versions of the organs in which they once resided (Huch and Koo, 2015). These so-called adult stem cell-derived organoids contain many of the cell types present in the organ from which they were derived, and can also recapitulate some degree of its spatial organization.

The first long-term feeder-free adult stem cell-derived organoid culture system to be developed was that of mouse small intestinal organoids, and it was the result of several key discoveries. As early as 1998, Korinek et al. reported that Wnt signaling is essential for maintenance of the stem cell compartment of the mouse small intestine (Korinek et al., 1998). The subsequent identification of the Wnt target gene leucine-rich repeat-containing G-protein-coupled receptor 5 (*Lgr5*) as a marker of stem cells in the small intestine and colon then led to the observation that intestinal stem cells (ISCs) are proliferative and display unlimited capacity to regenerate intestinal tissue *in vivo* (Barker et al., 2007). A third crucial element was the observation that ectopic expression of R-spondin – a Wnt agonist acting as an Lgr5 ligand (Carmon et al., 2011; de Lau et al., 2011) – induces crypt hyperplasia *in vivo* (Kim et al., 2005). These discoveries allowed the establishment of a culture system for the *in vitro* expansion of ISCs. In 2009, Sato et al. described the conditions for unlimited *in vitro* three-dimensional (3D) expansion of organotypic organoids from single Lgr5-positive ISCs (Sato et al., 2009). These organoids contained all cell types of the intestinal epithelium and were structured into proliferative 'crypt' and differentiated 'villus' compartments, thereby retaining the architecture of the native intestine (Sato et al., 2009). In addition to Wnt/R-spondin, epidermal growth factor (EGF) (Dignass and Sturm, 2001), noggin (BMP inhibitor) (Haramis et al., 2004) and an artificial laminin-rich extracellular matrix (provided by Matrigel) complemented the cocktail for successful *in vitro* propagation of mouse ISC-derived organoids (Sato et al., 2009). At the same time, Ootani et al. reported another Wnt-driven *in vitro* culture of ISCs (Ootani et al., 2009). In contrast to Sato et al., this culture was not based on defined growth factors. Instead, growth factors were provided by an underlying stromal component and organoids were cultured in collagen with an air-liquid interface (Ootani et al., 2009). Importantly, both studies 'rebuilt' the stem cell niche *in vitro* by either artificially providing the niche factors (Wnt, R-spondin, EGF and noggin) (Sato et al., 2009) (Fig. 1) or through the incorporation of a mesenchymal component (Ootani et al., 2009). Human small intestinal and colonic organoids additionally require nicotinamide, A83-01 [an ALK5 (TGFβR1) inhibitor] and SB202190 (a p38 MAP kinase inhibitor) (Jung et al., 2011; Sato et al., 2011). Differentiation towards specific lineages can be induced by modulating the Wnt and Notch signaling pathways (Jung et al., 2011; Sato et al., 2011; Yin et al., 2014). Importantly, and in stark contrast to cancer cell lines, organoids retain a stable genome while being cultured over long periods of time (Behjati et al., 2014; Huch et al., 2015; Sato et al., 2011). This was determined via serial passaging (Huch et al., 2015; Sato et al., 2011) and by whole-genome sequencing of early and late passages of the same organoid cultures (Behjati et al., 2014; Huch et al., 2015).

Based on insights into organ development and repair and on signaling pathways mutated in cancers, similar long-term adult stem cell-derived organoid cultures have subsequently been established for many other organs (Clevers, 2016). These include mouse and human liver (Huch et al., 2013a, 2015), pancreas (Boj et al., 2015; Huch et al., 2013b), stomach (Barker et al., 2010; Bartfeld et al., 2015), fallopian tube (Kessler et al., 2015), prostate (Chua et al., 2014; Karthaus et al., 2014), taste buds (Ren et al., 2014) and salivary gland (Maimets et al., 2016). Although not discussed in detail here, organoids can also be derived from pluripotent stem cells, including embryonic stem cells and induced pluripotent stem cells. Pluripotent stem cells have been extensively used as an independent approach to establish organoids representing a variety of tissues (Lancaster and Knoblich, 2014).

Importantly, organoid technology is versatile: cultures can be started from small tissue samples, typically obtained by biopsy or from surgical specimens. No stem cell purification is necessary: seeding of small, enzymatically disrupted tissue fragments results in rapid sealing of epithelial fragments to form cystic structures lined by a polarized epithelium. Not surprisingly, organoids are fast emerging as a powerful tool for both basic developmental biology and medical research, particularly for studies in which human tissue

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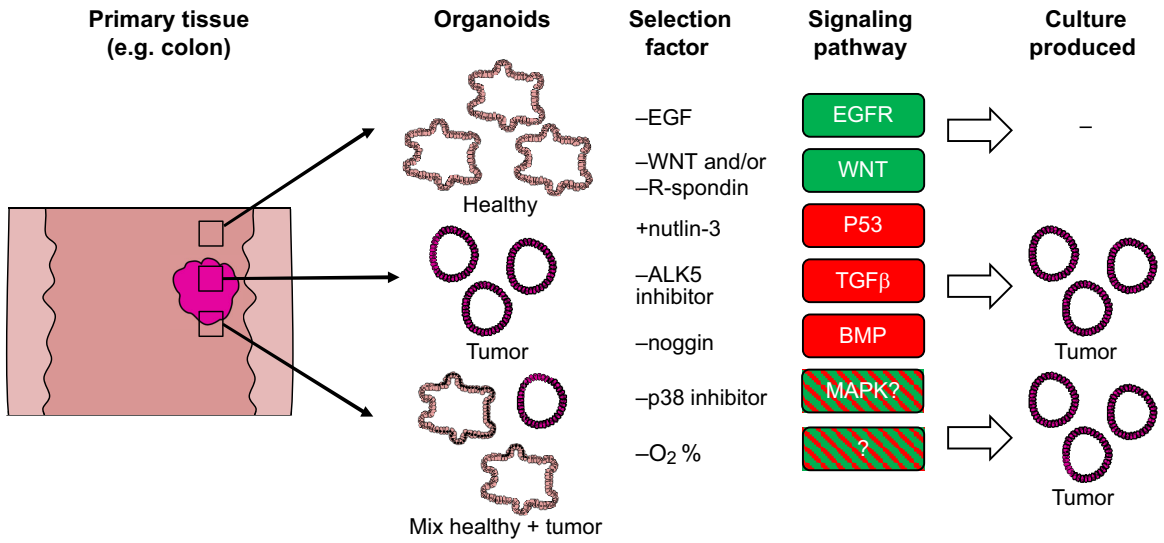

Fig. 1. Niche factor dependence of adult stem cell-derived organoids. Adult stem cell-derived organoids can be grown *in vitro* from a variety of tissues by mimicking the respective stem cell niche. Organoids derived from healthy human colonic tissue (pink) and colonic tumor tissue (purple) rely to varying extents on the presence of Wnt, R-spondin, noggin and EGF in the culture medium (Sato et al., 2011). Healthy tissue requires the presence of all of these elements in the medium (top), whereas, depending on their genetic background, organoids derived from tumor tissue can grow in the absence of at least one of these niche factors (middle) (Drost et al., 2015; Matano et al., 2015). This enables a functional selection strategy to obtain pure CRC organoid cultures from mixed input material. The majority of tumors contain mutations in the *P53* tumor suppressor gene, which can be selected for through addition of the MDM2 inhibitor nutlin-3 (Vassilev et al., 2004) to the culture medium. Treatment with nutlin-3 eradicates cells with wild-type *P53*. Sato and colleagues have demonstrated that certain tumors benefit from culturing in the absence of the p38 MAP kinase inhibitor or under hypoxic conditions (Fujii et al., 2016), although the signaling pathways that mediate this advantage remain unclear (question marks). Signaling pathways that are commonly inactivated in CRC are in red, those commonly activated in CRC in green; shading in both red and green indicates that the mutation underlying selective growth is not known.

is required. In this Review, we focus on adult stem cell-derived organoids and their putative medical applications. We first discuss the use of organoids for disease modeling and focus on genetic diseases, host-pathogen co-cultures and cancer modeling. We subsequently explore the feasibility of using organoid technology and tumor-derived organoid biobanks for personalized cancer therapy. Finally, we discuss the potential exploitation of adult stem cell-derived organoids for regenerative medicine.

Exploiting adult stem cell-derived organoids for *in vitro* disease modeling

Genetic diseases

The long-term *in vitro* expansion of patient-derived organoids allows disease modeling. Several studies have shown the value of adult stem cell-derived organoids for studying hereditary diseases. Beekman and colleagues modeled cystic fibrosis (CF) using intestinal organoids derived from rectal biopsies of CF patients (Dekkers et al., 2013). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes an anion channel expressed on the surface of epithelial cells. CFTR is responsible for fluid and electrolyte homeostasis. Mutations in *CFTR* result in the accumulation of viscous mucus in the respiratory and gastrointestinal tract due to defective epithelial ion transport (Ratjen and Döring, 2003). Forskolin, which activates CFTR through increasing intracellular cyclic AMP levels, induces rapid swelling of intestinal organoids derived from healthy tissue. By contrast, swelling is diminished or absent in intestinal organoids derived from CF patients (Dekkers et al., 2013). The technology has been successfully applied in predicting which drugs work most effectively in patients with different *CFTR* mutations (Dekkers et al., 2016) and as a tool to monitor functional drug levels in blood as an alternative to standard pharmacokinetic testing (Dekkers et al., 2015; Vijftigschild et al., 2016). All this suggests that *in vitro* measurement of CFTR function in CF patient-derived organoids can be used for the development of patient-specific treatment strategies.

In addition, our laboratory has used patient-derived liver organoids to model the disease pathology of two genetic liver disorders (Huch et al., 2015). α1-antitrypsin (A1AT) deficiency is a common genetic disorder caused by mutations in the *A1AT* (*SERPINA1*) gene, resulting in pulmonary fibrosis. Aggregation of misfolded A1AT protein occurs within the endoplasmic reticulum of A1AT-producing hepatocytes, leading to chronic liver failure and often requiring a liver transplant (Stoller and Aboussouan, 2005). Liver organoids from patients with A1AT deficiency generated intracellular A1AT protein aggregates resulting in increased apoptosis when hepatocyte differentiation was induced in the cultures (Huch et al., 2015). Similarly, liver organoids derived from Alagille syndrome (AGS) patients, which is caused by mutations in the Notch signaling pathway, failed to upregulate biliary markers upon differentiation, thereby resembling the paucity of bile ducts (‘biliary atresia’) seen in AGS patients (Huch et al., 2015; Kamath et al., 2013).

Host-pathogen interactions

Several studies have exploited adult stem cell-derived organoids to investigate host-pathogen interactions (Leslie and Young, 2016). Most pathogens colonize via the lumen of organs and make contact with the apical surface of the epithelium where the differentiated cells reside. One of the major advantages of using organoids for studying host-pathogen interactions is that they are either composed of, or can be differentiated into, virtually all the different cell types of a particular organ. They therefore closely recapitulate the *in vivo* environment, making them superior to the immortalized cell lines that have previously been widely used to model host-pathogen interactions. For instance, Paneth cells are terminally differentiated secretory cells located at the base of intestinal crypts and

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intermingled with ISCs. These structures are closely recapitulated in mouse intestinal organoid cultures (Sato et al., 2009). Co-cultures of mouse small intestinal organoids with *Escherichia coli* have been used to investigate the effects of antimicrobial products on the dynamics and function of Paneth cells (Farin et al., 2014). Moreover, human intestinal organoids were exploited to functionally characterize several cholera toxin inhibitors (Zomer-van Ommen et al., 2016), while two recent studies employed mouse intestinal organoids to model *Salmonella* infections (Wilson et al., 2015; Zhang et al., 2014). In addition to an NFκB-induced inflammatory response, the authors observed disruption of epithelial tight junctions and downregulation of ISC markers Lgr5 and Bmi1 in *Salmonella*-infected organoids (Zhang et al., 2014). Neefjes and colleagues recently exploited co-cultures of adult stem cell-derived gallbladder organoids with *Salmonella enterica* to determine the effects of infectious pathogens on the development of cancer (Scanu et al., 2015). Utilizing pre-transformed gallbladder organoids derived from mice deficient for the *Ink4a*/*Arf* (*Cdkn2a*) tumor suppressor locus, the authors found that *Salmonella* induces growth factor-independent growth and neoplastic transformation through activation of AKT and MAPK signaling (Scanu et al., 2015). Likewise, adult stem cell-derived human stomach organoids have been used to study *Helicobacter pylori* infections. Co-culture of gastric organoids and the microbe prompted an NFκB-induced inflammatory response (Bartfeld et al., 2015). Although poorly understood, chronic *Helicobacter* infection is implied as a risk factor in stomach cancer development (Salama et al., 2013). It would be interesting to model chronic infection by long-term co-culture of *Helicobacter* with human stomach organoids to gain a better understanding of the relationship between *Helicobacter* infections and stomach tumorigenesis.

In addition to the organoid-bacteria co-cultures described above, adult stem cell-derived organoids have also been exploited to model viral infections. Recently, rotavirus infection and antiviral therapy were modeled in mouse and human intestinal organoids. Rotaviruses not only efficiently replicated in the organoids, but infected organoids also produced infectious virus particles. Viral replication could be blocked by treatment of the infected organoids with the antiviral agents interferon-α or ribavirin (Yin et al., 2015). Another striking example of organoid-virus co-culture is the use of induced pluripotent stem cell (iPSC)-derived brain organoids to investigate the effects of Zika virus (ZIKV) infection on brain development (Qian et al., 2017). ZIKV infection during pregnancy has recently been linked to microcephaly, and several recent studies now show that ZIKV infection causes growth inhibition and increased apoptosis in iPSC-derived brain organoids, implying that ZIKV alters neurogenesis during brain development and is causative to microcephaly (Cugola et al., 2016; Dang et al., 2016; Garcez et al., 2016; Qian et al., 2016; Tang et al., 2016). Dang et al. further proposed that ZIKV-dependent growth inhibition can be relieved by inhibition of toll-like receptor 3 (TLR3), possibly providing a therapeutic target (Dang et al., 2016).

The accessibility of the organoid lumen can be a challenge in modeling pathogen infections. One possible way to overcome this is to inject the pathogens directly into the lumen of the organoid, instead of adding them to the culture medium. Alternatively, organoids can be dissociated prior to infection and subsequently replated in Matrigel. Finally, the generation of 2D monolayers from organoid cultures could also be exploited for these purposes (Moon et al., 2014). Kovbasnjuk and colleagues recently reported Transwell-based monolayers with an exposed apical surface that were established from human colonic epithelium-derived organoids and used them to model enterohemorrhagic *E. coli* infection (In et al., 2016). Particularly for high-throughput experiments and drug development, it will be of interest to develop and further optimize self-renewing 2D monolayer culture models for human primary epithelial tissues with an exposed apical surface. Also, the strict anaerobic nature of most gut microbes and the high oxygen concentration required by cells cultured *in vitro* is a technical challenge that needs to be overcome in order to mimic the native situation as closely as possible.

Immunotherapy

Although most tumors harbor significant numbers of mutations (Alexandrov et al., 2013a; Greenman et al., 2007), immune responses to the antigens that result from these mutations (neo-antigens) are limited in patients. Co-culture of organoids with intraepithelial lymphocytes (IELs) (Nozaki et al., 2016; Zumwalde et al., 2016) has opened up the possibility of exploiting organoids to expand immune cells *in vitro*, which could be used for immunotherapy purposes, for instance by predicting cytotoxicity when cultured together with patient-derived tumor organoids. IELs play important roles in local immune responses in the tissue in which they reside and could potentially be triggered to specifically attack tumor cells. Nozaki et al. established a novel strategy to maintain and expand IELs in a co-culture system with murine small intestinal organoids (Nozaki et al., 2016). Importantly, the authors showed that IEL migration dynamics are comparable to those of IELs residing in an *in vivo* setting, suggesting that IELs do not lose functional activity *in vitro*. Moreover, Gumperz and colleagues demonstrated that adult stem cell-derived organoids derived from non-cancerous primary human breast tissue contain IEL subtypes that can be primed to kill breast carcinoma cells (Zumwalde et al., 2016). Strønen et al. recently reported that naïve (unchallenged) T cells of healthy blood donors could provide a source of T cells that recognize neo-antigens (Stronen et al., 2016). These neo-antigen-recognizing T cells were shown to efficiently target patient-derived melanoma cells. However, only a subset of the predicted human leukocyte antigen-binding epitopes was recognized by T cells (Stronen et al., 2016). Co-culturing donor-derived T cells with tumor-derived organoids might therefore be used to predict the cytotoxicity of these T cells towards patient-derived tumor organoids, potentially predicting the *in vivo* patient response (Fig. 2). T cells that demonstrate *in vitro* cytotoxicity could subsequently be expanded *in vitro* and administrated to the patient to evoke an anti-tumor immune response. A similar strategy could be applied to autologous tumor-infiltrating lymphocytes from individual patients, which could be isolated, primed, tested and expanded using *in vitro* organoid cultures derived from the same patient (Fig. 2).

Organoid cancer progression models

The emergence of genomic instability is one of the hallmarks of cancer (Lengauer et al., 1998; Stratton et al., 2009). As a consequence, tumor cells typically contain large numbers of mutations, although there is wide variation in the number of mutations both between and within different types of cancer (Alexandrov et al., 2013a; Greenman et al., 2007). Only a few of these mutations, the so-called driver mutations, contribute to disease progression, whereas the remainder, the passenger mutations, do not (Stratton et al., 2009). This genetic heterogeneity complicates the identification of true disease-driving mutations, which could serve as potential therapeutic targets. Although cancer cell lines have proven their value for cancer research, they are not very suitable to

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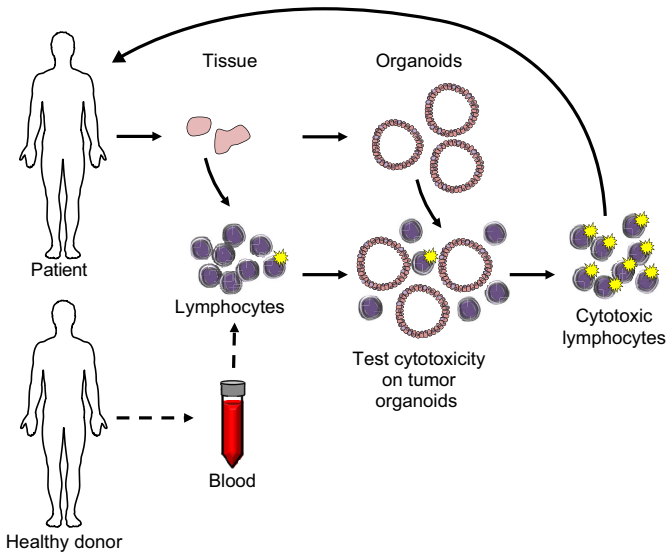

Fig. 2. Exploiting adult stem cell-derived organoids for immunotherapy. In parallel with establishing organoids, immune cells (e.g. lymphocytes) can be isolated from the same patient-derived tissue, expanded and potentially primed in an *in vitro* co-culture with organoids. Tumor-derived organoids can also be exploited to test immune cell cytotoxicity towards the tumor cells. Cytotoxic immune cells could subsequently be expanded and transplanted back into the patient. Similarly, blood from healthy donors has been shown to provide a source of neo-antigen-recognizing T cells, a specialized type of lymphocyte (Stronen et al., 2016). Co-culturing donor-derived T cells with tumor-derived organoids could be used to predict the cytotoxicity of these T cells towards patient-derived tumor organoids, potentially predicting the *in vivo* patient response. T cells that demonstrate *in vitro* cytotoxicity could subsequently be expanded *in vitro* and administrated to the patient to evoke an anti-tumor immune response. Solid arrows indicate biological material derived from the patient; dashed arrows indicate biological material derived from a healthy donor.

study the driver mutations required for tumor initiation and progression. The establishment of cancer cell lines from primary tissue is very inefficient, mainly because of the challenge in adapting to *in vitro* 2D culture conditions (Barretina et al., 2012; Lacroix and Leclercq, 2004). As only rare clones are able to survive and expand, the cultures do not faithfully recapitulate the genetic spectrum of the original tumor. Moreover, most cancer cell lines are derived from metastatic and fast growing tumors that already contain all the driver mutations required to become a full-blown metastatic tumor cell (Masters, 2000). As an alternative, potential oncogenic mutations can be generated in mice and interactions can be studied by interstrain crosses. Although widely used, this approach is time- and resource-consuming, and some might argue that genetically modified mouse models do not always faithfully phenocopy human cancers.

Two recent studies have established colorectal cancer (CRC) progression models starting from healthy human colon organoids (Drost et al., 2015; Matano et al., 2015). Four of the most frequently occurring CRC mutations in the adenoma-carcinoma sequence (Fearon, 2011; Fearon and Vogelstein, 1990) were sequentially introduced using CRISPR/Cas9 genome editing (Drost et al., 2015; Matano et al., 2015). These studies revealed a one-to-one correlation between oncogenic mutations and stem cell niche factor dependency. In contrast to healthy colonic organoids, organoids harboring inactivating mutations in *APC*, *P53* (*TP53*) and *SMAD4* and an activating mutation in *KRAS* (*KRAS*^{G12D}) were able to be grown in the absence of the stem cell niche factors Wnt/R-spondin, EGF and noggin (Fig. 1). Xenotransplantation further revealed that quadruple-mutant organoids grew as invasive adenocarcinomas. A common hallmark of CRC is the acquisition of chromosome instability (Rajagopalan et al., 2003). We found that loss of *APC* and *P53* is the main driver of chromosome instability and aneuploidy in CRC (Drost et al., 2015). Similar tumor progression models were established in air-liquid interface cultures of mouse pancreatic, gastric, small intestinal and colonic organoids (Li et al., 2014). However, the strategy of using RNA interference and cDNA overexpression in this system does not exactly emulate the specific genetic aberrations found in mutation hotspot regions within frequently mutated human cancer genes. Nonetheless, tamoxifen-treated organoids derived from *Villin-CreER;Apc*$^{flox/flox}$ mice that were subsequently infected with retroviruses encoding *KRAS*^{G12D}, P53 shRNA and SMAD4 shRNA showed histological features of invasive adenocarcinomas.

Finally, adult stem cell-derived organoids were shown to be genetically stable over long periods of time (Behjati et al., 2014; Huch et al., 2015; Sato et al., 2011). This opens the possibility of investigating the mutational effects of chemicals (e.g. chemotherapeutics) on the genome of both healthy and tumor cells. In addition, gene editing of specific genes (e.g. cancer predisposition genes) in healthy organoids might provide mutational signatures (Alexandrov et al., 2013a,b) that can be used in the clinic to identify patients with a high risk of developing particular genetic diseases, including cancer.

In conclusion, organoid tumor progression models closely recapitulate classical multi-hit CRC progression and might be useful for understanding the mutational effects of chemicals and possibly other environmental factors. We anticipate that combinatorial CRISPR-modified human organoids will serve as a valuable, high-throughput tool to evaluate potentially oncogenic mutations observed in genome-wide sequencing efforts on large tumor panels such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC).

Personalized cancer therapy

Large-scale drug screens on cancer cell lines have provided insights into correlations between drug sensitivity and the mutational background of a given tumor (Barretina et al., 2012; Garnett et al., 2012). As discussed above, cancer cell lines do not always histologically and genetically recapitulate human tumors, and the establishment of 2D cancer cell lines from primary tumor material is very inefficient (Barretina et al., 2012; Lacroix and Leclercq, 2004; Masters, 2000). The generation of organoid cultures from patient-derived tumor tissue may allow for the development of improved patient-specific treatment strategies. Indeed, organoids can be derived from freshly isolated primary and metastatic tumor tissue with relatively high efficiency (Clevers, 2016). Somewhat counterintuitively, tumor organoids tend to grow more slowly than their wild-type counterparts. As a consequence, wild-type organoids can overgrow the cultures unless specific measures are taken. Sato et al. first described the culture conditions for human colon adenomas and adenocarcinomas (Sato et al., 2011). In this case, the vast majority of CRCs carry inactivating mutations in the *APC* gene, resulting in active Wnt signaling, even in the absence of Wnt ligand binding (Clevers and Nusse, 2012). As such, pure CRC organoid cultures can be obtained using culture medium lacking Wnt and/or R-spondin, the lack of which impedes growth of wild-type tissue (Sato et al., 2011). Similar functional selection strategies can be exploited to select for other frequently mutated cancer genes (Fig. 1). For example, EGF is dispensable for tumors harboring

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activating mutations in the EGFR pathway, whereas tumor cells with inactivating mutations in the BMP or TGFβ signaling pathways can be grown in the absence of noggin or A83-01, respectively (Drost et al., 2015; Fujii et al., 2016; Matano et al., 2015). *P53* mutant tumors will grow in the presence of the small molecule nutlin-3, which kills cells harboring wild-type *P53* (Drost et al., 2015; Matano et al., 2015) (Fig. 1). Since not all tumors harbor mutations in these pathways, it is not always possible to employ functional selection strategies. Thus, it remains important to establish cultures from (nearly) pure tumor samples. So far, tumor organoids have also been generated from colon (Weeber et al., 2015) and prostate (Drost et al., 2016; Gao et al., 2014) cancer metastases, prostate cancer circulating tumor cells (Drost et al., 2016; Gao et al., 2014) and primary pancreatic tumors (Boj et al., 2015).

Using these approaches, living tumor organoid biobanks have been established from patient-derived tumor tissue. Van de Wetering et al. prospectively generated a biobank consisting of 20 tumor organoid cultures derived from CRC patients (van de Wetering et al., 2015). Genetic characterization and drug testing allowed for the correlation of mutations with drug responsiveness. For instance, only one of the tumor organoid cultures was sensitive to LGK974, an inhibitor of Wnt secretion (Chen et al., 2009). This particular tumor appeared not to carry an *APC* mutation, but instead a mutation was found in the negative Wnt regulator *RNF43*, which caused the sensitivity to LGK974 (Hao et al., 2012; Koo et al., 2012; van de Wetering et al., 2015). In addition, a previously reported drug-gene correlation between loss-of-function mutations in *P53* and resistance to the MDM2 inhibitor nutlin-3 was confirmed (van de Wetering et al., 2015; Vassilev et al., 2004). More recently, Sato and colleagues established an organoid collection derived from 55 colorectal tumors with a wide range of different histological subtypes (Fujii et al., 2016). They demonstrated that organoids could only be derived from certain CRCs using culture medium lacking p38 MAP kinase inhibitor or under hypoxic conditions (Fujii et al., 2016) (Fig. 1). Importantly, both studies showed that histological and genetic tumor characteristics are retained within the organoids (Fujii et al., 2016; van de Wetering et al., 2015). Thus, tumor-derived organoid biobanks hold great promise for revealing the rules underlying the correlation of tumor genetics with drug response. Another future direction could be the use of organoid technology for personalized *ex vivo* drug testing on cultured tumor tissue of individual patients. In a recent study by Snippert and colleagues, a CRC organoid biobank (van de Wetering et al., 2015) was used to investigate potential strategies to target mutant RAS (Verissimo et al., 2016). By evaluating the effect of different drugs and drug combinations on wild-type and *KRAS* mutant CRC organoids, the authors confirmed that organoid biobanks are a valuable tool for personalized drug testing. However, it will be an absolute prerequisite that tumor organoids can be rapidly established and expanded in order to allow drug screening in a clinically meaningful time window. Moreover, organoids could potentially be used to monitor the levels of functional drugs in the circulation of cancer patients, as was recently shown for drugs used in CF patients (Dekkers et al., 2015; Vijftigschild et al., 2016).

Regenerative medicine

Ex vivo expanded adult stem cell-derived organoids retain their organ identity and genome stability, and can be differentiated to virtually all cellular lineages present in the pertinent organ. Therefore, organoids could potentially serve as an unlimited source for replacing damaged tissues. This may complement the use of donor organs and additionally prevent an allogeneic immune

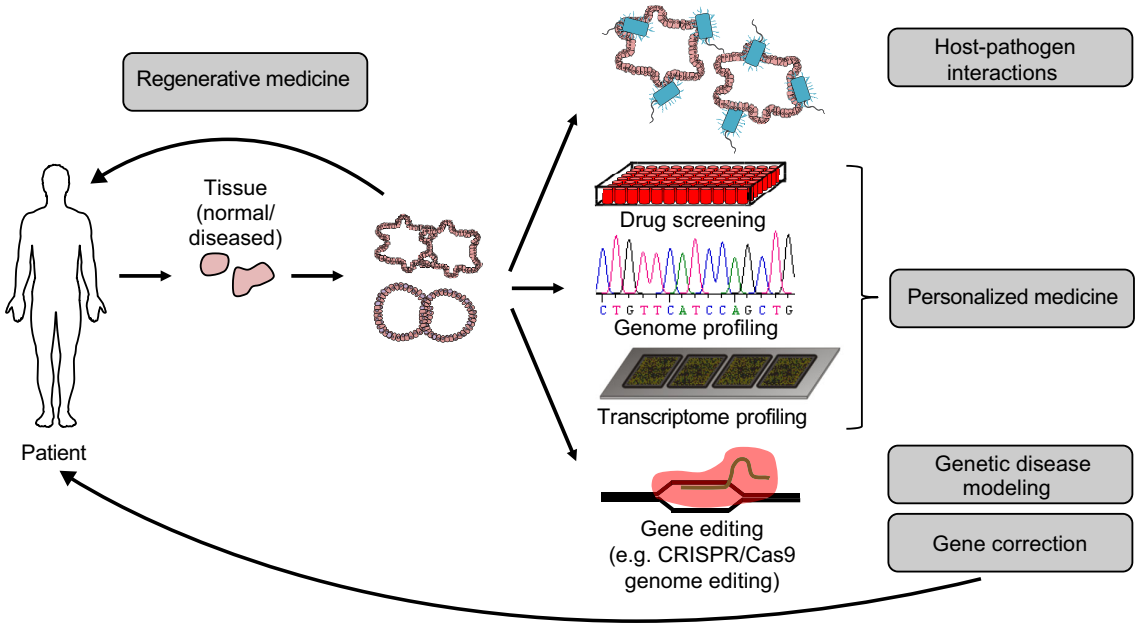

Fig. 3. Applications of adult stem cell-derived organoids. Organoids can be established from patient-derived tissue samples, including fresh biopsies and resected material. Organoids provide the unique opportunity for drug testing and correlation of drug functionality with genetic profile (by genome and transcriptome analysis) of individual tumors, potentially allowing the development of personalized treatment strategies. The ability to establish and expand organoids from single adult stem cell-derived organoids and subsequent transplantation into experimental animal models raises the possibility of using organoids in regenerative medicine. Transplantation of organoids in which disease-driving genetic events have been repaired using gene editing technologies (e.g. CRISPR/Cas9) could potentially be exploited to treat patients with the disease. Gene editing can also be exploited for disease modeling and for studying specific genetic alterations and signaling pathways. Host-pathogen interactions can be studied by co-culturing the organoids with the pathogen(s) of interest.

response upon transplantation, if organoids are used that were derived from healthy tissue of the same individual. Indeed, Watanabe and colleagues demonstrated that mouse colon organoids expanded *in vitro* from a single stem cell can, upon transplantation into multiple damaged mouse colons, engraft and develop into functional crypt units. Remarkably, epithelial barrier function was fully recovered in the transplanted mice (Yui et al., 2012). Similar results were obtained using adult stem cell-derived or fetal progenitor-derived small intestinal organoids (Fordham et al., 2013; Fukuda et al., 2014; Shaffiey et al., 2016). Moreover, single Lgr5-positive mouse liver stem cell-derived organoids were successfully transplanted into fumarylacetoacetate hydrolase mutant mice, a mouse model for tyrosinemia type I liver disease. Untreated mice die because of extensive liver failure, but the authors demonstrated that transplantation of *in vitro* differentiated liver organoids significantly increased the survival of the mice (Huch et al., 2013a). In addition, transplantation of human adult stem cell-derived liver organoids into immune-deficient mice in which the liver was chemically damaged yielded grafts containing functional hepatocytes (Huch et al., 2015). It should be noted that, in all cases, transplantation is inefficient at best and will require much optimization before clinical applications become feasible.

An 'ultimate' application in regenerative medicine would combine organoid technology with gene therapy. As a proof of concept for gene correction in patients with a single-gene hereditary defect, CRISPR/Cas9 gene editing was utilized to correct a *CFTR* mutation in small intestinal and rectal organoids from two CF patients (Schwank et al., 2013). Using the CF swelling assay (Dekkers et al., 2013), the authors demonstrated that the corrected allele was fully functional (Schwank et al., 2013).

Conclusions and outlook

We have attempted to provide a comprehensive overview of the applications – many of which are in their infancy – of adult stem cell-derived organoid cultures in disease modeling, personalized cancer therapy and regenerative medicine (Fig. 3). 3D organoid cultures can be established from a wide range of organs. As breast, skin and lung cancer are among the most common forms of cancer, it would be of particular interest for cancer research to establish long-term organoid culture protocols and organoid biobanks from these tissues as well. The recent establishment of a culture protocol allowing long-term (2-2.5 months) growth of murine mammary organoids (Jardé et al., 2016) potentially provides a lead towards optimized culture conditions for the growth of human mammary organoids. Lung organoids would also be of special interest for studying infectious respiratory diseases. To date, adult stem cell-derived organoid cultures have only been established from epithelial tissues. Instead, pluripotent stem cells have been successfully used to establish organoids from non-epithelial tissues, including brain and retina (Lancaster and Knoblich, 2014). Future studies will explore the feasibility of establishing adult stem cell-derived organoid cultures from non-epithelial tissues.

Although there is much enthusiasm for organoid technology and the possible applications thereof, it is important to recognize their potential limitations as well. Counterintuitively, organoids derived from progressed cancers generally grow poorly compared with those derived from normal tissue or early stage tumors. In most cases, culture conditions are optimized for growing normal tissue, which may be disadvantageous for growing tumors. Sato and colleagues recently showed that some CRC-derived organoids can be more efficiently grown in the absence of p38 MAP kinase inhibitor or under hypoxic conditions (Fujii et al., 2016), indeed suggesting that the optimal culture conditions vary between normal and tumor organoid cultures and even between tumor cultures. Moreover, organoid cultures lack stroma, immune cells and vasculature. The co-culture systems described in this Review could significantly improve the physiological relevance of organoid technology. The requirement of Matrigel (produced by mouse sarcoma cells) introduces an undefined factor into the organoid culture system, which complicates the use of organoids in regenerative medicine and drug testing. The development of artificial matrices that sustain organoid growth could potentially solve this issue.

In summary, the potential translational applications of adult stem cell-derived organoids are just beginning to be explored. Future research is required to determine the feasibility of these applications, to solve technical challenges, and to implement successful applications in the clinic.

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Competing interests

H.C. is named as inventor on several patents related to Lgr5-positive stem cell-based organoid technology.

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References

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L. et al. (2013a). Signatures of mutational processes in human cancer. *Nature* **500**, 415-421.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Campbell, P. J. and Stratton, M. R. (2013b). Deciphering signatures of mutational processes operative in human cancer. *Cell Rep.* **3**, 246-259.

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.

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. (2010). Lgr5 (+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25-36.

Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., Wilson, C. J., Lehár, J., Kryukov, G. V., Sonkin, D. et al. (2012). The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603-607.

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.

Boj, S. F., Hwang, C.-I., Baker, L. A., Chio, I. I. C., Engle, D. D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M. S. et al. (2015). Organoid models of human and mouse ductal pancreatic cancer. *Cell* **160**, 324-338.

Carmon, K. S., Gong, X., Lin, Q., Thomas, A. and Liu, Q. (2011). R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc. Natl. Acad. Sci. USA* **108**, 11452-11457.

Chen, B., Dodge, M. E., Tang, W., Lu, J., Ma, Z., Fan, C. W., Wei, S., Hao, W., Kilgore, J., Williams, N. S. et al. (2009). Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nat. Chem. Biol.* **5**, 100-107.

Chua, C. W., Shibata, M., Lei, M., Toivanen, R., Barlow, L. M. J., Bergren, S. K., Badani, K. K., McKiernan, J. M., Benson, M. C., Hibshoosh, H. et al. (2014). Single luminal epithelial progenitors can generate prostate organoids in culture. *Nat. Cell Biol.* **16**, 951-961.

Clevers, H. (2016). Modeling development and disease with organoids. *Cell* **165**, 1586-1597.

Clevers, H. and Nusse, R. (2012). Wnt/beta-catenin signaling and disease. *Cell* **149**, 1192-1205.
Cugola, F. R., Fernandes, I. R., Russo, F. B., Freitas, B. C., Dias, J. L., Guimarães, K. P., Benazzato, C., Almeida, N., Pignatari, G. C., Romero, S. et al. (2016). The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* **534**, 267-271. doi:10.1038/nature18296.
Dang, J., Tiwari, S. K., Lichinchi, G., Qin, Y., Patil, V. S., Eroshkin, A. M. and Rana, T. M. (2016). Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. *Cell Stem Cell* **19**, 258-265.
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. M., Bijvelds, M. J. C., Scholte, B. J. et al. (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939-945.
Dekkers, R., Vijftigschild, L. A. W., Vonk, A. M., Kruisselbrink, E., de Winter-de Groot, K. M., Janssens, H. M., van der Ent, C. K. and Beekman, J. M. (2015). A bioassay using intestinal organoids to measure CFTR modulators in human plasma. *J. Cyst. Fibros.* **14**, 178-181.
Dekkers, J. F., Berkers, G., Kruisselbrink, E., Vonk, A., de Jonge, H. R., Janssens, H. M., Bronsveld, I., van de Graaf, E. A., Nieuwenhuis, E. E., Houwen, R. H. et al. (2016). Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med.* **8**, 344ra384.
de Lau, W., Barker, N., Low, T. Y., Koo, B.-K., Li, V. S. W., 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.
Dignass, A. U. and Sturm, A. (2001). Peptide growth factors in the intestine. *Eur. J. Gastroenterol. Hepatol.* **13**, 763-770.
Drost, J., van Jaarsveld, R. H., Ponsioen, B., Zimberlin, C., van Boxtel, R., Buijs, A., Sachs, N., Overmeer, R. M., Offerhaus, G. J., Begthel, H. et al. (2015). Sequential cancer mutations in cultured human intestinal stem cells. *Nature* **521**, 43-47.
Drost, J., Karthaus, W. R., Gao, D., Driehuis, E., Sawyers, C. L., Chen, Y. and Clevers, H. (2016). Organoid culture systems for prostate epithelial and cancer tissue. *Nat. Protoc.* **11**, 347-358.
Farin, H. F., Karthaus, W. R., Kujala, P., Rakhshandehroo, M., Schwank, G., Vries, R. G. J., Kalkhoven, E., Nieuwenhuis, E. E. S. and Clevers, H. (2014). Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell-derived IFN-gamma. *J. Exp. Med.* **211**, 1393-1405.
Fearon, E. R. (2011). Molecular genetics of colorectal cancer. *Annu. Rev. Pathol.* **6**, 479-507.
Fearon, E. R. and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* **61**, 759-767.
Fordham, R. P., Yui, S., Hannan, N. R. F., Soendergaard, C., Madgwick, A., Schweiger, P. J., Nielsen, O. H., Vallier, L., Pedersen, R. A., Nakamura, T. et al. (2013). Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. *Cell Stem Cell* **13**, 734-744.
Fujii, M., Shimokawa, M., Date, S., Takano, A., Matano, M., Nanki, K., Ohta, Y., Toshimitsu, K., Nakazato, Y., Kawasaki, K. et al. (2016). A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* **18**, 827-838.
Fukuda, M., Mizutani, T., Mochizuki, W., Matsumoto, T., Nozaki, K., Sakamaki, Y., Ichinose, S., Okada, Y., Tanaka, T., Watanabe, M. et al. (2014). Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. *Genes Dev.* **28**, 1752-1757.
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.
Garcez, P. P., Loiola, E. C., Madeiro da Costa, R., Higa, L. M., Trindade, P., Delvecchio, R., Nascimento, J. M., Brindeiro, R., Tanuri, A. and Rehen, S. K. (2016). Zika virus impairs growth in human neurospheres and brain organoids. *Science* **352**, 816-818.
Garnett, M. J., Edelman, E. J., Heidorn, S. J., Greenman, C. D., Dastur, A., Lau, K. W., Greninger, P., Thompson, I. R., Luo, X., Soares, J. et al. (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* **483**, 570-575.
Greenman, C., Stephens, P., Smith, R., Dalgliesh, G. L., Hunter, C., Bignell, G., Davies, H., Teague, J., Butler, A., Stevens, C. et al. (2007). Patterns of somatic mutation in human cancer genomes. *Nature* **446**, 153-158.
Hao, H.-X., Xie, Y., Zhang, Y., Charlat, O., Oster, E., Avello, M., Lei, H., Mickanin, C., Liu, D., Ruffner, H. et al. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* **485**, 195-200.
Haramis, A.-P. G., Begthel, H., van den Born, M., van Es, J., Jonkheer, S., Offerhaus, G. J. and Clevers, H. (2004). De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science* **303**, 1684-1686.
Huch, M. and Koo, B.-K. (2015). Modeling mouse and human development using organoid cultures. *Development* **142**, 3113-3125.
Huch, M., Boj, S. F. and Clevers, H. (2013a). Lgr5(+) liver stem cells, hepatic organoids and regenerative medicine. *Regen. Med.* **8**, 385-387.
Huch, M., Bonfanti, P., Boj, S. F., Sato, T., Loomans, C. J. M., van de Wetering, M., Sojoodi, M., Li, V. S. W., Schuijers, J., Gracanin, A. et al. (2013b). Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J.* **32**, 2708-2721.
Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M. M. A., 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.
In, J., Foulke-Abel, J., Zachos, N. C., Hansen, A. M., Kaper, J. B., Bernstein, H. D., Halushka, M., Blutt, S., Estes, M. K., Donowitz, M. et al. (2016). Enterohemorrhagic Escherichia coli reduce mucus and intermicrovillar bridges in human stem cell-derived colonoids. *Cell. Mol. Gastroenterol. Hepatol.* **2**, 48-62 e43.
Jardé, T., Lloyd-Lewis, B., Thomas, M., Kendrick, H., Melchor, L., Bougaret, L., Watson, P. D., Ewan, K., Smalley, M. J. and Dale, T. C. (2016). Wnt and Neuregulin1/ErbB signalling extends 3D culture of hormone responsive mammary organoids. *Nat. Commun.* **7**, 13207.
Jung, P., Sato, T., Merlos-Suárez, 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. *Nat. Med.* **17**, 1225-1227.
Kamath, B. M., Spinner, N. B. and Rosenblum, N. D. (2013). Renal involvement and the role of Notch signalling in Alagille syndrome. *Nat. Rev. Nephrol.* **9**, 409-418.
Karthaus, W. R., Iaquinta, 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.
Kessler, M., Hoffmann, K., Brinkmann, V., Thieck, O., Jackisch, S., Toelle, B., Berger, H., Mollenkopf, H.-J., Mangler, M., Sehouli, J. et al. (2015). The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat. Commun.* **6**, 8989.
Kim, K.-A., Kakitani, M., Zhao, J., Oshima, T., Tang, T., Binnerts, M., Liu, Y., Boyle, B., Park, E., Emtage, P. et al. (2005). Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science* **309**, 1256-1259.
Koo, B.-K., Spit, M., Jordens, I., Low, T. Y., Stange, D. E., van de Wetering, M., van Es, J. H., Mohammed, S., Heck, A. J. R., Maurice, M. M. et al. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **488**, 665-669.
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. *Nat. Genet.* **19**, 379-383.
Lacroix, M. and Leclercq, G. (2004). Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Res. Treat.* **83**, 249-289.
Lancaster, M. A. and Knoblich, J. A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**, 1247125.
Lengauer, C., Kinzler, K. W. and Vogelstein, B. (1998). Genetic instabilities in human cancers. *Nature* **396**, 643-649.
Leslie, J. L. and Young, V. B. (2016). A whole new ball game: stem cell-derived epithelia in the study of host-microbe interactions. *Anaerobe* **37**, 25-28.
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.-M. et al. (2014). Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nat. Med.* **20**, 769-777.
Maimets, M., Rocchi, C., Bron, R., Pringle, S., Kuipers, J., Giepmans, B. N. G., Vries, R. G. J., Clevers, H., de Haan, G., van Os, R. et al. (2016). Long-term in vitro expansion of salivary gland stem cells driven by Wnt signals. *Stem Cell Rep.* **6**, 150-162.
Masters, J. R. W. (2000). Human cancer cell lines: fact and fantasy. *Nat. Rev.* **1**, 233-236.
Matano, M., Date, S., Shimokawa, M., Takano, A., Fujii, M., Ohta, Y., Watanabe, T., Kanai, T. and Sato, T. (2015). Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat. Med.* **21**, 256-262.
Moon, C., VanDussen, K. L., Miyoshi, H. and Stappenbeck, T. S. (2014). Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. *Mucosal. Immunol.* **7**, 818-828.
Nozaki, K., Mochizuki, W., Matsumoto, Y., Matsumoto, T., Fukuda, M., Mizutani, T., Watanabe, M. and Nakamura, T. (2016). Co-culture with intestinal epithelial organoids allows efficient expansion and motility analysis of intraepithelial lymphocytes. *J. Gastroenterol.* **51**, 206-213.
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. *Nat. Med.* **15**, 701-706.
Qian, X., Nguyen, H. N., Song, M. M., Hadiono, C., Ogden, S. C., Hammack, C., Yao, B., Hamersky, G. R., Jacob, F., Zhong, C. et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* **165**, 1238-1254.

Qian, X., Nguyen, H. N., Jacob, F., Song, H. and Ming, G.-l. (2017). Using brain organoids to understand Zika virus-induced microcephaly. *Development* **144**, 952-957.

Rajagopalan, H., Nowak, M. A., Vogelstein, B. and Lengauer, C. (2003). The significance of unstable chromosomes in colorectal cancer. *Nat. Rev. Cancer* **3**, 695-701.

Ratjen, F. and Döring, G. (2003). Cystic fibrosis. *Lancet* **361**, 681-689.

Ren, W., Lewandowski, B. C., Watson, J., Aihara, E., Iwatsuki, K., Bachmanov, A. A., Margolskee, R. F. and Jiang, P. (2014). Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. *Proc. Natl. Acad. Sci. USA* **111**, 16401-16406.

Salama, N. R., Hartung, M. L. and Müller, A. (2013). Life in the human stomach: persistence strategies of the bacterial pathogen Helicobacter pylori. *Nat. Rev. Microbiol.* **11**, 385-399.

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.

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G. J., 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.

Scanu, T., Spaapen, R. M., Bakker, J. M., Pratap, C. B., Wu, L.-e., Hofland, I., Broeks, A., Shukla, V. K., Kumar, M., Janssen, H. et al. (2015). Salmonella manipulation of host signaling pathways provokes cellular transformation associated with gallbladder carcinoma. *Cell Host Microbe* **17**, 763-774.

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.

Shaffiey, S. A., Jia, H., Keane, T., Costello, C., Wasserman, D., Quidgley, M., Dziki, J., Badylak, S., Sodhi, C. P., March, J. C. et al. (2016). Intestinal stem cell growth and differentiation on a tubular scaffold with evaluation in small and large animals. *Regen. Med.* **11**, 45-61.

Stoller, J. K. and Aboussouan, L. S. (2005). Alpha1-antitrypsin deficiency. *Lancet* **365**, 2225-2236.

Stratton, M. R., Campbell, P. J. and Futreal, P. A. (2009). The cancer genome. *Nature* **458**, 719-724.

Stronen, E., Toebes, M., Kelderman, S., van Buuren, M. M., Yang, W., van Rooij, N., Donia, M., Boschen, M.-L., Lund-Johansen, F., Olweus, J. et al. (2016). Targeting of cancer neoantigens with donor-derived T cell receptor repertoires. *Science* **352**, 1337-1341.

Tang, H., Hammack, C., Ogden, S. C., Wen, Z., Qian, X., Li, Y., Yao, B., Shin, J., Zhang, F., Lee, E. M. et al. (2016). Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* **18**, 587-590.

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.

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C. et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **303**, 844-848.

Verissimo, C. S., Overmeer, R. M., Ponsioen, B., Drost, J., Mertens, S., Verlaan-Klink, I., van Gerwen, B., van der Ven, M., Van de Wetering, M., Egan, D. A. et al. (2016). Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *Elife* **5**, e18489.

Vijftigschild, L. A. W., Berkers, G., Dekkers, J. F., Zomer-van Ommen, D. D., Matthes, E., Kruisselbrink, E., Vonk, A., Hensen, C. E., Heida-Michel, S., Geerdink, M. et al. (2016). beta2-Adrenergic receptor agonists activate CFTR in intestinal organoids and subjects with cystic fibrosis. *Eur. Respir. J.* **48**, 768-779.

Weeber, F., van de Wetering, M., Hoogstraat, M., Dijkstra, K. K., Krijgsman, O., Kuilman, T., Gadellaa-van Hooijdonk, C. G. M., van der Velden, D. L., Peeper, D. S., Cuppen, E. P. J. G. et al. (2015). Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc. Natl. Acad. Sci. USA* **112**, 13308-13311.

Wilson, S. S., Tocchi, A., Holly, M. K., Parks, W. C. and Smith, J. G. (2015). A small intestinal organoid model of non-invasive enteric pathogen-epithelial cell interactions. *Mucosal Immunol.* **8**, 352-361.

Yin, X., Farin, H. F., van Es, J. H., Clevers, H., Langer, R. and Karp, J. M. (2014). Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. *Nat. Methods* **11**, 106-112.

Yin, Y., Bijvelds, M., Dang, W., Xu, L., van der Eijk, A. A., Knipping, K., Tuysuz, N., Dekkers, J. F., Wang, Y., de Jonge, J. et al. (2015). Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. *Antiviral Res.* **123**, 120-131.

Yui, S., Nakamura, T., Sato, T., Nemoto, Y., Mizutani, T., Zheng, X., Ichinose, S., Nagaishi, T., Okamoto, R., Tsuchiya, K. et al. (2012). Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell. *Nat. Med.* **18**, 618-623.

Zhang, Y.-G., Wu, S., Xia, Y. and Sun, J. (2014). Salmonella-infected crypt-derived intestinal organoid culture system for host-bacterial interactions. *Physiol. Rep.* **2**, e12147.

Zomer-van Ommen, D. D., Pukin, A. V., Fu, O., Quarles van Ufford, L. H. C., Janssens, H. M., Beekman, J. M. and Pieters, R. J. (2016). Functional characterization of cholera toxin inhibitors using human intestinal organoids. *J. Med. Chem.* **59**, 6968-6972.

Zumwalde, N. A., Haag, J. D., Sharma, D., Mirrielees, J. A., Wilke, L. G., Gould, M. N. and Gumperz, J. E. (2016). Analysis of immune cells from human mammary ductal epithelial organoids reveals vdelta2+ T cells that efficiently target breast carcinoma cells in the presence of bisphosphonate. *Cancer Prev. Res. (Phila.)* **9**, 305-316.