



# Human stem cell-based disease modeling: prospects and challenges

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Human stem cell-based disease models have great promise to advance our understanding of human disease. These models can be derived from patients with genetic disorders and manipulated with genome editing and myriad differentiation protocols to model pathologies *in vitro*. However, several challenges have impeded the full potential of stem cell-based *in vitro* disease modeling. Many genetically predisposed diseases take time to manifest and occur in specific tissue microenvironments, and these parameters are often not adequately modeled using conventional shorter-term monolayer cultures. These challenges must be overcome especially for cases where animal models also incompletely recapitulate the complex pathologies found in humans. As prominent ways to tackle these challenges we discuss here how advanced genome editing tools in human stem cells and human organoid cultures, specifically the example of intestinal organoids, contribute genetically defined models that recapitulate phenotypes of disease.

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## The advent of stem cell-based disease modeling and current challenges

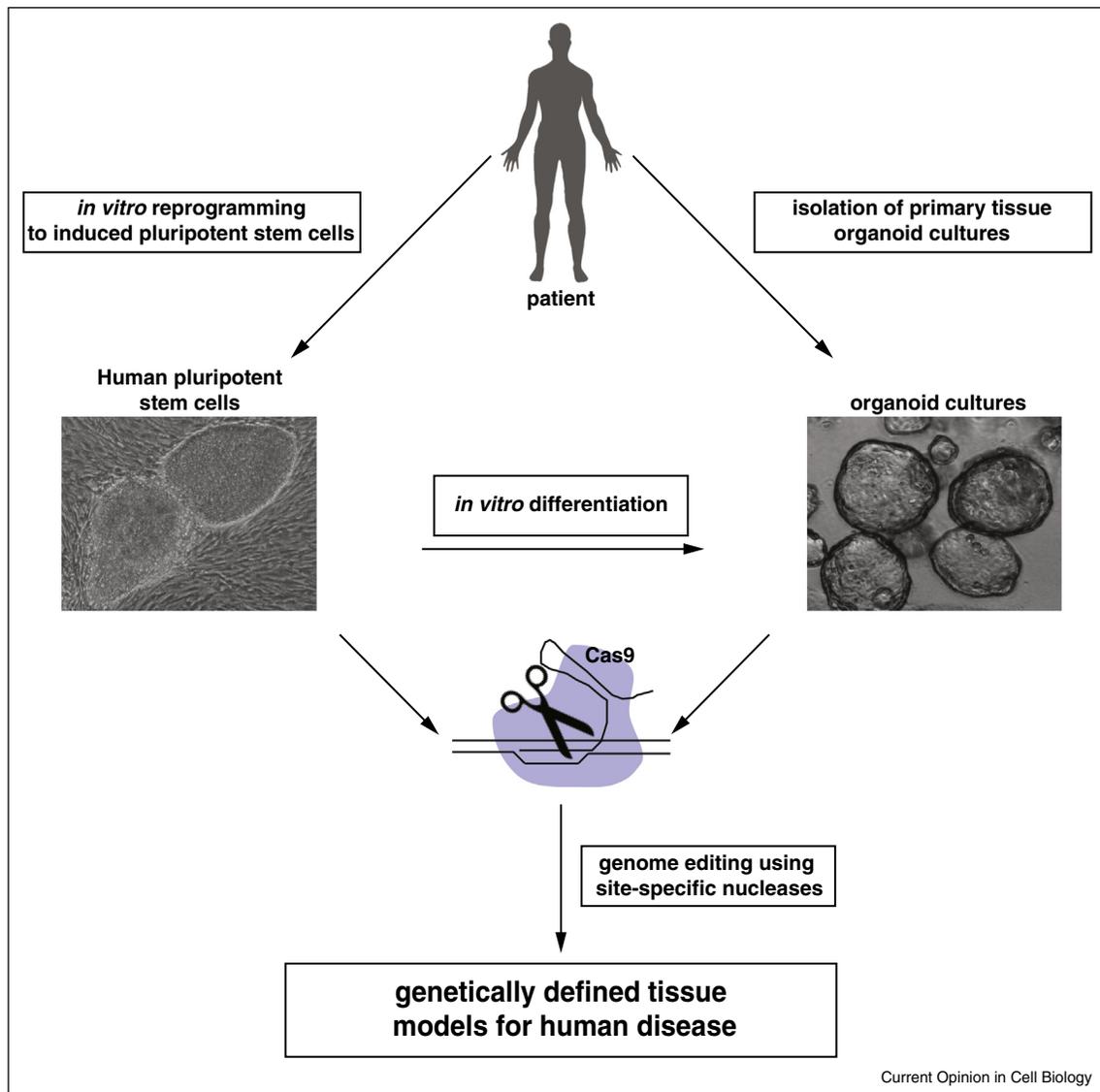
One of the most important developments in disease modeling was the generation of induced pluripotent stem cells (iPSCs) [1,2], which are functionally equivalent to embryonic stem cells (ESCs) [3–5] and are collectively referred to as pluripotent stem cells (PSCs). Cells taken from a patient with a genetic disease can be reprogrammed as human iPSCs and can subsequently be differentiated into disease-relevant cell types to uncover molecular and cellular mechanisms and to screen for drug treatment options (Figure 1).

A significant challenge in iPSC-based disease modeling lies in the fact that each disease-specific iPSC line is genetically distinct due to the genetic variability among patients [6]. As a consequence, phenotypes of iPSC disease models can show striking variability between individual patient-derived cell lines [7]. Moreover, variability can also be caused by the reprogramming process used to create the iPSCs [8,9]. This variability greatly challenges our ability to model disorders with mild or complex phenotypes. Recently, we and others have overcome this limitation by establishing the use of site-specific nucleases (reviewed in [10,11]) in hPSCs, allowing a level of genetic control previously limited to traditional model systems [12\*,13–15]. As a result, we can now perform targeted gene knock-outs, generate tissue-specific cell lineage reporters, overexpress genes from defined loci, and introduce and repair point mutations in hPSCs. This genetic amenability of hPSCs allows researchers to generate sets of isogenic cells that differ exclusively at the site of editing. Consequently, the phenotypes identified in these cells can be attributed to the disease-relevant mutation rather than the specific genetic background of a given patient.

A proof of concept for this approach in hPSCs was the genome editing-mediated correction of disease-causing mutations in  $\alpha$ -synuclein that cause a familial form of Parkinson's disease [16\*]. Comparing isogenic cortical neurons differentiated from these iPSCs identified that  $\alpha$ -synuclein mutations caused accumulation of nitrosative and endoplasmic reticulum stresses [17\*]. Furthermore, comparing these isogenic iPSCs in a similar approach showed that  $\alpha$ -synuclein disease-causing mutations predisposed iPSC-derived dopaminergic neurons to mitochondrial stresses from environmental toxins known to be associated with Parkinson's disease [18].

An elegant approach to increasing the efficiency of gene repair of disease alleles *in vitro* combines genome editing with the use of *piggyBac* transposase to correct patient-derived iPSCs for a point mutation in the  $\alpha$ (1)-antitrypsin gene, which causes  $\alpha$ (1)-antitrypsin deficiency [19]. This gene repair approach utilizes antibiotic selection of a zinc finger nuclease (ZFN)-mediated correction of the disease-causing allele using a selection cassette. Overexpression of *piggyBac* transposase can later be used to 'scarlessly excise' the selection cassette once a corrected hPSC clone is isolated and genotyped. This strategy yields efficient bi-allelic changes in patient-derived iPSCs, and restores enzymatic function of iPSC-derived and transplanted hepatocytes.

Fig. 1



Model systems to elucidate the mechanism of disease. Somatic cells derived from a patient afflicted with a genetically predisposed disease can be reprogrammed into induced pluripotent stem cells (iPSCs). These iPSCs can be differentiated into tissue-specific organoid cultures, which can also be derived from tissue samples of the patient. Modern genome editing technologies can be used in iPSCs and organoids to establish genetically defined models for disease. These model systems can be used to understand the influx of information from GWAS and then derive understanding of the epistatic relationship of genetic variants on pathology. Further work has allowed these model systems to employ the complexity of the organism in xenografts, which will facilitate the understanding of complex disease and will be important for the screening of clinically relevant drugs.

A more general translational application of genome editing that increased the versatility of iPSC-based disease modeling has been demonstrated for trisomy 21. Jiang *et al.* [20] showed that Down syndrome patient-derived iPSCs could be engineered to insert an inducible gene for the Xist lncRNA into chromosome 21. Induction of Xist in the edited iPSCs transcriptionally represses the third copy of chromosome 21 and thereby reverses cellular disease phenotypes *in vitro*.

Since these initial studies utilizing ZFNs [12\*,21\*\*] and transcription activator-like effector nucleases (TALENs) [13,22–24], the advent of the ‘Cas9 revolution’—the establishment of site specific nucleases based on the bacterial adaptive defense system CRISPR (Clustered Regularly Interspersed Short Palindromic Repeats)/Cas9 (Cas9)—has made genetic engineering of stem cells a widely available and standard tool in human disease modeling. Since the founding work by Jinek *et al.* [25\*], Cas9 has

been used to increase the feasibility of gene targeting, especially in the context of editing human cells [26–28]. The ease of Cas9-mediated genome editing and its ability to be arrayed with multiple guide RNA (gRNA) sequences [29] allow us to simultaneously interrogate several loci. This provides a platform to mechanistically understand the tremendous influx of human disease genetics identified by genome-wide association studies (GWAS). For example, we recently used this approach to elucidate the mechanism by which mutations in the promoter of the catalytic subunit of telomerase (TERT) [30,31], which are the most frequent non-coding mutations in human cancer identified by GWAS [32,33], facilitate tumorigenesis [34]. By introducing these promoter mutations and comparing otherwise isogenic cell lines, we demonstrated that hESCs were unable to silence TERT expression upon differentiation. Thus, we were able to use results of GWAS to guide our hypotheses and then use precise Cas9-mediated genome editing and differentiation protocols to elucidate the mechanism by which these mutations facilitate cancer.

Adaptations and improvements have increased the ease and scope of Cas9-mediated disease modeling in hPSCs. Cas9-mediated genome editing requires a gRNA that bears homology to the genomic locus to be modified. The target locus must have a short sequence motif called a protospacer-adjacent motif (PAM), 5'-NGG-3' in the case of Cas9 from *Streptococcus pyogenes* (spCas9), to be present 3 bases away from the cut site, limiting the number of potential cut sites in the genome. One approach for circumventing this limitation is to use CRISPR/CAS-systems from different organisms that respond to different PAM sequences [35–37]. An alternative approach is to engineer spCas9 to respond to alternative PAMs by structure-based engineering of Cas9 [38]. These orthogonal Cas9s are useful in designing editing strategies because of the mechanism of double-strand break repair in human cells [39,40]. Editing strategies that target sites closer to the intended modification are expected to significantly increase the efficiency of targeting [39,40], which is especially important in the correction or introduction of point mutations to generate isogenic cell lines.

Cas9 technologies can be used to introduce functional reporters, which when combined with phenotypes that can be robustly assayed may allow for the rapid determination of complex epistatic networks that govern disease phenotypes. To that end, Cas9 can be efficiently integrated into the AAVS1 safe harbor locus [12,13] as an inducible system in hPSCs [41] to rapidly and robustly generate triple knockout cell lines in one editing step [42]. This platform could be employed to study complex diseases and dissect complex epistasis by the simultaneous modification of several genomic loci. Additionally, Cas9 has been used in genome-wide screens with gRNA

libraries targeting multiple loci within all known protein-coding genes [43,44]. Such screening strategies are expected to become a standard tool to test for genome-wide loss-of-function phenotypes within the background of disease-modeling hPSCs.

### Genetically defined model systems of human tissues and microenvironments

Modeling complex diseases using an *in vitro* stem cell tissue culture system is dependent upon the establishment of robust differentiation protocols that permit the isolation of a large number of a discrete population of cells that can be fully differentiated into functional cells and tissue types of the adult human. While differentiation protocols exist for a limited number of cell types, the lack of protocols for many other cell types has curtailed efforts for modeling human pathologies. Furthermore, established protocols mostly focus on the isolation and purification of individual cell types rather than the derivation of a functional tissue that appropriately recapitulates the disease-relevant microenvironment. Thus, modeling the biology of human disease that manifests because of the complex interplay of different cell types in a tissue becomes a challenge. Consequently, current tissue culture models largely fail to recapitulate the milieu of changes that cells acquire over extended periods of disease progression.

One approach to overcome the challenges of monolayer cultures is to develop three-dimensional organoid cultures to study human disease. Organoids are a cellular culture system that generates multiple cell types of the corresponding organ spatially organized as they are *in vivo* and that recapitulates aspects of the tissue's function (reviewed in [45]). Such cultures can be derived from hPSCs or human tissue samples, and these model systems can be manipulated using the genome editing technologies described above. Organoid systems have so far been established to model a variety of organs such as the intestine [46], stomach [47,48], pancreas [49], mammary tissue [50], brain [51], liver [52], and lung [53]. Here we will review how these organoid technologies combined with genome editing have been used to model human disease with a focus on intestinal organoids.

### Genome editing in human organoids to elucidate intestinal biology

Intestinal biology significantly differs between mice and humans making it necessary to develop novel approaches to understand intestinal pathology in a human-derived *in vitro* context. Human intestinal organoids were first derived from adult intestinal stem cells [46], but later work showed that they could also be derived from hPSCs [54]. An alternative approach that our lab has recently developed used an intestinal stem cell marker, LGR5, labeled with GFP by genome editing [55]. Edited hESCs were injected into immune-compromised mice, allowed to

differentiate in the *in vivo* environment, and the labeled intestinal stem cell marker was used to isolate those cells that were then cultured to produce intestinal organoids in a 3D matrix.

Differentiation of hPSCs into intestinal tissue results in an immature transcriptional profile that resembles stages of early development and fetal tissue despite the ability to generate all of the major cell types of the epithelium [55,56]. Similar observations were made upon analysis of pancreatic beta cells derived from hPSCs [57] and in differentiation protocols that differentiate cells into hepatocytes [58,59]. Failure to differentiate into mature adult tissue poses a general challenge for disease modeling in hPSC-derived tissues. In the case of intestinal organoids, recent progress was made to overcome these challenges by developing xenograft transplantation protocols of growing human iPSC-derived intestinal organoids in the mouse kidney capsule [60]. Here the human cells matured and adopted a transcriptional profile more similar to that of adult tissue, suggesting that functional xenograft transplantation could be a general approach that allows for the necessary maturation and differentiation of hPSCs to model pathologies that manifest in adulthood.

An alternative to obviate the issue of ‘maturity’ in the *in vitro* systems is to use primary tissue-derived organoid cultures isolated from patients for disease modeling, as these already possess the developmental tissue properties of an adult donor. This has been done successfully with the intestine in the context of cystic fibrosis (CF) [61]. In intestinal organoid cultures isolated from CF patient samples the disease-causing allele in the CF transmembrane conductor receptor can be repaired using Cas9-mediated genome editing, restoring the function of the adult primary tissue-based intestinal organoids. Primary tissue-derived intestinal organoids [46] have also been used to understand the genetics of different stages of colorectal cancer [62,63]. The most common mutations of colorectal cancer found in *APC*, *P53*, *KRAS*, and *SMAD4* were combinatorially introduced to model the different stages of colorectal cancer. Mutations in *APC* and *P53* were sufficient to show hallmarks of tumor progression, but all four mutations allowed the tissue to show the most severe and physiologic cancer phenotype in xenografts [63]. Similar approaches might be used in the future to parse GWAS data sets for less well understood cancer mutations to determine which genes are important in the driving of disease, and which may be only passengers or important for later stages.

Addressing the challenge of modeling the tissue microenvironment involves introducing the *in vivo* complexity into a tissue culture system. Aspects of such complexity can be seen by culturing human intestinal organoids with

bacterial pathogens in order to model host–pathogen interactions. Interaction of *Clostridium difficile* [64] and *Salmonella enterica* serovar Typhimurium [65] with human intestinal organoids results in clinically relevant responses and phenotypes such as an appropriate inflammatory cytokine response [65].

Although these genetically amenable models offer much potential for understanding intestinal biology, they do not fully recapitulate the intestine, as it is a complex organ with essential links to the vasculature, enteric nervous system, immune system, and the microbiota. A potential solution is engraftment of intestinal organoids into a mammalian intestine, which would provide these missing physiological components. Initial studies already demonstrate allotransplantation of mouse organoid tissue [66], and these experiments were recently translated to the xenograftment of fetal human colon-derived organoids into the colon of mice [67]. Despite challenges of xenograftment, such as in many cases the need for immune-compromised hosts and potentially aberrant interactions of host factors with human tissue, we predict that it will become a powerful system to understand disease and the activity of putative drugs in a complex human tissue context.

## Conclusions

In summary, there have been tremendous advancements in the modeling of human disease. These include the still ongoing discovery of disease alleles from GWAS, genome editing and its implications in stem cell genetics, and the ability to induce patients’ cells to pluripotency. These advancements are of interest because the mechanistic insight gleaned from studies using these approaches can directly influence drug design and clinical strategies. Applying these tools to organoid cultures that more closely recapitulate the *in vivo* microenvironment and that ideally can functionally engraft into mammalian model organisms will greatly facilitate our ability to understand and treat human disease.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S: **Induction of pluripotent stem cells from adult human fibroblasts by defined factors.** *Cell* 2007, **131**:861–872.

2. Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.** *Cell* 2006, **126**:663-676.
  3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: **Embryonic stem cell lines derived from human blastocysts.** *Science* 1998, **282**:1145-1147.
  4. Zhao X-y, Li W, Lv Z, Liu L, Tong M, Hai T, Hao J, Guo C-I, Ma Q-w, Wang L: **iPS cells produce viable mice through tetraploid complementation.** *Nature* 2009, **461**:86-90.
  5. Kang L, Wang J, Zhang Y, Kou Z, Gao S: **iPS cells can support full-term development of tetraploid blastocyst-complemented embryos.** *Cell Stem Cell* 2009, **5**:135-138.
  6. Vitale AM, Matigian NA, Ravishankar S, Bellette B, Wood SA, Wolvetang EJ, Mackay-Sim A: **Variability in the generation of induced pluripotent stem cells: importance for disease modeling.** *Stem Cells Transl Med* 2012, **1**:641-650.
  7. Reinhardt P, Schmid B, Burbulla LF, Schondorf DC, Wagner L, Glatza M, Hoing S, Hargus G, Heck SA, Dhingra A *et al.*: **Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression.** *Cell Stem Cell* 2013, **12**:354-367.
  8. Guenther MG, Frampton GM, Soldner F, Hockemeyer D, Mitalipova M, Jaenisch R, Young RA: **Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells.** *Cell Stem Cell* 2010, **7**:249-257.
  9. Carey BW, Markoulaki S, Hanna JH, Faddah DA, Buganim Y, Kim J, Ganz K, Steine EJ, Cassady JP, Creighton MP: **Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells.** *Cell Stem Cell* 2011, **9**:588-598.
  10. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD: **Genome editing with engineered zinc finger nucleases.** *Nat Rev Genet* 2010, **11**:636-646.
  11. Carroll D: **Genome engineering with targetable nucleases.** *Annu Rev Biochem* 2014, **83**:409-439.
  12. Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, DeKaveler RC, Katibah GE, Amora R, Boydston EA, Zeitler B *et al.*: **Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases.** *Nat Biotechnol* 2009, **27**:851-857.
- This paper establishes that use of site-specific nucleases to edit human pluripotent stem cells.
13. Hockemeyer D, Wang H, Kiani S, Lai CS, Gao Q, Cassady JP, Cost GJ, Zhang L, Santiago Y, Miller JC *et al.*: **Genetic engineering of human pluripotent cells using TALE nucleases.** *Nat Biotechnol* 2011, **29**:731-734.
  14. Zou J, Maeder ML, Mali P, Pruetz-Miller SM, Thibodeau-Beganny S, Chou BK, Chen G, Ye Z, Park IH, Daley GQ *et al.*: **Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells.** *Cell Stem Cell* 2009, **5**:97-110.
  15. Sexton AN, Regalado SG, Lai CS, Cost GJ, O'Neil CM, Urnov FD, Gregory PD, Jaenisch R, Collins K, Hockemeyer D: **Genetic and molecular identification of three human TPP1 functions in telomerase action: recruitment, activation, and homeostasis set point regulation.** *Genes Dev* 2014, **28**:1885-1899.
  16. Soldner F, Laganieri J, Cheng AW, Hockemeyer D, Gao Q, Alagappan R, Khurana V, Golbe LI, Myers RH, Lindquist S *et al.*: **Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations.** *Cell* 2011, **146**:318-331.
- These studies provide the proof of concept for the generation of isogenic induced pluripotent stem cell disease models using genome editing.
17. Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzer Y, Cho S *et al.*: **Identification and rescue of alpha-synuclein toxicity in Parkinson patient-derived neurons.** *Science* 2013, **342**:983-987.
- These studies provide the proof of concept for the generation of isogenic induced pluripotent stem cell disease models using genome editing.
18. Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M *et al.*: **Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription.** *Cell* 2013, **155**:1351-1364.
  19. Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu PQ, Paschon DE, Miranda E, Ordonez A, Hannan NR, Rouhani FJ *et al.*: **Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells.** *Nature* 2011, **478**:391-394.
  20. Jiang J, Jing Y, Cost GJ, Chiang JC, Kolpa HJ, Cotton AM, Carone DM, Carone BR, Shivak DA, Guschin DY *et al.*: **Translating dosage compensation to trisomy 21.** *Nature* 2013, **500**:296-300.
  21. Urnov FD, Miller JC, Lee Y-L, Beausejour CM, Rock JM, Augustus S, Jamieson AC, Porteus MH, Gregory PD, Holmes MC: **Highly efficient endogenous human gene correction using designed zinc-finger nucleases.** *Nature* 2005, **435**:646-651.
- This is a milestone paper that provides proof of concept for using genome editing, specifically ZFNs, in order to correct a disease allele at the endogenous locus in a human cell context.
22. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ: **A TALE nuclease architecture for efficient genome editing.** *Nat Biotechnol* 2011, **29**:143-148.
  23. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U: **Breaking the code of DNA binding specificity of TAL-type III effectors.** *Science* 2009, **326**:1509-1512.
  24. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF: **Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting.** *Nucleic Acids Res* 2011:gkr218.
  25. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E: **A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.** *Science* 2012, **337**:816-821.
- This study allowed the use of site-specific nucleases for genome editing to be much more financially feasible relative to the technologies of zinc finger nucleases and transcription-like effector nucleases, allowing Cas9 to effectively replace these technologies for many applications.
26. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J: **RNA-programmed genome editing in human cells.** *Elife* 2013, **2**:e00471.
  27. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM: **RNA-guided human genome engineering via Cas9.** *Science* 2013, **339**:823-826.
  28. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F: **Genome engineering using the CRISPR-Cas9 system.** *Nat Protoc* 2013, **8**:2281-2308.
  29. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA *et al.*: **Multiplex genome engineering using CRISPR/Cas systems.** *Science* 2013, **339**:819-823.
  30. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K *et al.*: **TERT promoter mutations in familial and sporadic melanoma.** *Science* 2013, **339**:959-961.
  31. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA: **Highly recurrent TERT promoter mutations in human melanoma.** *Science* 2013, **339**:957-959.
  32. Fredriksson NJ, Ny L, Nilsson JA, Larsson E: **Systematic analysis of noncoding somatic mutations and gene expression alterations across 14 tumor types.** *Nat Genet* 2014, **46**:1258-1263.
  33. Weinhold N, Jacobsen A, Schultz N, Sander C, Lee W: **Genome-wide analysis of noncoding regulatory mutations in cancer.** *Nat Genet* 2014, **46**:1160-1165.

34. Chiba K, Johnson JZ, Vogan JM, Wagner T, Boyle JM, Hockemeyer D: **Cancer-associated TERT promoter mutations abrogate telomerase silencing.** *Elife* 2015:4.  
This paper elucidates the mechanism of the most common non-coding mutations in cancer, as described by GWAS, by comparing Cas9-edited isogenic pluripotent stem cells.
35. Hou Z, Zhang Y, Propson NE, Howden SE, Chu LF, Sontheimer EJ, Thomson JA: **Efficient genome engineering in human pluripotent stem cells using Cas9 from *Neisseria meningitidis*.** *Proc Natl Acad Sci U S A* 2013, **110**:15644-15649.
36. Esvelt KM, Mali P, Braff JL, Moosburner M, Yaung SJ, Church GM: **Orthogonal Cas9 proteins for RNA-guided gene regulation and editing.** *Nat Methods* 2013, **10**:1116-1121.
37. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A: **Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system.** *Cell* 2015, **163**:759-771.
38. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR *et al.*: **Engineered CRISPR-Cas9 nucleases with altered PAM specificities.** *Nature* 2015, **523**:481-485.
39. Kan Y, Ruis B, Lin S, Hendrickson EA: **The mechanism of gene targeting in human somatic cells.** *PLoS Genet* 2014, **10**:e1004251.
40. Elliott B, Richardson C, Winderbaum J, Nickoloff JA, Jasin M: **Gene conversion tracts from double-strand break repair in mammalian cells.** *Mol Cell Biol* 1998, **18**:93-101.  
Elliott *et al.* increased our understanding of the basic biology of double-strand break repair in mammalian cells, which greatly informed subsequent efforts to perform genome editing in these cells.
41. DeKolver RC, Choi VM, Moehle EA, Paschon DE, Hockemeyer D, Meijising SH, Sancak Y, Cui X, Steine EJ, Miller JC *et al.*: **Functional genomics, proteomics, and regulatory DNA analysis in isogenic settings using zinc finger nuclease-driven transgenesis into a safe harbor locus in the human genome.** *Genome Res* 2010, **20**:1133-1142.
42. Gonzalez F, Zhu Z, Shi ZD, Lelli K, Verma N, Li QV, Huangfu D: **An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells.** *Cell Stem Cell* 2014, **15**:215-226.
43. Shalem O, Sanjana NE, Zhang F: **High-throughput functional genomics using CRISPR-Cas9.** *Nat Rev Genet* 2015, **16**:299-311.
44. Wang T, Wei JJ, Sabatini DM, Lander ES: **Genetic screens in human cells using the CRISPR-Cas9 system.** *Science* 2014, **343**:80-84.
45. Lancaster MA, Knoblich JA: **Organogenesis in a dish: modeling development and disease using organoid technologies.** *Science* 2014, **345**:1247125.
46. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ *et al.*: **Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche.** *Nature* 2009, **459**:262-265.  
This study demonstrates that *in vitro* intestinal organoid cultures can be established from genetically marked mouse cells.
47. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M *et al.*: **Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro.** *Cell Stem Cell* 2010, **6**:25-36.
48. McCracken KW, Cata EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, Tsai YH, Mayhew CN, Spence JR, Zavros Y *et al.*: **Modelling human development and disease in pluripotent stem-cell-derived gastric organoids.** *Nature* 2014, **516**:400-404.
49. Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V, Jager M, Ponz-Sarvisé M, Tiriach H, Spector MS *et al.*: **Organoid models of human and mouse ductal pancreatic cancer.** *Cell* 2015, **160**:324-338.
50. Liao MJ, Zhang CC, Zhou B, Zimonjic DB, Mani SA, Kaba M, Gifford A, Reinhardt F, Popescu NC, Guo W *et al.*: **Enrichment of a population of mammary gland cells that form mammospheres and have in vivo repopulating activity.** *Cancer Res* 2007, **67**:8131-8138.
51. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurler ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA: **Cerebral organoids model human brain development and microcephaly.** *Nature* 2013, **501**:373-379.
52. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ *et al.*: **In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration.** *Nature* 2013, **494**:247-250.
53. Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, Wells JM, Mayhew CN, Nattiv R, Klein OD *et al.*: **In vitro generation of human pluripotent stem cell derived lung organoids.** *Elife* 2015:4.
54. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM *et al.*: **Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro.** *Nature* 2011, **470**:105-109.  
This study establishes protocols to differentiate hPSCs into intestinal organoids.
55. Forster R, Chiba K, Schaeffer L, Regalado SG, Lai CS, Gao Q, Kiani S, Farin HF, Clevers H, Cost GJ *et al.*: **Human intestinal tissue with adult stem cell properties derived from pluripotent stem cells.** *Stem Cell Rep* 2014, **2**:838-852.
56. Finkbeiner SR, Hill DR, Altheim CH, Dedhia PH, Taylor MJ, Tsai YH, Chin AM, Mahe MM, Watson CL, Freeman JJ *et al.*: **Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo.** *Stem Cell Rep* 2015, **4**:1140-1155.
57. Hrvatin S, O'Donnell CW, Deng F, Millman JR, Pagliuca FW, Dilorio P, Reznana A, Gifford DK, Melton DA: **Differentiated human stem cells resemble fetal, not adult, beta cells.** *Proc Natl Acad Sci U S A* 2014, **111**:3038-3043.
58. Zhu S, Rezvani M, Harbell J, Mattis AN, Wolfe AR, Benet LZ, Willenbring H, Ding S: **Mouse liver repopulation with hepatocytes generated from human fibroblasts.** *Nature* 2014, **508**:93-97.
59. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N *et al.*: **Vascularized and functional human liver from an iPSC-derived organ bud transplant.** *Nature* 2013, **499**:481-484.
60. Watson CL, Mahe MM, Munera J, Howell JC, Sundaram N, Poling HM, Schweitzer JI, Vallance JE, Mayhew CN, Sun Y *et al.*: **An in vivo model of human small intestine using pluripotent stem cells.** *Nat Med* 2014, **20**:1310-1314.
61. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK *et al.*: **Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients.** *Cell Stem Cell* 2013, **13**:653-658.  
This report demonstrates the use of genome editing to correct the CF disease-causing allele in organoids isolated from primary tissue of CF patients.
62. Drost J, van Jaarsveld RH, Ponsioen B, Zimmerlin C, van Boxtel R, Buijs A, Sachs N, Overmeer RM, Offerhaus GJ, Begthel H *et al.*: **Sequential cancer mutations in cultured human intestinal stem cells.** *Nature* 2015, **521**:43-47.
63. Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y, Watanabe T, Kanai T, Sato T: **Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids.** *Nat Med* 2015, **21**:256-262.
64. Leslie JL, Huang S, Opp JS, Nagy MS, Kobayashi M, Young VB, Spence JR: **Persistence and toxin production by *Clostridium difficile* within human intestinal organoids result in disruption of epithelial paracellular barrier function.** *Infect Immun* 2015, **83**:138-145.

65. Forbester JL, Goulding D, Vallier L, Hannan N, Hale C, Pickard D, Mukhopadhyay S, Dougan G: **Interaction of *Salmonella enterica* serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells.** *Infect Immun* 2015, **83**:2926-2934.
66. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, Ichinose S, Nagaishi T, Okamoto R, Tsuchiya K *et al.*: **Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell.** *Nat Med* 2012, **18**:618-623.
67. Fordham RP, Yui S, Hannan NR, Soendergaard C, Madgwick A, Schweiger PJ, Nielsen OH, Vallier L, Pedersen RA, Nakamura T *et al.*: **Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury.** *Cell Stem Cell* 2013, **13**:734-744.