CRISPR and Stem Cell Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of prokaryotic DNA containing short, repetitive base sequences. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA. Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences. CRISPR-Cas9 is a new powerful technique for the gene editing target. This article introduces recent research reports as references in the related studies.
[Ma H, Young M, Zhu Y, Yang Y, Zhu H. **CRISPR and Stem Cell Research Literatures.** *Stem Cell* 2016;7(4):64-116]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 13. doi:10.7537/marsscj070416.13

Key words: stem cell; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of prokaryotic DNA containing short, repetitive base sequences. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA. Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences. CRISPR-Cas9 is a new powerful technique for the gene editing target. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Alapati, D. and E. E. Morrisey "Gene Editing and Genetic Lung Disease: Basic Research Meets Therapeutic Application." Am J Respir Cell Mol Biol. 2016 Oct 25.

CRISPR/Cas9 gene editing technology is a promising approach to repair or inactivate disease causing mutations. Recent reports have provided proof of concept by using CRISPR/Cas9 to successfully repair or inactivate mutations in animal models of monogenic human diseases. Potential pulmonary applications of CRISPR/Cas9 gene editing include gene correction of monogenic diseases in pre- or postnatal lungs and ex vivo gene editing of patient specific airway stem cells followed by autologous cell transplant. Strategies to enhance gene editing efficiency and eliminate off target effects by targeting pulmonary stem/progenitor cells and assessment of short term and long term effects of gene editing are important considerations as the field advances. If methods continue to advance rapidly, CRISPR/Cas9 mediated gene editing may provide a novel opportunity to correct monogenic diseases of the respiratory system.

Amabile, A., A. Migliara, et al. "Inheritable Silencing of Endogenous Genes by Hit-and-Run Targeted Epigenetic Editing." Cell. 2016 Sep 22;167(1):219-232.e14. doi: 10.1016/j.cell.2016.09.006.

Gene silencing is instrumental to interrogate gene function and holds promise for therapeutic applications. Here, we repurpose the endogenous

retroviruses' silencing machinery of embryonic stem cells to stably silence three highly expressed genes in somatic cells by epigenetics. This was achieved by transiently expressing combinations of engineered transcriptional repressors that bind to and synergize at the target locus to instruct repressive histone marks and de novo DNA methylation, thus ensuring long-term memory of the repressive epigenetic state. Silencing was highly specific, as shown by genome-wide analyses, sharply confined to the targeted locus without spreading to nearby genes, resistant to activation induced by cytokine stimulation, and relieved only by targeted DNA demethylation. We demonstrate the portability of this technology by multiplex gene silencing, adopting different DNA binding platforms and interrogating thousands of genomic loci in different cell types, including primary T lymphocytes. Targeted epigenome editing might have broad application in research and medicine.

Andrey, G. and M. Spielmann "CRISPR/Cas9 Genome Editing in Embryonic Stem Cells." Methods Mol Biol. 2017;1468:221-34. doi: 10.1007/978-1-4939-4035-6_15.

Targeted mutagenesis is required to evaluate the function of DNA segments across the genome. In recent years the CRISPR/Cas9 technology has been widely used for functional genome studies and is partially replacing classical homologous recombination methods in different aspects. CRISPR/Cas9-derived tools indeed allow the production of a wide-range of engineered mutations: from point mutations to large chromosomal rearrangements such as deletions, duplications and inversions. Here we present a protocol to engineer Embryonic Stem Cells (ESC) with desired mutations using transfection of custom-made CRISPR/Cas9 vectors. These methods allow the in vivo modeling of congenital mutations and the functional interrogation of DNA sequences.

Arbab, M. and R. I. Sherwood "Self-Cloning CRISPR." Curr Protoc Stem Cell Biol. 2016 Aug 17;38:5B.5.1-5B.5.16. doi: 10.1002/cpsc.14.

CRISPR/Cas9-gene editing has emerged as a revolutionary technology to easily modify specific genomic loci by designing complementary sgRNA sequences and introducing these into cells along with Cas9. Self-cloning CRISPR/Cas9 (scCRISPR) uses a self-cleaving palindromic sgRNA plasmid (sgPal) that recombines with short PCR-amplified site-specific sgRNA sequences within the target cell by homologous recombination to circumvent the process of sgRNA plasmid construction. Through this mechanism, scCRISPR enables gene editing within 2 hr once sgRNA oligos are available, with high efficiency equivalent to conventional sgRNA targeting: >90% gene knockout in both mouse and human embryonic stem cells and cancer cell lines. Furthermore, using PCR-based addition of short homology arms, we achieve efficient site-specific knock-in of transgenes such as GFP without traditional plasmid cloning or genome-integrated selection cassette (2% to 4% knock-in rate). The methods in this paper describe the most rapid and efficient means of CRISPR gene editing. (c) 2016 by John Wiley & Sons, Inc.

Arya, D., S. P. Sachithanandan, et al. "MiRNA182 regulates percentage of myeloid and erythroid cells in chronic myeloid leukemia." Cell Death Dis. 2017 Jan 12;8(1):e2547. doi: 10.1038/cddis.2016.471.

The deregulation of lineage control programs is often associated with the progression of haematological malignancies. The molecular regulators of lineage choices in the context of tyrosine kinase inhibitor (TKI) resistance remain poorly understood in chronic myeloid leukemia (CML). To find a potential molecular regulator contributing to lineage distribution and TKI resistance, we undertook an RNA-sequencing approach for identifying microRNAs (miRNAs). Following an unbiased screen, elevated miRNA182-5p levels were detected in Bcr-Abl-inhibited K562 cells (CML blast crisis cell line) and in a panel of CML patients. Earlier, miRNA182-5p upregulation was reported in several solid tumours and haematological malignancies. We undertook a strategy involving transient modulation and CRISPR/Cas9 (clustered regularly interspersed short palindromic repeats)-mediated knockout of the MIR182 locus in CML cells. The lineage contribution was assessed by methylcellulose colony formation assay. The transient modulation of miRNA182-5p revealed a biased phenotype. Strikingly, Delta182 cells (homozygous deletion of MIR182 locus) produced a marked shift in lineage distribution. The phenotype was rescued by ectopic expression of miRNA182-5p in Delta182 cells. A bioinformatic analysis and Hes1 modulation data suggested that Hes1 could be a putative target of miRNA182-5p. A reciprocal relationship between miRNA182-5p and Hes1 was seen in the context of TK inhibition. In conclusion, we reveal a key role for miRNA182-5p in restricting the myeloid development of leukemic cells. We propose that the Delta182 cell line will be valuable in designing experiments for next-generation pharmacological interventions.

Avellino, R., M. Havermans, et al. "An autonomous CEBPA enhancer specific for myeloid-lineage priming and neutrophilic differentiation." Blood. 2016 Jun 16;127(24):2991-3003. doi: 10.1182/blood-2016-01-695759. Epub 2016 Mar 10.

Neutrophilic differentiation is dependent on CCAAT enhancer-binding protein alpha (C/EBPalpha), a transcription factor expressed in multiple organs including the bone marrow. Using functional genomic technologies in combination with clustered regularly-interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 genome editing and in vivo mouse modeling, we show that CEBPA is located in a 170-kb topological-associated domain that contains 14 potential enhancers. Of these, 1 enhancer located +42 kb from CEBPA is active and engages with the CEBPA promoter in myeloid cells only. Germ line deletion of the homologous enhancer in mice in vivo reduces Cebpa levels exclusively in hematopoietic stem cells (HSCs) and myeloid-primed progenitor cells leading to severe defects in the granulocytic lineage, without affecting any other Cebpa-expressing organ studied. The enhancer-deleted progenitor cells lose their myeloid transcription program and are blocked in differentiation. Deletion of the enhancer also causes loss of HSC maintenance. We conclude that a single +42-kb enhancer is essential for CEBPA expression in myeloid cells only.

Bai, M., Y. Wu, et al. "Generation and application of mammalian haploid embryonic stem cells." J Intern Med. 2016 Sep;280(3):236-45. doi: 10.1111/joim.12503. Epub 2016 May 3.

Haploid cells contain one set of chromosomes and are amenable for genetic analyses. In mammals, haploidy exists only in gametes. An intriguing question is whether haploid cells can be derived from gametes. Recently, by application of haploid cell enrichment using fluorescence-activated cell sorting, stable haploid embryonic stem cells (haESCs) have been successfully derived from oocyte-derived parthenogenetic and sperm-derived androgenetic embryos from several species. Whilst both parthenogenetic and androgenetic (AG)-haESCs enable whole-genome genetic screening at the cellular level, such as screening of drug resistance or disease-related genes, AG-haESCs, after intracytoplasmic injection into oocytes, can also be used to produce alive semi-cloned mice. Nevertheless, one major drawback associated with wild-type AG-haESCs is the very low birth rate of healthy semi-cloned mice. Of interest, after inhibiting the expression of two paternally imprinted genes (H19 and Gtl2) in AG-haESCs by removal of their differentially DNA methylated regions, double-knockout AG-haESCs can efficiently and stably support the generation of healthy semi-cloned pups. Importantly, double-knockout AG-haESCs are feasible for multiple genetic manipulations, followed by efficient generation of semi-cloned mice carrying multiple genetic traits; thus they could be used to validate candidate loci that have been identified in genome-wide association studies of multigenic diseases by generation of mouse models carrying multiple alterations. Of note, by combining a CRISPR-Cas9 library and double-knockout AG-haESCs, semi-cloned mice carrying different mutant genes can be efficiently generated in one step, enabling functional mutagenic screening in mice. HaESCs, therefore, provide a powerful tool for genetic analyses in mammals at both the cellular and organismal levels.

Basiri, M., M. Behmanesh, et al. "The Convenience of Single Homology Arm Donor DNA and CRISPR/Cas9-Nickase for Targeted Insertion of Long DNA Fragment." Cell J. 2017 Winter;18(4):532-539. Epub 2016 Sep 26.

OBJECTIVE: CRISPR/Cas9 technology provides a powerful tool for targeted modification of genomes. In this system, a donor DNA harboring two flanking homology arms is mostly used for targeted insertion of long exogenous DNA. Here, we introduced an alternative design for the donor DNA by incorporation of a single short homology arm into a circular plasmid. MATERIALS AND METHODS: In this experimental study, single homology arm donor was applied along with a single guide RNA (sgRNA) specific to the homology region, and either Cas9 or its mutant nickase variant (Cas9n). Using Pdx1 gene as the target locus the functionality of this system was evaluated in MIN6 cell line and murine embryonic stem cells (ESCs). RESULTS: Both wild type Cas9 and Cas9n could conduct the knock-in process with this system. We successfully applied this strategy with Cas9n for generation of Pdx1GFP knock-in mouse ESC lines. Altogether, our results demonstrated that a combination of a single homology arm donor, a single guide RNA and Cas9n is capable of precisely incorporating DNA fragments of multiple kilo base pairs into the targeted genomic locus. CONCLUSION: While taking advantage of low off-target mutagenesis of the Cas9n, our new design strategy may facilitate the targeting process. Consequently, this strategy can be applied in knock-in or insertional inactivation studies.

Bassuk, A. G., A. Zheng, et al. "Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells." Sci Rep. 2016 Jan 27;6:19969. doi: 10.1038/srep19969.

Induced pluripotent stem cells (iPSCs) generated from patient fibroblasts could potentially be used as a source of autologous cells for transplantation in retinal disease. Patient-derived iPSCs, however, would still harbor disease-causing mutations. To generate healthy patient-derived cells, mutations might be repaired with new gene-editing technology based

on the bacterial system of clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9, thereby yielding grafts that require no patient immunosuppression. We tested whether CRISPR/Cas9 could be used in patient-specific iPSCs to precisely repair an RPGR point mutation that causes X-linked retinitis pigmentosa (XLRP). Fibroblasts cultured from a skin-punch biopsy of an XLRP patient were transduced to produce iPSCs carrying the patient's c.3070G > T mutation. The iPSCs were transduced with CRISPR guide RNAs, Cas9 endonuclease, and a donor homology template. Despite the gene's repetitive and GC-rich sequences, 13% of RPGR gene copies showed mutation correction and conversion to the wild-type allele. This is the first report using CRISPR to correct a pathogenic mutation in iPSCs derived from a patient with photoreceptor degeneration. This important proof-of-concept finding supports the development of personalized iPSC-based transplantation therapies for retinal disease.

Beer, N. L. and A. L. Gloyn "Genome-edited human stem cell-derived beta cells: a powerful tool for drilling down on type 2 diabetes GWAS biology." F1000Res. 2016 Jul 15;5. pii: F1000 Faculty Rev-1711. doi: 10.12688/f1000research.8682.1. eCollection 2016.

Type 2 diabetes (T2D) is a disease of pandemic proportions, one defined by a complex aetiological mix of genetic, epigenetic, environmental, and lifestyle risk factors. Whilst the last decade of T2D genetic research has identified more than 100 loci showing strong statistical association with disease susceptibility, our inability to capitalise upon these signals reflects, in part, a lack of appropriate human cell models for study. This review discusses the impact of two complementary, state-of-the-art technologies on T2D genetic research: the generation of stem cell-derived, endocrine pancreas-lineage cells and the editing of their genomes. Such models facilitate investigation of diabetes-associated genomic perturbations in a physiologically representative cell context and allow the role of both developmental and adult islet dysfunction in T2D pathogenesis to be investigated. Accordingly, we interrogate the role that patient-derived induced pluripotent stem cell models are playing in understanding cellular dysfunction in monogenic diabetes, and how site-specific nucleases such as the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system are helping to confirm genes crucial to human endocrine pancreas development. We also highlight the novel biology gleaned in the absence of patient lines, including an ability to model the whole phenotypic spectrum of diabetes phenotypes occurring both in utero and in adult cells, interrogating the non-coding 'islet regulome' for disease-causing perturbations, and understanding the role of other islet cell types in aberrant glycaemia. This article aims to reinforce the importance of investigating T2D signals in cell models reflecting appropriate species, genomic context, developmental time point, and tissue type.

Berger, R. P., Y. H. Sun, et al. "ST8SIA4-Dependent Polysialylation is Part of a Developmental Program Required for Germ Layer Formation from Human Pluripotent Stem Cells." Stem Cells. 2016 Jul;34(7):1742-52. doi: 10.1002/stem.2379. Epub 2016 May 3.

Polysialic acid (PSA) is a carbohydrate polymer of repeating alpha-2,8 sialic acid residues that decorates multiple targets, including neural cell adhesion molecule (NCAM). PST and STX encode the two enzymes responsible for PSA modification of target proteins in mammalian cells, but despite widespread polysialylation in embryonic development, the majority of studies have focused strictly on the role of PSA in neurogenesis. Using human pluripotent stem cells (hPSCs), we have revisited the developmental role of PST and STX and show that early progenitors of the three embryonic germ layers are polysialylated on their cell surface. Changes in polysialylation can be attributed to lineage-specific expression of polysialyltransferase genes; PST is elevated in endoderm and mesoderm, while STX is elevated in ectoderm. In hPSCs, PST and STX genes are epigenetically marked by overlapping domains of H3K27 and H3K4 trimethylation, indicating that they are held in a "developmentally-primed" state. Activation of PST transcription during early mesendoderm differentiation is under control of the T-Goosecoid transcription factor network, a key regulatory axis required for early cell fate decisions in the vertebrate embryo. This establishes polysialyltransferase genes as part of a developmental program associated with germ layer establishment. Finally, we show by shRNA knockdown and CRISPR-Cas9 genome editing that PST-dependent cell surface polysialylation is essential for endoderm specification. This is the first report to demonstrate a role for a glycosyltransferase in hPSC lineage specification. Stem Cells 2016;34:1742-1752.

Bertero, A., M. Pawlowski, et al. "Optimized inducible shRNA and CRISPR/Cas9 platforms for in vitro studies of human development using hPSCs." Development. 2016 Dec 1;143(23):4405-4418.

Inducible loss of gene function experiments are necessary to uncover mechanisms underlying development, physiology and disease. However, current methods are complex, lack robustness and do not work in multiple cell types. Here we address these limitations by developing single-step optimized

inducible gene knockdown or knockout (sOPTiKD or sOPTiKO) platforms. These are based on genetic engineering of human genomic safe harbors combined with an improved tetracycline-inducible system and CRISPR/Cas9 technology. We exemplify the efficacy of these methods in human pluripotent stem cells (hPSCs), and show that generation of sOPTiKD/KO hPSCs is simple, rapid and allows tightly controlled individual or multiplexed gene knockdown or knockout in hPSCs and in a wide variety of differentiated cells. Finally, we illustrate the general applicability of this approach by investigating the function of transcription factors (OCT4 and T), cell cycle regulators (cyclin D family members) and epigenetic modifiers (DPY30). Overall, sOPTiKD and sOPTiKO provide a unique opportunity for functional analyses in multiple cell types relevant for the study of human development.

Blair, J. D., H. S. Bateup, et al. "Establishment of Genome-edited Human Pluripotent Stem Cell Lines: From Targeting to Isolation." J Vis Exp. 2016 Feb 2;(108):e53583. doi: 10.3791/53583.

Genome-editing of human pluripotent stem cells (hPSCs) provides a genetically controlled and clinically relevant platform from which to understand human development and investigate the pathophysiology of disease. By employing site-specific nucleases (SSNs) for genome editing, the rapid derivation of new hPSC lines harboring specific genetic alterations in an otherwise isogenic setting becomes possible. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 are the most commonly used SSNs. All of these nucleases function by introducing a double stranded DNA break at a specified site, thereby promoting precise gene editing at a genomic locus. SSN-meditated genome editing exploits two of the cell's endogenous DNA repair mechanisms, non-homologous end joining (NHEJ) and homology directed repair (HDR), to either introduce insertion/deletion mutations or alter the genome using a homologous repair template at the site of the double stranded break. Electroporation of hPSCs is an efficient means of transfecting SSNs and repair templates that incorporate transgenes such as fluorescent reporters and antibiotic resistance cassettes. After electroporation, it is possible to isolate only those hPSCs that incorporated the repair construct by selecting for antibiotic resistance. Mechanically separating hPSC colonies and confirming proper integration at the target site through genotyping allows for the isolation of correctly targeted and genetically homogeneous cell lines. The validity of this protocol is demonstrated here by using all three SSN platforms to incorporate EGFP and a puromycin resistance construct into the AAVS1 safe harbor locus in human pluripotent stem cells.

Bonthron, D. T. and W. D. Foulkes "Genetics meets pathology - an increasingly important relationship." J Pathol. 2017 Jan;241(2):119-122. doi: 10.1002/path.4849.

The analytical power of modern methods for DNA analysis has outstripped our capability to interpret and understand the data generated. To make good use of this genomic data in a biomedical setting (whether for research or diagnosis), it is vital that we understand the mechanisms through which mutations affect biochemical pathways and physiological systems. This lies at the centre of what genetics is all about, and it is the reason why genetics and genomics should go hand in hand whenever possible. In this Annual Review Issue of The Journal of Pathology, we have assembled a collection of 16 expert reviews covering a wide range of topics. Through these, we illustrate the power of genetic analysis to improve our understanding of normal physiology and disease pathology, and thereby to think in rational ways about clinical management. Copyright (c) 2016 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Booth, C., H. B. Gaspar, et al. "Treating Immunodeficiency through HSC Gene Therapy." Trends Mol Med. 2016 Apr;22(4):317-27. doi: 10.1016/j.molmed.2016.02.002. Epub 2016 Mar 15.

Haematopoietic stem cell (HSC) gene therapy has been successfully employed as a therapeutic option to treat specific inherited immune deficiencies, including severe combined immune deficiencies (SCID) over the past two decades. Initial clinical trials using first-generation gamma-retroviral vectors to transfer corrective DNA demonstrated clinical benefit for patients, but were associated with leukemogenesis in a number of cases. Safer vectors have since been developed, affording comparable efficacy with an improved biosafety profile. These vectors are now in Phase I/II clinical trials for a number of immune disorders with more preclinical studies underway. Targeted gene editing allowing precise DNA correction via platforms such as ZFNs, TALENs and CRISPR/Cas9 may now offer promising strategies to improve the safety and efficacy of gene therapy in the future.

Brunger, J. M., A. Zutshi, et al. "CRISPR/Cas9 editing of induced pluripotent stem cells for engineering inflammation-resistant tissues." Arthritis Rheumatol. 2016 Nov 3. doi: 10.1002/art.39982.

OBJECTIVE: Pro-inflammatory cytokines

such as interleukin 1 (IL-1) are elevated in diseased or injured tissues and promote rapid tissue degradation while preventing stem cell differentiation. The goals of this study were to engineer inflammation-resistant induced pluripotent stem cells (iPSCs) through deletion of the IL-1 signaling pathway and to demonstrate the utility of these cells for engineering replacements for diseased or damaged tissues. METHODS: Targeted deletion of the interleukin 1 receptor 1 (Il1r1) gene in murine iPSCs was achieved using the RNA-guided, site-specific CRISPR/Cas9 genome engineering system. Clonal cell populations with homozygous and heterozygous deletions were isolated, and loss of receptor expression and cytokine signaling was confirmed by flow cytometry and transcriptional reporter assays, respectively. Cartilage was engineered from edited iPSCs and tested for its ability to resist IL-1-mediated degradation in gene expression, histological, and biomechanical assays after a three day treatment with 1 ng/ml IL-1alpha. RESULTS: Three of 41 clones isolated possessed the Il1r1+/- genotype. Four clones possessed the Il1r1-/- genotype, and flow cytometry confirmed loss of Il1r1 on the surface of these cells and led to an absence of NF-kappaB transcriptional activation after IL-1alpha treatment. Cartilage engineered from homozygous null clones was resistant to cytokine-mediated tissue degradation. By contrast, cartilage derived from wild-type and heterozygous clones exhibited significant degradative responses, highlighting the need for complete IL-1 blockade. CONCLUSION: This work demonstrates proof-of-concept of the ability to engineer custom-designed stem cells that are immune to pro-inflammatory cytokines (i.e., IL-1) as a potential cell source for cartilage tissue engineering. This article is protected by copyright. All rights reserved.

Chen, J., T. Cai, et al. "MicroRNA-202 maintains spermatogonial stem cells by inhibiting cell cycle regulators and RNA binding proteins." Nucleic Acids Res. 2016 Dec 19. pii: gkw1287. doi: 10.1093/nar/gkw1287.

miRNAs play important roles during mammalian spermatogenesis. However, the function of most miRNAs in spermatogenesis and the underlying mechanisms remain unknown. Here, we report that miR-202 is highly expressed in mouse spermatogonial stem cells (SSCs), and is oppositely regulated by Glial cell-Derived Neurotrophic Factor (GDNF) and retinoic acid (RA), two key factors for SSC self-renewal and differentiation. We used inducible CRISPR-Cas9 to knockout miR-202 in cultured SSCs, and found that the knockout SSCs initiated premature differentiation accompanied by reduced stem cell activity and increased mitosis and apoptosis. Target genes were identified with iTRAQ-based proteomic analysis and RNA sequencing, and are enriched with cell cycle regulators and RNA-binding proteins. Rbfox2 and Cpeb1 were found to be direct targets of miR-202 and Rbfox2 but not Cpeb1, is essential for the differentiation of SSCs into meiotic cells. Accordingly, an SSC fate-regulatory network composed of signaling molecules of GDNF and RA, miR-202 and diverse downstream effectors has been identified.

Cheung, M. B., V. Sampayo-Escobar, et al. "Respiratory Syncytial Virus-Infected Mesenchymal Stem Cells Regulate Immunity via Interferon Beta and Indoleamine-2,3-Dioxygenase." PLoS One. 2016 Oct 3;11(10):e0163709. doi: 10.1371/journal.pone.0163709. eCollection 2016.

Respiratory syncytial virus (RSV) has been reported to infect human mesenchymal stem cells (MSCs) but the consequences are poorly understood. MSCs are present in nearly every organ including the nasal mucosa and the lung and play a role in regulating immune responses and mediating tissue repair. We sought to determine whether RSV infection of MSCs enhances their immune regulatory functions and contributes to RSV-associated lung disease. RSV was shown to replicate in human MSCs by fluorescence microscopy, plaque assay, and expression of RSV transcripts. RSV-infected MSCs showed differentially altered expression of cytokines and chemokines such as IL-1beta, IL6, IL-8 and SDF-1 compared to epithelial cells. Notably, RSV-infected MSCs exhibited significantly increased expression of IFN-beta (~100-fold) and indoleamine-2,3-dioxygenase (IDO) (~70-fold) than in mock-infected MSCs. IDO was identified in cytosolic protein of infected cells by Western blots and enzymatic activity was detected by tryptophan catabolism assay. Treatment of PBMCs with culture supernatants from RSV-infected MSCs reduced their proliferation in a dose dependent manner. This effect on PBMC activation was reversed by treatment of MSCs with the IDO inhibitors 1-methyltryptophan and vitamin K3 during RSV infection, a result we confirmed by CRISPR/Cas9-mediated knockout of IDO in MSCs. Neutralizing IFN-beta prevented IDO expression and activity. Treatment of MSCs with an endosomal TLR inhibitor, as well as a specific inhibitor of the TLR3/dsRNA complex, prevented IFN-beta and IDO expression. Together, these results suggest that RSV infection of MSCs alters their immune regulatory function by upregulating IFN-beta and IDO, affecting immune cell proliferation, which may account for the lack of protective RSV immunity and for chronicity of RSV-associated lung diseases such as asthma and COPD.

Choi, H., J. Song, et al. "Modeling of Autism Using Organoid Technology." Mol Neurobiol. 2016 Nov 14.

Autism is a neurodevelopmental disease caused by multiple mutations during development. However, a suitable disease model to study the molecular pathway of disease onset and progression is not available. Although many studies have used human stem cells such as induced pluripotent stem cells and embryonic stem cells to investigate the disease pathogenesis, these stem cell techniques are limited in their abilities to study the pathology and mechanism of pathogenesis of neurodevelopmental diseases such as autism. Therefore, researchers are focusing on the strengths of three-dimensional (3D) structures mimicking organs, organoids, for modeling autism. In this review, we highlight the advantages of 3D organoid systems to investigate the mechanisms of the pathogenesis of autism. Further, because the onset of autism is determined by genetic background, we suggest the application of the clustered regularly interspersed short palindromic repeat-associated protein 9 (CRISPR/Cas9) technique for genome editing in 3D organoid systems to study mutations that cause autism. We propose that 3D organoid systems combined with the CRISPR/Cas9 technique may advance autism research.

Chu, L. F., N. Leng, et al. "Single-cell RNA-seq reveals novel regulators of human embryonic stem cell differentiation to definitive endoderm." Genome Biol. 2016 Aug 17;17(1):173. doi: 10.1186/s13059-016-1033-x.

BACKGROUND: Human pluripotent stem cells offer the best available model to study the underlying cellular and molecular mechanisms of human embryonic lineage specification. However, it is not fully understood how individual stem cells exit the pluripotent state and transition towards their respective progenitor states. RESULTS: Here, we analyze the transcriptomes of human embryonic stem cell-derived lineage-specific progenitors by single-cell RNA-sequencing (scRNA-seq). We identify a definitive endoderm (DE) transcriptomic signature that leads us to pinpoint a critical time window when DE differentiation is enhanced by hypoxia. The molecular mechanisms governing the emergence of DE are further examined by time course scRNA-seq experiments, employing two new statistical tools to identify stage-specific genes over time (SCPattern) and to reconstruct the differentiation trajectory from the pluripotent state through mesendoderm to DE (Wave-Crest). Importantly, presumptive DE cells can be detected during the transitory phase from Brachyury (T) (+) mesendoderm toward a CXCR4 (+) DE state. Novel regulators are identified within this time window and are functionally validated on a screening platform with a T-2A-EGFP knock-in reporter engineered by CRISPR/Cas9. Through loss-of-function and gain-of-function experiments, we demonstrate that KLF8 plays a pivotal role modulating mesendoderm to DE differentiation. CONCLUSIONS: We report the analysis of 1776 cells by scRNA-seq covering distinct human embryonic stem cell-derived progenitor states. By reconstructing a differentiation trajectory at single-cell resolution, novel regulators of the mesendoderm transition to DE are elucidated and validated. Our strategy of combining single-cell analysis and genetic approaches can be applied to uncover novel regulators governing cell fate decisions in a variety of systems.

Chung, K. M., E. J. Jeong, et al. "Mediation of Autophagic Cell Death by Type 3 Ryanodine Receptor (RyR3) in Adult Hippocampal Neural Stem Cells." Front Cell Neurosci. 2016 May 6;10:116. doi: 10.3389/fncel.2016.00116. eCollection 2016.

Cytoplasmic Ca(2+) actively engages in diverse intracellular processes from protein synthesis, folding and trafficking to cell survival and death. Dysregulation of intracellular Ca(2+) levels is observed in various neuropathological states including Alzheimer's and Parkinson's diseases. Ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP3Rs), the main Ca(2+) release channels located in endoplasmic reticulum (ER) membranes, are known to direct various cellular events such as autophagy and apoptosis. Here we investigated the intracellular Ca(2+)-mediated regulation of survival and death of adult hippocampal neural stem (HCN) cells utilizing an insulin withdrawal model of autophagic cell death (ACD). Despite comparable expression levels of RyR and IP3R transcripts in HCN cells at normal state, the expression levels of RyRs-especially RyR3-were markedly upregulated upon insulin withdrawal. While treatment with the RyR agonist caffeine significantly promoted the autophagic death of insulin-deficient HCN cells, treatment with its inhibitor dantrolene prevented the induction of autophagy following insulin withdrawal. Furthermore, CRISPR/Cas9-mediated knockout of the RyR3 gene abolished ACD of HCN cells. This study delineates a distinct, RyR3-mediated ER Ca(2+) regulation of autophagy and programmed cell death in neural stem cells. Our findings provide novel insights into the critical, yet understudied mechanisms underlying the regulatory function of ER Ca(2+) in neural stem cell biology.

Cowan, P. J. "The use of CRISPR/Cas associated technologies for cell transplant applications." Curr Opin Organ Transplant. 2016 Oct;21(5):461-6. doi: 10.1097/MOT.0000000000000347.

PURPOSE OF REVIEW: In this review, I will summarize recent developments in the use of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) genome editing system for cell transplant applications, ranging from transplantation of corrected autologous patient stem cells to treat inherited diseases, to the tailoring of donor pigs for cell xenotransplantation. Rational engineering of the Cas9 nuclease to improve its specificity will also be discussed. RECENT FINDINGS: Over the past year, CRISPR/Cas9 has been used in preclinical studies to correct mutations in a rapidly increasing spectrum of diseases including hematological, neuromuscular, and respiratory disorders. The growing popularity of CRISPR/Cas9 over earlier genome editing platforms is partly due to its ease of use and flexibility, which is evident from the success of complex manipulations such as specific deletion of up to 725 kb in patient-derived stem cells, and simultaneous disruption of up to 62 endogenous retrovirus loci in pig cells. In addition, high-fidelity variants of Cas9 with greatly increased specificity are now available. SUMMARY: CRISPR/Cas9 is a fast-evolving technology that is likely to have a significant impact on autologous, allogeneic, and xenogeneic cell transplantation.

De Ravin, S. S., L. Li, et al. "CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease." Sci Transl Med. 2017 Jan 11;9(372). pii: eaah3480. doi: 10.1126/scitranslmed.aah3480.

Gene repair of CD34+ hematopoietic stem and progenitor cells (HSPCs) may avoid problems associated with gene therapy, such as vector-related mutagenesis and dysregulated transgene expression. We used CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated 9) to repair a mutation in the CYBB gene of CD34+ HSPCs from patients with the immunodeficiency disorder X-linked chronic granulomatous disease (X-CGD). Sequence-confirmed repair of >20% of HSPCs from X-CGD patients restored the function of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and superoxide radical production in myeloid cells differentiated from these progenitor cells in vitro. Transplant of gene-repaired X-CGD HSPCs into NOD (nonobese diabetic) SCID (severe combined immunodeficient) gammac-/- mice resulted in efficient engraftment and production of functional mature human myeloid and lymphoid cells for up to 5 months. Whole-exome sequencing detected no indels outside of the CYBB gene after gene correction. CRISPR-mediated gene editing of HSPCs may be applicable to other CGD mutations and other monogenic disorders of the hematopoietic system.

Dehghani, M. and P. Lasko "C-terminal residues specific to Vasa among DEAD-box helicases are required for its functions in piRNA biogenesis and embryonic patterning." Dev Genes Evol. 2016 Nov;226(6):401-412. Epub 2016 Aug 29.

The DEAD-box RNA helicase Vasa (Vas, also known as DDX4) is required for germ cell development. In Drosophila, analysis of hypomorphic mutations has implicated maternally expressed Vas in germ cell formation and posterior embryonic patterning. vas-null females, which rarely complete oogenesis, exhibit defects in mitotic progression of germline stem cells, Piwi-interacting RNA (piRNA)-mediated transposon silencing, and translation of Gurken (Grk), an EGFR ligand. The carboxy-terminal region of Vas orthologs throughout the animal kingdom consists of several acidic residues as well as an invariant tryptophan in the penultimate or ultimate position (Trp660 in Drosophila melanogaster). Using CRISPR/Cas9 gene editing, we made a substitution mutant in this residue. Replacing Trp660 by Glu (W660E) abolishes the ability of Vas to support germ cell formation and embryonic patterning and greatly reduces Vas activity in piRNA biogenesis, as measured by transposon silencing, and in activating Grk translation. A conservative substitution (W660F) has much milder phenotypic consequences. In addition, females expressing only a form of Vas in which the seven C-terminal amino acids were replaced with the corresponding residues from Belle (Bel, also known as DDX3) show defects in perinuclear nuage assembly and transposon silencing. Oogenesis in females expressing only the chimeric Vas arrests early; however, in a vas 1 background, in which early expression of endogenous Vas supports oogenesis, the chimeric protein supports posterior patterning and germ cell specification. These results indicate that the unique C-terminus of Vas is essential for its function in piRNA biogenesis and that the conserved Trp660 residue has an important functional role.

Denning, C., V. Borgdorff, et al. "Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform." Biochim Biophys Acta. 2016 Jul;1863(7 Pt B):1728-48. doi: 10.1016/j.bbamcr.2015.10.014. Epub 2015 Oct 31.

Cardiomyocytes from human pluripotent stem cells (hPSCs-CMs) could revolutionise biomedicine. Global burden of heart failure will soon reach USD $90bn, while unexpected cardiotoxicity underlies 28% of drug withdrawals. Advances in hPSC isolation, Cas9/CRISPR genome engineering and hPSC-CM differentiation have improved patient care, progressed drugs to clinic and opened a new era in safety pharmacology. Nevertheless, predictive

cardiotoxicity using hPSC-CMs contrasts from failure to almost total success. Since this likely relates to cell immaturity, efforts are underway to use biochemical and biophysical cues to improve many of the ~30 structural and functional properties of hPSC-CMs towards those seen in adult CMs. Other developments needed for widespread hPSC-CM utility include subtype specification, cost reduction of large scale differentiation and elimination of the phenotyping bottleneck. This review will consider these factors in the evolution of hPSC-CM technologies, as well as their integration into high content industrial platforms that assess structure, mitochondrial function, electrophysiology, calcium transients and contractility. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Integration of Developmental and Environmental Cues in the Heart edited by Marcus Schaub and Hughes Abriel.

Dever, D. P., R. O. Bak, et al. "CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells." Nature. 2016 Nov 17;539(7629):384-389. doi: 10.1038/nature20134. Epub 2016 Nov 7.

The beta-haemoglobinopathies, such as sickle cell disease and beta-thalassaemia, are caused by mutations in the beta-globin (HBB) gene and affect millions of people worldwide. Ex vivo gene correction in patient-derived haematopoietic stem cells followed by autologous transplantation could be used to cure beta-haemoglobinopathies. Here we present a CRISPR/Cas9 gene-editing system that combines Cas9 ribonucleoproteins and adeno-associated viral vector delivery of a homologous donor to achieve homologous recombination at the HBB gene in haematopoietic stem cells. Notably, we devise an enrichment model to purify a population of haematopoietic stem and progenitor cells with more than 90% targeted integration. We also show efficient correction of the Glu6Val mutation responsible for sickle cell disease by using patient-derived stem and progenitor cells that, after differentiation into erythrocytes, express adult beta-globin (HbA) messenger RNA, which confirms intact transcriptional regulation of edited HBB alleles. Collectively, these preclinical studies outline a CRISPR-based methodology for targeting haematopoietic stem cells by homologous recombination at the HBB locus to advance the development of next-generation therapies for beta-haemoglobinopathies.

Diao, Y., B. Li, et al. "A new class of temporarily phenotypic enhancers identified by CRISPR/Cas9-mediated genetic screening." Genome Res. 2016 Mar;26(3):397-405. doi: 10.1101/gr.197152.115. Epub 2016 Jan 26.

With <2% of the human genome coding for proteins, a major challenge is to interpret the function of the noncoding DNA. Millions of regulatory sequences have been predicted in the human genome through analysis of DNA methylation, chromatin modification, hypersensitivity to nucleases, and transcription factor binding, but few have been shown to regulate transcription in their native contexts. We have developed a high-throughput CRISPR/Cas9-based genome-editing strategy and used it to interrogate 174 candidate regulatory sequences within the 1-Mbp POU5F1 locus in human embryonic stem cells (hESCs). We identified two classical regulatory elements, including a promoter and a proximal enhancer, that are essential for POU5F1 transcription in hESCs. Unexpectedly, we also discovered a new class of enhancers that contribute to POU5F1 transcription in an unusual way: Disruption of such sequences led to a temporary loss of POU5F1 transcription that is fully restored after a few rounds of cell division. These results demonstrate the utility of high-throughput screening for functional characterization of noncoding DNA and reveal a previously unrecognized layer of gene regulation in human cells.

Dolatshad, H., A. Pellagatti, et al. "Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes." Leukemia. 2016 Dec;30(12):2322-2331. doi: 10.1038/leu.2016.149. Epub 2016 May 23.

The splicing factor SF3B1 is the most frequently mutated gene in myelodysplastic syndromes (MDS), and is strongly associated with the presence of ring sideroblasts (RS). We have performed a systematic analysis of cryptic splicing abnormalities from RNA sequencing data on hematopoietic stem cells (HSCs) of SF3B1-mutant MDS cases with RS. Aberrant splicing events in many downstream target genes were identified and cryptic 3' splice site usage was a frequent event in SF3B1-mutant MDS. The iron transporter ABCB7 is a well-recognized candidate gene showing marked downregulation in MDS with RS. Our analysis unveiled aberrant ABCB7 splicing, due to usage of an alternative 3' splice site in MDS patient samples, giving rise to a premature termination codon in the ABCB7 mRNA. Treatment of cultured SF3B1-mutant MDS erythroblasts and a CRISPR/Cas9-generated SF3B1-mutant cell line with the nonsense-mediated decay (NMD) inhibitor cycloheximide showed that the aberrantly spliced ABCB7 transcript is targeted by NMD. We describe cryptic splicing events in the HSCs of SF3B1-mutant MDS, and our data support a model in which NMD-induced downregulation of the iron exporter ABCB7 mRNA transcript resulting from aberrant splicing caused by mutant SF3B1 underlies the increased

mitochondrial iron accumulation found in MDS patients with RS.

Flaherty, E. K. and K. J. Brennand "Using hiPSCs to model neuropsychiatric copy number variations (CNVs) has potential to reveal underlying disease mechanisms." Brain Res. 2017 Jan 15;1655:283-293. doi: 10.1016/j.brainres.2015.11.009. Epub 2015 Nov 12.

Schizophrenia is a neuropsychological disorder with a strong heritable component; genetic risk for schizophrenia is conferred by both common variants of relatively small effect and rare variants with high penetrance. Genetically engineered mouse models can recapitulate rare variants, displaying some behavioral defects associated with schizophrenia; however, these mouse models cannot recapitulate the full genetic architecture underlying the disorder. Patient-derived human induced pluripotent stem cells (hiPSCs) present an alternative approach for studying rare variants, in the context of all other risk alleles. Genome editing technologies, such as CRISPR-Cas9, enable the generation of isogenic hiPSC lines with which to examine the functional contribution of single variants within any genetic background. Studies of these rare variants using hiPSCs have the potential to identify commonly disrupted pathways in schizophrenia and allow for the identification of new therapeutic targets. This article is part of a Special Issue entitled SI:StemsCellsinPsychiatry.

Frieda, K. L., J. M. Linton, et al. "Synthetic recording and in situ readout of lineage information in single cells." Nature. 2017 Jan 5;541(7635):107-111. doi: 10.1038/nature20777. Epub 2016 Nov 21.

Reconstructing the lineage relationships and dynamic event histories of individual cells within their native spatial context is a long-standing challenge in biology. Many biological processes of interest occur in optically opaque or physically inaccessible contexts, necessitating approaches other than direct imaging. Here we describe a synthetic system that enables cells to record lineage information and event histories in the genome in a format that can be subsequently read out of single cells in situ. This system, termed memory by engineered mutagenesis with optical in situ readout (MEMOIR), is based on a set of barcoded recording elements termed scratchpads. The state of a given scratchpad can be irreversibly altered by CRISPR/Cas9-based targeted mutagenesis, and later read out in single cells through multiplexed single-molecule RNA fluorescence hybridization (smFISH). Using MEMOIR as a proof of principle, we engineered mouse embryonic stem cells to contain multiple scratchpads and other recording components. In these cells, scratchpads were altered in a progressive and stochastic fashion as the cells proliferated. Analysis of the final states of scratchpads in single cells in situ enabled reconstruction of lineage information from cell colonies. Combining analysis of endogenous gene expression with lineage reconstruction in the same cells further allowed inference of the dynamic rates at which embryonic stem cells switch between two gene expression states. Finally, using simulations, we show how parallel MEMOIR systems operating in the same cell could enable recording and readout of dynamic cellular event histories. MEMOIR thus provides a versatile platform for information recording and in situ, single-cell readout across diverse biological systems.

Ge, X., H. Xi, et al. "CRISPR/Cas9-AAV Mediated Knock-in at NRL Locus in Human Embryonic Stem Cells." Mol Ther Nucleic Acids. 2016 Nov 29;5(11):e393. doi: 10.1038/mtna.2016.100.

Clustered interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated genome engineering technologies are sparking a new revolution in biological research. This technology efficiently induces DNA double strand breaks at the targeted genomic sequence and results in indel mutations by the error-prone process of nonhomologous end joining DNA repair or homologous recombination with a DNA repair template. The efficiency of genome editing with CRISPR/Cas9 alone in human embryonic stem cells is still low. Gene targeting with adeno-associated virus (AAV) vectors has been demonstrated in multiple human cell types with maximal targeting frequencies without engineered nucleases. However, whether CRISPR/Cas9-mediated double strand breaks and AAV based donor DNA mediated homologous recombination approaches could be combined to create a novel CRISPR/Cas9-AAV genetic tool for highly specific gene editing is not clear. Here we demonstrate that using CRISPR/Cas9-AAV, we could successfully knock-in a DsRed reporter gene at the basic motifleucine zipper transcription factor (NRL) locus in human embryonic stem cells. For the first time, this study provides the proof of principle that these two technologies can be used together. CRISPR/Cas9-AAV, a new genome editing tool, offers a platform for the manipulation of human genome.

Genga, R. M., N. A. Kearns, et al. "Controlling transcription in human pluripotent stem cells using CRISPR-effectors." Methods. 2016 May 15;101:36-42. doi: 10.1016/j.ymeth.2015.10.014. Epub 2015 Oct 23.

The ability to manipulate transcription in human pluripotent stem cells (hPSCs) is fundamental for the discovery of key genes and mechanisms governing cellular state and differentiation. Recently

developed CRISPR-effector systems provide a systematic approach to rapidly test gene function in mammalian cells, including hPSCs. In this review, we discuss recent advances in CRISPR-effector technologies that have been employed to control transcription through gene activation, gene repression, and epigenome engineering. We describe an application of CRISPR-effector mediated transcriptional regulation in hPSCs by targeting a synthetic promoter driving a GFP transgene, demonstrating the ease and effectiveness of CRISPR-effector mediated transcriptional regulation in hPSCs.

Gerlai, R. "Gene Targeting Using Homologous Recombination in Embryonic Stem Cells: The Future for Behavior Genetics?" Front Genet. 2016 Apr 11;7:43. doi: 10.3389/fgene.2016.00043. eCollection 2016.

Gene targeting with homologous recombination in embryonic stem cells created a revolution in the analysis of the function of genes in behavioral brain research. The technology allowed unprecedented precision with which one could manipulate genes and study the effect of this manipulation on the central nervous system. With gene targeting, the uncertainty inherent in psychopharmacology regarding whether a particular compound would act only through a specific target was removed. Thus, gene targeting became highly popular. However, with this popularity came the realization that like other methods, gene targeting also suffered from some technical and principal problems. For example, two decades ago, issues about compensatory changes and about genetic linkage were raised. Since then, the technology developed, and its utility has been better delineated. This review will discuss the pros and cons of the technique along with these advancements from the perspective of the neuroscientist user. It will also compare and contrast methods that may represent novel alternatives to the homologous recombination based gene targeting approach, including the TALEN and the CRISPR/Cas9 systems. The goal of the review is not to provide detailed recipes, but to attempt to present a short summary of these approaches a behavioral geneticist or neuroscientist may consider for the analysis of brain function and behavior.

Gonzalez, F. "CRISPR/Cas9 genome editing in human pluripotent stem cells: Harnessing human genetics in a dish." Dev Dyn. 2016 Jul;245(7):788-806. doi: 10.1002/dvdy.24414. Epub 2016 Jun 9.

Because of their extraordinary differentiation potential, human pluripotent stem cells (hPSCs) can differentiate into virtually any cell type of the human body, providing a powerful platform not only for generating relevant cell types useful for cell replacement therapies, but also for modeling human development and disease. Expanding this potential, structures resembling human organs, termed organoids, have been recently obtained from hPSCs through tissue engineering. Organoids exhibit multiple cell types self-organizing into structures recapitulating in part the physiology and the cellular interactions observed in the organ in vivo, offering unprecedented opportunities for human disease modeling. To fulfill this promise, tissue engineering in hPSCs needs to be supported by robust and scalable genome editing technologies. With the advent of the CRISPR/Cas9 technology, manipulating the genome of hPSCs has now become an easy task, allowing modifying their genome with superior precision, speed, and throughput. Here we review current and potential applications of the CRISPR/Cas9 technology in hPSCs and how they contribute to establish hPSCs as a model of choice for studying human genetics. Developmental Dynamics 245:788-806, 2016. (c) 2016 Wiley Periodicals, Inc.

Granata, A., F. Serrano, et al. "An iPSC-derived vascular model of Marfan syndrome identifies key mediators of smooth muscle cell death." Nat Genet. 2017 Jan;49(1):97-109. doi: 10.1038/ng.3723. Epub 2016 Nov 28.

Marfan syndrome (MFS) is a heritable connective tissue disorder caused by mutations in FBN1, which encodes the extracellular matrix protein fibrillin-1. To investigate the pathogenesis of aortic aneurysms in MFS, we generated a vascular model derived from human induced pluripotent stem cells (MFS-hiPSCs). Our MFS-hiPSC-derived smooth muscle cells (SMCs) recapitulated the pathology seen in Marfan aortas, including defects in fibrillin-1 accumulation, extracellular matrix degradation, transforming growth factor-beta (TGF-beta) signaling, contraction and apoptosis; abnormalities were corrected by CRISPR-based editing of the FBN1 mutation. TGF-beta inhibition rescued abnormalities in fibrillin-1 accumulation and matrix metalloproteinase expression. However, only the noncanonical p38 pathway regulated SMC apoptosis, a pathological mechanism also governed by Kruppel-like factor 4 (KLF4). This model has enabled us to dissect the molecular mechanisms of MFS, identify novel targets for treatment (such as p38 and KLF4) and provided an innovative human platform for the testing of new drugs.

Gundry, M. C., L. Brunetti, et al. "Highly Efficient Genome Editing of Murine and Human Hematopoietic Progenitor Cells by CRISPR/Cas9." Cell Rep. 2016 Oct 25;17(5):1453-1461. doi: 10.1016/j.celrep.2016.09.092.

Our understanding of the mechanisms that regulate hematopoietic stem/progenitor cells (HSPCs) has been advanced by the ability to genetically manipulate mice; however, germline modification is time consuming and expensive. Here, we describe fast, efficient, and cost-effective methods to directly modify the genomes of mouse and human HSPCs using the CRISPR/Cas9 system. Using plasmid and virus-free delivery of guide RNAs alone into Cas9-expressing HSPCs or Cas9-guide RNA ribonucleoprotein (RNP) complexes into wild-type cells, we have achieved extremely efficient gene disruption in primary HSPCs from mouse (>60%) and human (approximately 75%). These techniques enabled rapid evaluation of the functional effects of gene loss of Eed, Suz12, and DNMT3A. We also achieved homology-directed repair in primary human HSPCs (>20%). These methods will significantly expand applications for CRISPR/Cas9 technologies for studying normal and malignant hematopoiesis.

Guo, H., S. Cooper, et al. "In Vivo Deletion of the Cebpa +37 kb Enhancer Markedly Reduces Cebpa mRNA in Myeloid Progenitors but Not in Non-Hematopoietic Tissues to Impair Granulopoiesis." PLoS One. 2016 Mar 3;11(3):e0150809. doi: 10.1371/journal.pone.0150809. eCollection 2016.

The murine Cebpa gene contains a +37 kb, evolutionarily conserved 440 bp enhancer that directs high-level expression to myeloid progenitors in transgenic mice. The enhancer is bound and activated by Runx1, Scl, GATA2, C/EBPalpha, c-Myb, Pu.1, and additional Ets factors in myeloid cells. CRISPR/Cas9-mediated replacement of the wild-type enhancer with a variant mutant in its seven Ets sites leads to 20-fold reduction of Cebpa mRNA in the 32Dcl3 myeloid cell line. To determine the effect of deleting the enhancer in vivo, we now characterize C57BL/6 mice in which loxP sites flank a 688 bp DNA segment containing the enhancer. CMV-Cre mediated germline deletion resulted in diminution of the expected number of viable Enh(f/f);CMV-Cre offspring, with 28-fold reduction in marrow Cebpa mRNA but normal levels in liver, lung, adipose, intestine, muscle, and kidney. Cre-transduction of lineage-negative marrow cells in vitro reduced Cebpa mRNA 12-fold, with impairment of granulocytic maturation, morphologic blast accumulation, and IL-3 dependent myeloid colony replating for >12 generations. Exposure of Enh(f/f);Mx1-Cre mice to pIpC led to 14-fold reduction of Cebpa mRNA in GMP or CMP, 30-fold reduction in LSK, and <2-fold reduction in the LSK/SLAM subset. FACS analysis of marrow from these mice revealed 10-fold reduced neutrophils, 3-fold decreased GMP, and 3-fold increased LSK cells. Progenitor cell cycle progression was mildly impaired. Granulocyte and B lymphoid colony forming units were reduced while monocytic and erythroid colonies were increased, with reduced Pu.1 and Gfi1 and increased Egr1 and Klf4 in GMP. Finally, competitive transplantation indicated preservation of functional long-term hematopoietic stem cells upon enhancer deletion and confirmed marrow-intrinsic impairment of granulopoiesis and B cell generation with LSK and monocyte lineage expansion. These findings demonstrate a critical role for the +37 kb Cebpa enhancer for hematopoietic-specific Cebpa expression, with enhancer deletion leading to impaired myelopoiesis and potentially preleukemic progenitor expansion.

Haggarty, S. J., M. C. Silva, et al. "Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models." Mol Cell Neurosci. 2016 Jun;73:104-15. doi: 10.1016/j.mcn.2016.01.011. Epub 2016 Jan 28.

Compelling clinical, social, and economic reasons exist to innovate in the process of drug discovery for neuropsychiatric disorders. The use of patient-specific, induced pluripotent stem cells (iPSCs) now affords the ability to generate neuronal cell-based models that recapitulate key aspects of human disease. In the context of neuropsychiatric disorders, where access to physiologically active and relevant cell types of the central nervous system for research is extremely limiting, iPSC-derived in vitro culture of human neurons and glial cells is transformative. Potential applications relevant to early stage drug discovery, include support of quantitative biochemistry, functional genomics, proteomics, and perhaps most notably, high-throughput and high-content chemical screening. While many phenotypes in human iPSC-derived culture systems may prove adaptable to screening formats, addressing the question of which in vitro phenotypes are ultimately relevant to disease pathophysiology and therefore more likely to yield effective pharmacological agents that are disease-modifying treatments requires careful consideration. Here, we review recent examples of studies of neuropsychiatric disorders using human stem cell models where cellular phenotypes linked to disease and functional assays have been reported. We also highlight technical advances using genome-editing technologies in iPSCs to support drug discovery efforts, including the interpretation of the functional significance of rare genetic variants of unknown significance and for the purpose of creating cell type- and pathway-selective functional reporter assays. Additionally, we evaluate the potential of in vitro stem cell models to investigate early events of disease pathogenesis, in an effort to understand the underlying molecular mechanism, including the basis of selective

cell-type vulnerability, and the potential to create new cell-based diagnostics to aid in the classification of patients and subsequent selection for clinical trials. A number of key challenges remain, including the scaling of iPSC models to larger cohorts and integration with rich clinicopathological information and translation of phenotypes. Still, the overall use of iPSC-based human cell models with functional cellular and biochemical assays holds promise for supporting the discovery of next-generation neuropharmacological agents for the treatment and ultimately prevention of a range of severe mental illnesses.

Han, K., L. Liang, et al. "Generation of Hoxc13 knockout pigs recapitulates human ectodermal dysplasia-9." Hum Mol Genet. 2016 Dec 22. pii: ddw378. doi: 10.1093/hmg/ddw378.

Atrichia and sparse hair phenotype cause distress to many patients. Ectodermal dysplasia-9 (ED-9) is a congenital condition characterized by hypotrichosis and nail dystrophy without other disorders, and Hoxc13 is a pathogenic gene for ED-9. However, mice carrying Hoxc13 mutation present several other serious disorders, such as skeletal defects, progressive weight loss and low viability. Mouse models cannot faithfully mimic human ED-9. In this study, we generated an ED-9 pig model via Hoxc13 gene knockout through single-stranded oligonucleotides (c.396C > A) combined with CRISPR/Cas9 and somatic cell nuclear transfer. Eight cloned piglets with three types of biallelic mutations (five piglets with Hoxc13c.396C > A/c.396C > A, two piglets with Hoxc13c.396C > A/c.396C > A + 1 and one piglet with Hoxc13Delta40/Delta40) were obtained. Hoxc13 was not expressed in pigs with all three mutation types, and the expression levels of Hoxc13-regulated genes, namely, Foxn1, Krt85 and Krt35, were decreased. The hair follicles displayed various abnormal phenotypes, such as reduced number of follicles and disarrayed hair follicle cable without normal hair all over the body. By contrast, the skin structure, skeleton phenotype, body weight gain and growth of Hoxc13 knockout pigs were apparently normal. The phenotypes of Hoxc13 mutation in pigs were similar to those in ED-9 patients. Therefore, Hoxc13 knockout pigs could be utilized as a model for ED-9 pathogenesis and as a hairless model for hair regeneration research. Moreover, the hairless pigs without other major abnormal phenotypes generated in this study could be effective models for other dermatological research because of the similarity between pig and human skins.

Hay, E. A., C. Knowles, et al. "Using the CRISPR/Cas9 system to understand neuropeptide biology and regulation." Neuropeptides. 2016 Dec 3. pii: S0143-4179(16)30169-X. doi: 10.1016/j.npep.2016.11.010.

Neuropeptides and their receptors play a role in physiological responses such as appetite, stress and inflammatory pain. With neuropeptides having such diverse and important physiological roles, knocking-out the genes encoding them, their receptors, parts of their regulatory sequences, or reproducing disease associated polymorphic variants are important steps in studying neuropeptides and how they may contribute to disease. Previously, knock-outs were generated using methods such as targeted homologous recombination in embryonic stem cells but this method is costly and time-consuming. The CRISPR/Cas9 system has rapidly taken over the genome editing field and will advance our understanding of neuropeptide genes and their regulation. With CRISPR/Cas9 technology, the time and costs involved in producing transgenic animal models, is greatly reduced. In this review, we describe how the system can be used to manipulate genomic sequences by "knock-out" or "knock-in" mutations in cell lines or in animal models. We also discuss the specificity of the system and methods to limit off-target effects. When combined with the availability of genome sequences, CRISPR/Cas9 directed genome editing in vitro and in vivo, promises to provide a deeper understanding of the biology of the neuropeptides in health and disease than has ever been available before.

Hayashi, M., K. Maehara, et al. "Chd5 Regulates MuERV-L/MERVL Expression in Mouse Embryonic Stem Cells Via H3K27me3 Modification and Histone H3.1/H3.2." J Cell Biochem. 2016 Mar;117(3):780-92. doi: 10.1002/jcb.25368. Epub 2015 Sep 29.

Chd5 is an essential factor for neuronal differentiation and spermatogenesis and is a known tumor suppressor. H3K27me3 and H3K4un are modifications recognized by Chd5; however, it remains unclear how Chd5 remodels chromatin structure. We completely disrupted the Chd5 locus using the CRISPR-Cas9 system to generate a 52 kbp long deletion and analyzed Chd5 function in mouse embryonic stem cells. Our findings show that Chd5 represses murine endogenous retrovirus-L (MuERV-L/MERVL), an endogenous retrovirus-derived retrotransposon, by regulating H3K27me3 and H3.1/H3.2 function.

He, X., C. Tan, et al. "Knock-in of large reporter genes in human cells via CRISPR/Cas9-induced homology-dependent and independent DNA repair." Nucleic Acids Res. 2016 May 19;44(9):e85. doi: 10.1093/nar/gkw064. Epub 2016 Feb 4.

CRISPR/Cas9-induced site-specific DNA

double-strand breaks (DSBs) can be repaired by homology-directed repair (HDR) or non-homologous end joining (NHEJ) pathways. Extensive efforts have been made to knock-in exogenous DNA to a selected genomic locus in human cells; which, however, has focused on HDR-based strategies and was proven inefficient. Here, we report that NHEJ pathway mediates efficient rejoining of genome and plasmids following CRISPR/Cas9-induced DNA DSBs, and promotes high-efficiency DNA integration in various human cell types. With this homology-independent knock-in strategy, integration of a 4.6 kb promoterless ires-eGFP fragment into the GAPDH locus yielded up to 20% GFP+ cells in somatic LO2 cells, and 1.70% GFP+ cells in human embryonic stem cells (ESCs). Quantitative comparison further demonstrated that the NHEJ-based knock-in is more efficient than HDR-mediated gene targeting in all human cell types examined. These data support that CRISPR/Cas9-induced NHEJ provides a valuable new path for efficient genome editing in human ESCs and somatic cells.

Helsley, R. N., Y. Sui, et al. "Targeting IkappaB kinase beta in Adipocyte Lineage Cells for Treatment of Obesity and Metabolic Dysfunctions." Stem Cells. 2016 Jul;34(7):1883-95. doi: 10.1002/stem.2358. Epub 2016 Mar 28.

IkappaB kinase beta (IKKbeta), a central coordinator of inflammation through activation of nuclear factor-kappaB, has been identified as a potential therapeutic target for the treatment of obesity-associated metabolic dysfunctions. In this study, we evaluated an antisense oligonucleotide (ASO) inhibitor of IKKbeta and found that IKKbeta ASO ameliorated diet-induced metabolic dysfunctions in mice. Interestingly, IKKbeta ASO also inhibited adipocyte differentiation and reduced adiposity in high-fat (HF)-fed mice, indicating an important role of IKKbeta signaling in the regulation of adipocyte differentiation. Indeed, CRISPR/Cas9-mediated genomic deletion of IKKbeta in 3T3-L1 preadipocytes blocked these cells differentiating into adipocytes. To further elucidate the role of adipose progenitor IKKbeta signaling in diet-induced obesity, we generated mice that selectively lack IKKbeta in the white adipose lineage and confirmed the essential role of IKKbeta in mediating adipocyte differentiation in vivo. Deficiency of IKKbeta decreased HF-elicited adipogenesis in addition to reducing inflammation and protected mice from diet-induced obesity and insulin resistance. Further, pharmacological inhibition of IKKbeta also blocked human adipose stem cell differentiation. Our findings establish IKKbeta as a pivotal regulator of adipogenesis and suggest that overnutrition-mediated IKKbeta activation serves as an initial signal that triggers adipose progenitor cell differentiation in response to HF feeding. Inhibition of IKKbeta with antisense therapy may represent as a novel therapeutic approach to combat obesity and metabolic dysfunctions. Stem Cells 2016;34:1883-1895.

Heman-Ackah, S. M., A. R. Bassett, et al. "Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons." Sci Rep. 2016 Jun 24;6:28420. doi: 10.1038/srep28420.

The ability to reprogram adult somatic cells into induced pluripotent stem cells (iPSCs) and the subsequent development of protocols for their differentiation into disease-relevant cell types have enabled in-depth molecular analyses of multiple disease states as hitherto impossible. Neurons differentiated from patient-specific iPSCs provide a means to recapitulate molecular phenotypes of neurodegenerative diseases in vitro. However, it remains challenging to conduct precise manipulations of gene expression in iPSC-derived neurons towards modeling complex human neurological diseases. The application of CRISPR/Cas9 to mammalian systems is revolutionizing the utilization of genome editing technologies in the study of molecular contributors to the pathogenesis of numerous diseases. Here, we demonstrate that CRISPRa and CRISPRi can be used to exert precise modulations of endogenous gene expression in fate-committed iPSC-derived neurons. This highlights CRISPRa/i as a major technical advancement in accessible tools for evaluating the specific contributions of critical neurodegenerative disease-related genes to neuropathogenesis.

Henao-Mejia, J., A. Williams, et al. "Generation of Genetically Modified Mice Using the CRISPR-Cas9 Genome-Editing System." Cold Spring Harb Protoc. 2016 Feb 1;2016(2):pdb.prot090704. doi: 10.1101/pdb.prot090704.

Genetically modified mice are extremely valuable tools for studying gene function and human diseases. Although the generation of mice with specific genetic modifications through traditional methods using homologous recombination in embryonic stem cells has been invaluable in the last two decades, it is an extremely costly, time-consuming, and, in some cases, uncertain technology. The recently described CRISPR-Cas9 genome-editing technology significantly reduces the time and the cost that are required to generate genetically engineered mice, allowing scientists to test more precise and bold hypotheses in vivo. Using this revolutionary methodology we have generated more than 100 novel genetically engineered mouse strains. In the current protocol, we describe in detail the optimal conditions

to generate mice carrying point mutations, chromosomal deletions, conditional alleles, fusion tags, or endogenous reporters.

Herrera-Carrillo, E. and B. Berkhout "Attacking HIV-1 RNA versus DNA by sequence-specific approaches: RNAi versus CRISPR-Cas." Biochem Soc Trans. 2016 Oct 15;44(5):1355-1365.

Human immunodeficiency virus type 1 (HIV-1) infection can be effectively controlled by potent antiviral drugs, but this never results in a cure. The patient should therefore take these drugs for the rest of his/her life, which can cause drug-resistance and adverse effects. Therefore, more durable therapeutic strategies should be considered, such as a stable gene therapy to protect the target T cells against HIV-1 infection. The development of potent therapeutic regimens based on the RNA interference (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR-Cas) mechanisms will be described, which can be delivered by lentiviral vectors. These mechanisms attack different forms of the viral genome, the RNA and DNA, respectively, but both mechanisms act in a strictly sequence-specific manner. Early RNAi experiments demonstrated profound virus inhibition, but also indicated that viral escape is possible. Such therapy failure can be prevented by the design of a combinatorial RNAi attack on the virus and this gene therapy is currently being tested in a preclinical humanized mouse model. Recent CRISPR-Cas studies also document robust virus inhibition, but suggest a novel viral escape route that is induced by the cellular nonhomologous end joining DNA repair pathway, which is activated by CRISPR-Cas-induced DNA breaks. We will compare these two approaches for durable HIV-1 suppression and discuss the respective advantages and disadvantages. The potential for future clinical applications will be described.

Ho, Y. K., L. H. Zhou, et al. "Enhanced non-viral gene delivery by coordinated endosomal release and inhibition of beta-tubulin deactylase." Nucleic Acids Res. 2016 Nov 29. pii: gkw1143.

Efficient non-viral gene delivery is highly desirable but often unattainable with some cell-types. We report here that non-viral DNA polyplexes can efficiently transfect differentiated neuronal and stem cells. Polyplex transfection centrifugation protocols was enhanced by including a simultaneous treatment with a DOPE/CHEMS lipid suspension and a microtubule inhibitor, Tubastatin A. Lipoplex transfection protocols were not improved by this treatment. This mechanism of action was unravelled by systematically identifying and rationally mitigating barriers limiting high transfection efficiency, allowing unexpected improvements in the transfection of mesenchymal stem cells (MSC), primary neuron and several hard-to-transfect cell types beyond what are currently achievable using cationic polymers. The optimized formulation and method achieved high transfection efficiency with no adverse effects on cell viability, cell proliferation or differentiation. High efficiency modification of MSC for cytokine overexpression, efficient generation of dopaminergic neuron using neural stem cells and enhanced genome editing with CRISPR-Cas9 were demonstrated. In summary, this study described a cost-effective method for efficient, rapid and scalable workflow for ex vivo gene delivery using a myriad of nucleic acids including plasmid DNA, mRNA, siRNA and shRNA.

Hoban, M. D., D. Lumaquin, et al. "CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells." Mol Ther. 2016 Sep;24(9):1561-9. doi: 10.1038/mt.2016.148. Epub 2016 Jul 29.

Targeted genome editing technology can correct the sickle cell disease mutation of the beta-globin gene in hematopoietic stem cells. This correction supports production of red blood cells that synthesize normal hemoglobin proteins. Here, we demonstrate that Transcription Activator-Like Effector Nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 nuclease system can target DNA sequences around the sickle-cell mutation in the beta-globin gene for site-specific cleavage and facilitate precise correction when a homologous donor template is codelivered. Several pairs of TALENs and multiple CRISPR guide RNAs were evaluated for both on-target and off-target cleavage rates. Delivery of the CRISPR/Cas9 components to CD34+ cells led to over 18% gene modification in vitro. Additionally, we demonstrate the correction of the sickle cell disease mutation in bone marrow derived CD34+ hematopoietic stem and progenitor cells from sickle cell disease patients, leading to the production of wild-type hemoglobin. These results demonstrate correction of the sickle mutation in patient-derived CD34+ cells using CRISPR/Cas9 technology.

Hockemeyer, D. and R. Jaenisch "Induced Pluripotent Stem Cells Meet Genome Editing." Cell Stem Cell. 2016 May 5;18(5):573-86. doi: 10.1016/j.stem.2016.04.013.

It is extremely rare for a single experiment to be so impactful and timely that it shapes and forecasts the experiments of the next decade. Here, we review how two such experiments-the generation of human induced pluripotent stem cells (iPSCs) and the development of CRISPR/Cas9 technology-have fundamentally reshaped our approach to biomedical research, stem cell biology, and human genetics. We

will also highlight the previous knowledge that iPSC and CRISPR/Cas9 technologies were built on as this groundwork demonstrated the need for solutions and the benefits that these technologies provided and set the stage for their success.

Hofherr, A., T. Busch, et al. "Efficient genome editing of differentiated renal epithelial cells." Pflugers Arch. 2017 Feb;469(2):303-311. doi: 10.1007/s00424-016-1924-4. Epub 2016 Dec 16.

Recent advances in genome editing technologies have enabled the rapid and precise manipulation of genomes, including the targeted introduction, alteration, and removal of genomic sequences. However, respective methods have been described mainly in non-differentiated or haploid cell types. Genome editing of well-differentiated renal epithelial cells has been hampered by a range of technological issues, including optimal design, efficient expression of multiple genome editing constructs, attainable mutation rates, and best screening strategies. Here, we present an easily implementable workflow for the rapid generation of targeted heterozygous and homozygous genomic sequence alterations in renal cells using transcription activator-like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat (CRISPR) system. We demonstrate the versatility of established protocols by generating novel cellular models for studying autosomal dominant polycystic kidney disease (ADPKD). Furthermore, we show that cell culture-validated genetic modifications can be readily applied to mouse embryonic stem cells (mESCs) for the generation of corresponding mouse models. The described procedure for efficient genome editing can be applied to any cell type to study physiological and pathophysiological functions in the context of precisely engineered genotypes.

Hofsteen, P., A. M. Robitaille, et al. "Quantitative proteomics identify DAB2 as a cardiac developmental regulator that inhibits WNT/beta-catenin signaling." Proc Natl Acad Sci U S A. 2016 Jan 26;113(4):1002-7. doi: 10.1073/pnas.1523930113. Epub 2016 Jan 11.

To reveal the molecular mechanisms involved in cardiac lineage determination and differentiation, we quantified the proteome of human embryonic stem cells (hESCs), cardiac progenitor cells (CPCs), and cardiomyocytes during a time course of directed differentiation by label-free quantitative proteomics. This approach correctly identified known stage-specific markers of cardiomyocyte differentiation, including SRY-box2 (SOX2), GATA binding protein 4 (GATA4), and myosin heavy chain 6 (MYH6). This led us to determine whether our proteomic screen could reveal previously unidentified mediators of heart development. We identified Disabled 2 (DAB2) as one of the most dynamically expressed proteins in hESCs, CPCs, and cardiomyocytes. We used clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) mutagenesis in zebrafish to assess whether DAB2 plays a functional role during cardiomyocyte differentiation. We found that deletion of Dab2 in zebrafish embryos led to a significant reduction in cardiomyocyte number and increased endogenous WNT/beta-catenin signaling. Furthermore, the Dab2-deficient defects in cardiomyocyte number could be suppressed by overexpression of dickkopf 1 (DKK1), an inhibitor of WNT/beta-catenin signaling. Thus, inhibition of WNT/beta-catenin signaling by DAB2 is essential for establishing the correct number of cardiomyocytes in the developing heart. Our work demonstrates that quantifying the proteome of human stem cells can identify previously unknown developmental regulators.

Hong, S. N., J. C. Dunn, et al. "Concise Review: The Potential Use of Intestinal Stem Cells to Treat Patients With Intestinal Failure." Stem Cells Transl Med. 2016 Sep 16. pii: sctm.2016-0153.

: Intestinal failure is a rare life-threatening condition that results in the inability to maintain normal growth and hydration status by enteral nutrition alone. Although parenteral nutrition and whole organ allogeneic transplantation have improved the survival of these patients, current therapies are associated with a high risk for morbidity and mortality. Development of methods to propagate adult human intestinal stem cells (ISCs) and pluripotent stem cells raises the possibility of using stem cell-based therapy for patients with monogenic and polygenic forms of intestinal failure. Organoids have demonstrated the capacity to proliferate indefinitely and differentiate into the various cellular lineages of the gut. Genome-editing techniques, including the overexpression of the corrected form of the defective gene, or the use of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 to selectively correct the monogenic disease-causing variant within the stem cell, make autologous ISC transplantation a feasible approach. However, numerous techniques still need to be further optimized, including more robust ex vivo ISC expansion, native ISC ablation, and engraftment protocols. Large-animal models can to be used to develop such techniques and protocols and to establish the safety of autologous ISC transplantation because outcomes in such models can be extrapolated more readily to humans. SIGNIFICANCE: The field of intestinal stem cell biology has exploded over the past 5 years with discoveries related to in vivo and in vitro stem cell identity and function. The goal of this review

article is to highlight the potential use of these cells to treat various epithelial disorders of the gut and discuss the various roadblocks that will be encountered in the coming years.

Hosain, S. B., S. K. Khiste, et al. "Inhibition of glucosylceramide synthase eliminates the oncogenic function of p53 R273H mutant in the epithelial-mesenchymal transition and induced pluripotency of colon cancer cells." Oncotarget. 2016 Sep 13;7(37):60575-60592. doi: 10.18632/oncotarget.11169.

Missense mutation of tumor suppressor p53, which exhibits oncogenic gain-of-function (GOF), not only promotes tumor progression, but also diminishes therapeutic efficacies of cancer treatments. However, it remains unclear how a p53 missense mutant contributes to induced pluripotency of cancer stem cells (CSCs) in tumors exposed to chemotherapeutic agents. More importantly, it may be possible to abrogate the GOF by restoring wild-type p53 activity, thereby overcoming the deleterious effects resulting from heterotetramer formation, which often compromises the efficacies of current approaches being used to reactivate p53 function. Herewith, we report that p53 R273H missense mutant urges cancer cells to spawn CSCs. SW48/TP53 cells, which heterozygously carry the p53 R273H hot-spot mutant (R273H/+, introduced by a CRISPR/Casp9 system), were subchronically exposed to doxorubicin in cell culture and in tumor-bearing mice. We found that p53-R273H (TP53-Dox) cells were drug-resistant and exhibited epithelial-mesenchymal transition (EMT) and increased numbers of CSCs (CD44v6+/CD133+), which resulted in enhanced wound healing and tumor formation. Inhibition of glucosylceramide synthase with d-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) sensitized p53-R273H cancer cells and tumor xenografts to doxorubicin treatments. Intriguingly, PDMP treatments restored wild-type p53 expression in heterozygous R273H mutant cells and in tumors, decreasing CSCs and sensitizing cells and tumors to treatments. This study demonstrated that p53-R273H promotes EMT and induced pluripotency of CSCs in cancer cells exposed to doxorubicin, mainly through Zeb1 and beta-catenin transcription factors. Our results further indicate that restoration of p53 through inhibition of ceramide glycosylation might be an effective treatment approach for targeting cancers heterozygously harboring TP53 missense mutations.

Howden, S. E., B. McColl, et al. "A Cas9 Variant for Efficient Generation of Indel-Free Knockin or Gene-Corrected Human Pluripotent Stem Cells." Stem Cell Reports. 2016 Sep 13;7(3):508-17. doi: 10.1016/j.stemcr.2016.07.001. Epub 2016 Aug 4.

While Cas9 nucleases permit rapid and efficient generation of gene-edited cell lines, the CRISPR-Cas9 system can introduce undesirable "on-target" mutations within the second allele of successfully modified cells via non-homologous end joining (NHEJ). To address this, we fused the Streptococcus pyogenes Cas9 (SpCas9) nuclease to a peptide derived from the human Geminin protein (SpCas9-Gem) to facilitate its degradation during the G1 phase of the cell cycle, when DNA repair by NHEJ predominates. We also use mRNA transfection to facilitate low and transient expression of modified and unmodified versions of Cas9. Although the frequency of homologous recombination was similar for SpCas9-Gem and SpCas9, we observed a marked reduction in the capacity for SpCas9-Gem to induce NHEJ-mediated indels at the target locus. Moreover, in contrast to native SpCas9, we demonstrate that transient SpCas9-Gem expression enables reliable generation of both knockin reporter cell lines and genetically repaired patient-specific induced pluripotent stem cell lines free of unwanted mutations at the targeted locus.

Hu, X. "CRISPR/Cas9 system and its applications in human hematopoietic cells." Blood Cells Mol Dis. 2016 Nov;62:6-12. doi: 10.1016/j.bcmd.2016.09.003. Epub 2016 Oct 2.

Since 2012, the CRISPR-Cas9 system has been quickly and successfully tested in a broad range of organisms and cells including hematopoietic cells. The application of CRISPR-Cas9 in human hematopoietic cells mainly involves the genes responsible for HIV infection, beta-thalassemia and sickle cell disease (SCD). The successful disruption of CCR5 and CXCR4 genes in T cells by CRISPR-Cas9 promotes the prospect of the technology in the functional cure of HIV. More recently, eliminating CCR5 and CXCR4 in induced pluripotent stem cells (iPSCs) derived from patients and targeting the HIV genome have been successfully carried out in several laboratories. The outcome from these approaches bring us closer to the goal of eradicating HIV infection. For hemoglobinopathies the ability to produce iPSC-derived from patients with the correction of hemoglobin (HBB) mutations by CRISPR-Cas9 has been tested in a number of laboratories. These corrected iPSCs also show the potential to differentiate into mature erythrocytes expressing high-level and normal HBB. In light of the initial success of CRESPR-Cas9 in target mutated gene(s) in the iPSCs, a combination of genomic editing and autogenetic stem cell transplantation would be the best strategy for root treatment of the diseases, which could replace traditional allogeneic stem cell transplantation.

Hunt, C. P., C. W. Pouton, et al. "Characterising the developmental profile of hESC-derived medium spiny neuron progenitors and assessing mature neuron function using a CRISPR-generated human DARPP-32WT/eGFP-AMP reporter line." Neurochem Int. 2017 Jan 5. pii: S0197-0186(17)30003-7. doi: 10.1016/j.neuint.2017.01.003.

In the developing ventral telencephalon, cells of the lateral ganglionic eminence (LGE) give rise to all medium spiny neurons (MSNs). This development occurs in response to a highly orchestrated series of morphogenetic stimuli that pattern the resultant neurons as they develop. Striatal MSNs are characterised by expression of dopamine receptors, dopamine-and cyclic AMP-regulated phosphoprotein (DARPP32) and the neurotransmitter GABA. In this study, we demonstrate that fine tuning WNT and SHH signaling early in human embryonic stem cell differentiation can induce a subpallial progenitor molecular profile. Stimulation of TGFbeta signaling pathway by activin-A further supports patterning of progenitors to striatal precursors which adopt an LGE-specific gene signature. Moreover, we report that these MSNs also express markers associated with mature neuron function(cannabinoid, adenosine and dopamine receptors). To facilitate live-cell identification we generated a human embryonic stem cell line using CRISPR-mediated gene editing at the DARPP32 locus (DARPP32WT/eGFP-AMP-LacZ). The addition of dopamine to MSNs either increased, decreased or had no effect on intracellular calcium, indicating the presence of multiple dopamine receptor subtypes. In summary, we demonstrate greater control over early fate decisions using activin-A, Wnt and SHH to direct differentiation into MSNs. We also generate a DARPP32 reporter line that enables deeper pharmacological profiling and interrogation of complex receptor interactions in human MSNs.

Ishikawa, T., K. Imamura, et al. "Genetic and pharmacological correction of aberrant dopamine synthesis using patient iPSCs with BH4 metabolism disorders." Hum Mol Genet. 2016 Oct 18. pii: ddw339. doi: 10.1093/hmg/ddw339.

Dopamine (DA) is a neurotransmitter in the brain, playing a central role in several disease conditions, including tetrahydrobiopterin (BH4) metabolism disorders and Parkinson's disease (PD). BH4 metabolism disorders present a variety of clinical manifestations including motor disturbance via altered DA metabolism, since BH4 is a cofactor for tyrosine hydroxylase (TH), a rate-limiting enzyme for DA synthesis. Genetically, BH4 metabolism disorders are, in an autosomal recessive pattern, caused by a variant in genes encoding enzymes for BH4 synthesis or recycling, including 6-pyruvoyltetrahydropterin synthase (PTPS) or dihydropteridine reductase (DHPR), respectively. Although BH4 metabolism disorders and its metabolisms have been studied, it is unclear how gene variants cause aberrant DA synthesis in patient neurons. Here, we generated induced pluripotent stem cells (iPSCs) from BH4 metabolism disorder patients with PTPS or DHPR variants, corrected the gene variant in the iPSCs using the CRISPR/Cas9 system, and differentiated the BH4 metabolism disorder patient- and isogenic control iPSCs into midbrain DA neurons. We found that by the gene correction, the BH4 amount, TH protein level and extracellular DA level were restored in DA neuronal culture using PTPS deficiency iPSCs. Furthermore, the pharmacological correction by BH4 precursor sepiapterin treatment also improved the phenotypes of PTPS deficiency. These results suggest that patient iPSCs with BH4 metabolism disorders provide an opportunity for screening substances for treating aberrant DA synthesis-related disorders.

Jang, Y. Y. and Z. Ye "Gene correction in patient-specific iPSCs for therapy development and disease modeling." Hum Genet. 2016 Sep;135(9):1041-58. doi: 10.1007/s00439-016-1691-5. Epub 2016 Jun 2.

The discovery that mature cells can be reprogrammed to become pluripotent and the development of engineered endonucleases for enhancing genome editing are two of the most exciting and impactful technology advances in modern medicine and science. Human pluripotent stem cells have the potential to establish new model systems for studying human developmental biology and disease mechanisms. Gene correction in patient-specific iPSCs can also provide a novel source for autologous cell therapy. Although historically challenging, precise genome editing in human iPSCs is becoming more feasible with the development of new genome-editing tools, including ZFNs, TALENs, and CRISPR. iPSCs derived from patients of a variety of diseases have been edited to correct disease-associated mutations and to generate isogenic cell lines. After directed differentiation, many of the corrected iPSCs showed restored functionality and demonstrated their potential in cell replacement therapy. Genome-wide analyses of gene-corrected iPSCs have collectively demonstrated a high fidelity of the engineered endonucleases. Remaining challenges in clinical translation of these technologies include maintaining genome integrity of the iPSC clones and the differentiated cells. Given the rapid advances in genome-editing technologies, gene correction is no longer the bottleneck in developing iPSC-based gene and cell therapies; generating functional and transplantable cell types from iPSCs remains the biggest challenge needing to be addressed

by the research field.

Jiang, J., L. Zhang, et al. "Induction of site-specific chromosomal translocations in embryonic stem cells by CRISPR/Cas9." Sci Rep. 2016 Feb 22;6:21918. doi: 10.1038/srep21918.

Chromosomal translocation is the most common form of chromosomal abnormality and is often associated with congenital genetic disorders, infertility, and cancers. The lack of cellular and animal models for chromosomal translocations, however, has hampered our ability to understand the underlying disease mechanisms and to develop new therapies. Here, we show that site-specific chromosomal translocations can be generated in mouse embryonic stem cells (mESCs) via CRISPR/Cas9. Mouse ESCs carrying translocated chromosomes can be isolated and expanded to establish stable cell lines. Furthermore, chimeric mice can be generated by injecting these mESCs into host blastocysts. The establishment of ESC-based cellular and animal models of chromosomal translocation by CRISPR/Cas9 provides a powerful platform for understanding the effect of chromosomal translocation and for the development of new therapeutic strategies.

Jiang, L., J. Shan, et al. "Androgen/androgen receptor axis maintains and promotes cancer cell stemness through direct activation of Nanog transcription in hepatocellular carcinoma." Oncotarget. 2016 Jun 14;7(24):36814-36828. doi: 10.18632/oncotarget.9192.

Hepatocellular carcinoma (HCC) is one of the most common and malignant cancers. The HCC incidence gets a strong sexual dimorphism as men are the major sufferers in this disaster. Although several studies have uncovered the presentative correlation between the axis of androgen/androgen receptor (AR) and HCC incidence, the mechanism is still largely unknown. Cancer stem cells (CSCs) are a small subgroup of cancer cells contributing to multiple tumors malignant behaviors, which play an important role in oncogenesis of various cancers including HCC. However, whether androgen/AR axis involves in regulation of HCC cells stemness remains unclear. Our previous study had identified that the pluripotency factor Nanog is not only a stemness biomarker, but also a potent regulator of CSCs in HCC. In this study, we revealed androgen/AR axis can promote HCC cells stemness by transcriptional activation of Nanog expression through directly binding to its promoter. In HCC tissues, we found that AR expression was abnormal high and got correlation with Nanog. Then, by labeling cellular endogenous Nanog with green fluorescent protein (GFP) through CRISPR/Cas9 system, it verified the co-localization of AR and Nanog in HCC cells. With in vitro experiments, we demonstrated the axis can promote HCC cells stemness, which effect is in a Nanog-dependent manner and through activating its transcription. And the xenografted tumor experiments confirmed the axis effect on tumorigenesis facilitation in vivo. Above all, we revealed a new sight of androgen/AR axis roles in HCC and provided a potential way for suppressing the axis in HCC therapy.

Jin, L. F. and J. S. Li "Generation of genetically modified mice using CRISPR/Cas9 and haploid embryonic stem cell systems." Dongwuxue Yanjiu. 2016 Jul 18;37(4):205-13. doi: 10.13918/j.issn.2095-8137.2016.4.205.

With the development of high-throughput sequencing technology in the post-genomic era, researchers have concentrated their efforts on elucidating the relationships between genes and their corresponding functions. Recently, important progress has been achieved in the generation of genetically modified mice based on CRISPR/Cas9 and haploid embryonic stem cell (haESC) approaches, which provide new platforms for gene function analysis, human disease modeling, and gene therapy. Here, we review the CRISPR/Cas9 and haESC technology for the generation of genetically modified mice and discuss the key challenges in the application of these approaches.

Jung, C. J., J. Zhang, et al. "Efficient gene targeting in mouse zygotes mediated by CRISPR/Cas9-protein." Transgenic Res. 2016 Nov 30.

The CRISPR/Cas9 system has rapidly advanced targeted genome editing technologies. However, its efficiency in targeting with constructs in mouse zygotes via homology directed repair (HDR) remains low. Here, we systematically explored optimal parameters for targeting constructs in mouse zygotes via HDR using mouse embryonic stem cells as a model system. We characterized several parameters, including single guide RNA cleavage activity and the length and symmetry of homology arms in the construct, and we compared the targeting efficiency between Cas9, Cas9nickase, and dCas9-FokI. We then applied the optimized conditions to zygotes, delivering Cas9 as either mRNA or protein. We found that Cas9 nucleo-protein complex promotes highly efficient, multiplexed targeting of circular constructs containing reporter genes and floxed exons. This approach allows for a one-step zygote injection procedure targeting multiple genes to generate conditional alleles via homologous recombination, and simultaneous knockout of corresponding genes in non-targeted alleles via non-homologous end joining.

Kafer, G. R., X. Li, et al. "5-Hydroxymethylcytosine

Marks Sites of DNA Damage and Promotes Genome Stability." Cell Rep. 2016 Feb 16;14(6):1283-92. doi: 10.1016/j.celrep.2016.01.035. Epub 2016 Feb 4.

5-hydroxymethylcytosine (5hmC) is a DNA base created during active DNA demethylation by the recently discovered TET enzymes. 5hmC has essential roles in gene expression and differentiation. Here, we demonstrate that 5hmC also localizes to sites of DNA damage and repair. 5hmC accumulates at damage foci induced by aphidicolin and microirradiation and colocalizes with major DNA damage response proteins 53BP1 and gammaH2AX, revealing 5hmC as an epigenetic marker of DNA damage. Deficiency for the TET enzymes eliminates damage-induced 5hmC accumulation and elicits chromosome segregation defects in response to replication stress. Our results indicate that the TET enzymes and 5hmC play essential roles in ensuring genome integrity.

Kalebic, N., E. Taverna, et al. "CRISPR/Cas9-induced disruption of gene expression in mouse embryonic brain and single neural stem cells in vivo." EMBO Rep. 2016 Mar;17(3):338-48. doi: 10.15252/embr.201541715. Epub 2016 Jan 12.

We have applied the CRISPR/Cas9 system in vivo to disrupt gene expression in neural stem cells in the developing mammalian brain. Two days after in utero electroporation of a single plasmid encoding Cas9 and an appropriate guide RNA (gRNA) into the embryonic neocortex of Tis21::GFP knock-in mice, expression of GFP, which occurs specifically in neural stem cells committed to neurogenesis, was found to be nearly completely (approximately 90%) abolished in the progeny of the targeted cells. Importantly, upon in utero electroporation directly of recombinant Cas9/gRNA complex, near-maximal efficiency of disruption of GFP expression was achieved already after 24 h. Furthermore, by using microinjection of the Cas9 protein/gRNA complex into neural stem cells in organotypic slice culture, we obtained disruption of GFP expression within a single cell cycle. Finally, we used either Cas9 plasmid in utero electroporation or Cas9 protein complex microinjection to disrupt the expression of Eomes/Tbr2, a gene fundamental for neocortical neurogenesis. This resulted in a reduction in basal progenitors and an increase in neuronal differentiation. Thus, the present in vivo application of the CRISPR/Cas9 system in neural stem cells provides a rapid, efficient and enduring disruption of expression of specific genes to dissect their role in mammalian brain development.

Kalra, S., F. Montanaro, et al. "Can Human Pluripotent Stem Cell-Derived Cardiomyocytes Advance Understanding of Muscular Dystrophies?" J Neuromuscul Dis. 2016 Aug 30;3(3):309-332.

Muscular dystrophies (MDs) are clinically and molecularly a highly heterogeneous group of single-gene disorders that primarily affect striated muscles. Cardiac disease is present in several MDs where it is an important contributor to morbidity and mortality. Careful monitoring of cardiac issues is necessary but current management of cardiac involvement does not effectively protect from disease progression and cardiac failure. There is a critical need to gain new knowledge on the diverse molecular underpinnings of cardiac disease in MDs in order to guide cardiac treatment development and assist in reaching a clearer consensus on cardiac disease management in the clinic. Animal models are available for the majority of MDs and have been invaluable tools in probing disease mechanisms and in pre-clinical screens. However, there are recognized genetic, physiological, and structural differences between human and animal hearts that impact disease progression, manifestation, and response to pharmacological interventions. Therefore, there is a need to develop parallel human systems to model cardiac disease in MDs. This review discusses the current status of cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSC) to model cardiac disease, with a focus on Duchenne muscular dystrophy (DMD) and myotonic dystrophy (DM1). We seek to provide a balanced view of opportunities and limitations offered by this system in elucidating disease mechanisms pertinent to human cardiac physiology and as a platform for treatment development or refinement.

Kawasaki, Y., H. Oda, et al. "Pluripotent cell-based phenotypic dissection identifies a high-frequency somatic NLRC4 mutation as a cause of autoinflammation." Arthritis Rheumatol. 2016 Oct 27. doi: 10.1002/art.39960.

Objective To elucidate the genetic background of a patient with neonatal-onset multisystem inflammatory disease (NOMID) who does not carry any NLRP3 mutation. Methods A Japanese male diagnosed as NOMID was recruited. The patient had no NLRP3 mutation even as low frequency mosaicism. We performed whole exome sequencing (WES) of the patient and his parents. Induced pluripotent stem cells (iPSCs) were established from the fibroblasts of the patient. iPSCs were then differentiated into monocytic lineage to evaluate the cytokine profile. Results We established multiple iPSC clones from an NOMID patient and incidentally found that the phenotype of monocytes from iPSC clones were heterogeneous, and could be grouped into "diseased" and "normal" phenotype. Because each iPSC clone was derived from a single somatic cell, we hypothesized the patient had somatic mosaicism of an

IL-1beta-related gene. WES of both representative iPSC clones and patient's blood identified a novel heterozygous NLRC4 mutation, p.T177A (c.529A>G), as a specific mutation in "diseased" iPSC clones. Knockout of the NLRC4 gene using CRISPR/Cas9 system in a mutant iPSC clone abrogated the pathogenic phenotype. Conclusion We concluded the patient as having somatic mosaicism of a novel NLRC4 mutation. To our knowledge, this is the first case showing somatic NLRC4 mutation causes autoinflammatory symptoms compatible to NOMID. The present study demonstrates the significance of prospective genetic screening combined with iPSC-based phenotypic dissection for individualized diagnoses. This article is protected by copyright. All rights reserved.

Kehler, J., M. Greco, et al. "RNA-Generated and Gene-Edited Induced Pluripotent Stem Cells for Disease Modeling and Therapy." J Cell Physiol. 2016 Sep 15. doi: 10.1002/jcp.25597.

Cellular reprogramming by epigenomic remodeling of chromatin holds great promise in the field of human regenerative medicine. As an example, human-induced Pluripotent Stem Cells (iPSCs) obtained by reprograming of patient somatic cells are sufficiently similar to embryonic stem cells (ESCs) and can generate all cell types of the human body. Clinical use of iPSCs is dependent on methods that do not utilize genome altering transgenic technologies that are potentially unsafe and ethically unacceptable. Transient delivery of exogenous RNA into cells provides a safer reprogramming system to transgenic approaches that rely on exogenous DNA or viral vectors. RNA reprogramming may prove to be more suitable for clinical applications and provide stable starting cell lines for gene-editing, isolation, and characterization of patient iPSC lines. The introduction and rapid evolution of CRISPR/Cas9 gene-editing systems has provided a readily accessible research tool to perform functional human genetic experiments. Similar to RNA reprogramming, transient delivery of mRNA encoding Cas9 in combination with guide RNA sequences to target specific points in the genome eliminates the risk of potential integration of Cas9 plasmid constructs. We present optimized RNA-based laboratory procedure for making and editing iPSCs. In the near-term these two powerful technologies are being harnessed to dissect mechanisms of human development and disease in vitro, supporting both basic, and translational research. J. Cell. Physiol. 9999: 1-8, 2016. (c) 2016 Wiley Periodicals, Inc.

Kelava, I. and M. A. Lancaster "Dishing out mini-brains: Current progress and future prospects in brain organoid research." Dev Biol. 2016 Dec 15;420(2):199-209. doi: 10.1016/j.ydbio.2016.06.037. Epub 2016 Jul 9.

The ability to model human brain development in vitro represents an important step in our study of developmental processes and neurological disorders. Protocols that utilize human embryonic and induced pluripotent stem cells can now generate organoids which faithfully recapitulate, on a cell-biological and gene expression level, the early period of human embryonic and fetal brain development. In combination with novel gene editing tools, such as CRISPR, these methods represent an unprecedented model system in the field of mammalian neural development. In this review, we focus on the similarities of current organoid methods to in vivo brain development, discuss their limitations and potential improvements, and explore the future venues of brain organoid research.

Kim, B. Y., S. Jeong, et al. "Concurrent progress of reprogramming and gene correction to overcome therapeutic limitation of mutant ALK2-iPSC." Exp Mol Med. 2016 Jun 3;48(6):e237. doi: 10.1038/emm.2016.43.

Fibrodysplasia ossificans progressiva (FOP) syndrome is caused by mutation of the gene ACVR1, encoding a constitutive active bone morphogenetic protein type I receptor (also called ALK2) to induce heterotopic ossification in the patient. To genetically correct it, we attempted to generate the mutant ALK2-iPSCs (mALK2-iPSCs) from FOP-human dermal fibroblasts. However, the mALK2 leads to inhibitory pluripotency maintenance, or impaired clonogenic potential after single-cell dissociation as an inevitable step, which applies gene-correction tools to induced pluripotent stem cells (iPSCs). Thus, current iPSC-based gene therapy approach reveals a limitation that is not readily applicable to iPSCs with ALK2 mutation. Here we developed a simplified one-step procedure by simultaneously introducing reprogramming and gene-editing components into human fibroblasts derived from patient with FOP syndrome, and genetically treated it. The mixtures of reprogramming and gene-editing components are composed of reprogramming episomal vectors, CRISPR/Cas9-expressing vectors and single-stranded oligodeoxynucleotide harboring normal base to correct ALK2 c.617G>A. The one-step-mediated ALK2 gene-corrected iPSCs restored global gene expression pattern, as well as mineralization to the extent of normal iPSCs. This procedure not only helps save time, labor and costs but also opens up a new paradigm that is beyond the current application of gene-editing methodologies, which is hampered by inhibitory pluripotency-maintenance requirements, or vulnerability of single-cell-dissociated iPSCs.

Koferle, A., K. Worf, et al. "CORALINA: a universal method for the generation of gRNA libraries for CRISPR-based screening." BMC Genomics. 2016 Nov 14;17(1):917.

BACKGROUND: The bacterial CRISPR system is fast becoming the most popular genetic and epigenetic engineering tool due to its universal applicability and adaptability. The desire to deploy CRISPR-based methods in a large variety of species and contexts has created an urgent need for the development of easy, time- and cost-effective methods enabling large-scale screening approaches. RESULTS: Here we describe CORALINA (comprehensive gRNA library generation through controlled nuclease activity), a method for the generation of comprehensive gRNA libraries for CRISPR-based screens. CORALINA gRNA libraries can be derived from any source of DNA without the need of complex oligonucleotide synthesis. We show the utility of CORALINA for human and mouse genomic DNA, its reproducibility in covering the most relevant genomic features including regulatory, coding and non-coding sequences and confirm the functionality of CORALINA generated gRNAs. CONCLUSIONS: The simplicity and cost-effectiveness make CORALINA suitable for any experimental system. The unprecedented sequence complexities obtainable with CORALINA libraries are a necessary pre-requisite for less biased large scale genomic and epigenomic screens.

Koo, T. and J. S. Kim "Therapeutic applications of CRISPR RNA-guided genome editing." Brief Funct Genomics. 2016 Aug 25. pii: elw032.

The rapid development of programmable nuclease-based genome editing technologies has enabled targeted gene disruption and correction both in vitro and in vivo This revolution opens up the possibility of precise genome editing at target genomic sites to modulate gene function in animals and plants. Among several programmable nucleases, the type II clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated nuclease 9 (Cas9) system has progressed remarkably in recent years, leading to its widespread use in research, medicine and biotechnology. In particular, CRISPR-Cas9 shows highly efficient gene editing activity for therapeutic purposes in systems ranging from patient stem cells to animal models. However, the development of therapeutic approaches and delivery methods remains a great challenge for biomedical applications. Herein, we review therapeutic applications that use the CRISPR-Cas9 system and discuss the possibilities and challenges ahead.

Labott, A. T. and V. Lopez-Pajares "Epidermal differentiation gene regulatory networks controlled by MAF and MAFB." Cell Cycle. 2016 Jun 2;15(11):1405-9. doi: 10.1080/15384101.2016.1172148. Epub 2016 Apr 20.

Numerous regulatory factors in epidermal differentiation and their role in regulating different cell states have been identified in recent years. However, the genetic interactions between these regulators over the dynamic course of differentiation have not been studied. In this Extra-View article, we review recent work by Lopez-Pajares et al. that explores a new regulatory network in epidermal differentiation. They analyze the changing transcriptome throughout epidermal regeneration to identify 3 separate gene sets enriched in the progenitor, early and late differentiation states. Using expression module mapping, MAF along with MAFB, are identified as transcription factors essential for epidermal differentiation. Through double knock-down of MAF:MAFB using siRNA and CRISPR/Cas9-mediated knockout, epidermal differentiation was shown to be impaired both in-vitro and in-vivo, confirming MAF:MAFB's role to activate genes that drive differentiation. Lopez-Pajares and collaborators integrated 42 published regulator gene sets and the MAF:MAFB gene set into the dynamic differentiation gene expression landscape and found that lncRNAs TINCR and ANCR act as upstream regulators of MAF:MAFB. Furthermore, ChIP-seq analysis of MAF:MAFB identified key transcription factor genes linked to epidermal differentiation as downstream effectors. Combined, these findings illustrate a dynamically regulated network with MAF:MAFB as a crucial link for progenitor gene repression and differentiation gene activation.

Li, C., L. Ding, et al. "Novel HDAd/EBV Reprogramming Vector and Highly Efficient Ad/CRISPR-Cas Sickle Cell Disease Gene Correction." Sci Rep. 2016 Jul 27;6:30422. doi: 10.1038/srep30422.

CRISPR/Cas enhanced correction of the sickle cell disease (SCD) genetic defect in patient-specific induced Pluripotent Stem Cells (iPSCs) provides a potential gene therapy for this debilitating disease. An advantage of this approach is that corrected iPSCs that are free of off-target modifications can be identified before differentiating the cells into hematopoietic progenitors for transplantation. In order for this approach to be practical, iPSC generation must be rapid and efficient. Therefore, we developed a novel helper-dependent adenovirus/Epstein-Barr virus (HDAd/EBV) hybrid reprogramming vector, rCLAE-R6, that delivers six reprogramming factors episomally. HDAd/EBV transduction of keratinocytes from SCD patients

resulted in footprint-free iPSCs with high efficiency. Subsequently, the sickle mutation was corrected by delivering CRISPR/Cas9 with adenovirus followed by nucleoporation with a 70 nt single-stranded oligodeoxynucleotide (ssODN) correction template. Correction efficiencies of up to 67.9% (beta(A)/[beta(S)+beta(A)]) were obtained. Whole-genome sequencing (WGS) of corrected iPSC lines demonstrated no CRISPR/Cas modifications in 1467 potential off-target sites and no modifications in tumor suppressor genes or other genes associated with pathologies. These results demonstrate that adenoviral delivery of reprogramming factors and CRISPR/Cas provides a rapid and efficient method of deriving gene-corrected, patient-specific iPSCs for therapeutic applications.

Li, C. H., L. Z. Yan, et al. "Long-term propagation of tree shrew spermatogonial stem cells in culture and successful generation of transgenic offspring." Cell Res. 2016 Dec 23. doi: 10.1038/cr.2016.156.

Tree shrews have a close relationship to primates and have many advantages over rodents in biomedical research. However, the lack of gene manipulation methods has hindered the wider use of this animal. Spermatogonial stem cells (SSCs) have been successfully expanded in culture to permit sophisticated gene editing in the mouse and rat. Here, we describe a culture system for the long-term expansion of tree shrew SSCs without the loss of stem cell properties. In our study, thymus cell antigen 1 was used to enrich tree shrew SSCs. RNA-sequencing analysis revealed that the Wnt/beta-catenin signaling pathway was active in undifferentiated SSCs, but was downregulated upon the initiation of SSC differentiation. Exposure of tree shrew primary SSCs to recombinant Wnt3a protein during the initial passages of culture enhanced the survival of SSCs. Use of tree shrew Sertoli cells, but not mouse embryonic fibroblasts, as feeder was found to be necessary for tree shrew SSC proliferation, leading to a robust cell expansion and long-term culture. The expanded tree shrew SSCs were transfected with enhanced green fluorescent protein (EGFP)-expressing lentiviral vectors. After transplantation into sterilized adult male tree shrew's testes, the EGFP-tagged SSCs were able to restore spermatogenesis and successfully generate transgenic offspring. Moreover, these SSCs were suitable for the CRISPR/Cas9-mediated gene modification. The development of a culture system to expand tree shrew SSCs in combination with a gene editing approach paves the way for precise genome manipulation using the tree shrew.Cell Research advance online publication 23 December 2016; doi:10.1038/cr.2016.156.

Li, H., S. L. Bielas, et al. "Biallelic Mutations in Citron Kinase Link Mitotic Cytokinesis to Human Primary Microcephaly." Am J Hum Genet. 2016 Aug 4;99(2):501-10. doi: 10.1016/j.ajhg.2016.07.004. Epub 2016 Jul 21.

Cell division terminates with cytokinesis and cellular separation. Autosomal-recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by a reduction in brain and head size at birth in addition to non-progressive intellectual disability. MCPH is genetically heterogeneous, and 16 loci are known to be associated with loss-of-function mutations predominantly affecting centrosomal-associated proteins, but the multiple roles of centrosomes in cellular function has left questions about etiology. Here, we identified three families affected by homozygous missense mutations in CIT, encoding citron rho-interacting kinase (CIT), which has established roles in cytokinesis. All mutations caused substitution of conserved amino acid residues in the kinase domain and impaired kinase activity. Neural progenitors that were differentiated from induced pluripotent stem cells (iPSCs) derived from individuals with these mutations exhibited abnormal cytokinesis with delayed mitosis, multipolar spindles, and increased apoptosis, rescued by CRISPR/Cas9 genome editing. Our results highlight the importance of cytokinesis in the pathology of primary microcephaly.

Liang, X., J. Potter, et al. "Enhanced CRISPR/Cas9-mediated precise genome editing by improved design and delivery of gRNA, Cas9 nuclease, and donor DNA." J Biotechnol. 2017 Jan 10;241:136-146. doi: 10.1016/j.jbiotec.2016.11.011. Epub 2016 Nov 11.

While CRISPR-based gene knock out in mammalian cells has proven to be very efficient, precise insertion of genetic elements via the cellular homology directed repair (HDR) pathway remains a rate-limiting step to seamless genome editing. Under the conditions described here, we achieved up to 56% targeted integration efficiency with up to a six-nucleotide insertion in HEK293 cells. In induced pluripotent stem cells (iPSCs), we achieved precise genome editing rates of up to 45% by co-delivering the Cas9 RNP and donor DNA. In addition, the use of a short double stranded DNA oligonucleotide with 3' overhangs allowed integration of a longer FLAG epitope tag along with a restriction site at rates of up to 50%. We propose a model that favors the design of donor DNAs with the change as close to the cleavage site as possible. For small changes such as SNPs or short insertions, asymmetric single stranded donor molecules with 30 base homology arms 3' to the insertion/repair cassette and greater than 40 bases of homology on the 5' end seems to be favored. For larger

insertions such as an epitope tag, a dsDNA donor with protruding 3' homology arms of 30 bases is favored. In both cases, protecting the ends of the donor DNA with phosphorothioate modifications improves the editing efficiency.

Limpitikul, W. B., I. E. Dick, et al. "A Precision Medicine Approach to the Rescue of Function on Malignant Calmodulinopathic Long-QT Syndrome." Circ Res. 2017 Jan 6;120(1):39-48. doi: 10.1161/CIRCRESAHA.116.309283. Epub 2016 Oct 20.

RATIONALE: Calmodulinopathies comprise a new category of potentially life-threatening genetic arrhythmia syndromes capable of producing severe long-QT syndrome (LQTS) with mutations involving CALM1, CALM2, or CALM3. The underlying basis of this form of LQTS is a disruption of Ca2+/calmodulin (CaM)-dependent inactivation of L-type Ca2+ channels. OBJECTIVE: To gain insight into the mechanistic underpinnings of calmodulinopathies and devise new therapeutic strategies for the treatment of this form of LQTS. METHODS AND RESULTS: We generated and characterized the functional properties of induced pluripotent stem cell-derived cardiomyocytes from a patient with D130G-CALM2-mediated LQTS, thus creating a platform with which to devise and test novel therapeutic strategies. The patient-derived induced pluripotent stem cell-derived cardiomyocytes display (1) significantly prolonged action potentials, (2) disrupted Ca2+ cycling properties, and (3) diminished Ca2+/CaM-dependent inactivation of L-type Ca2+ channels. Next, taking advantage of the fact that calmodulinopathy patients harbor a mutation in only 1 of 6 redundant CaM-encoding alleles, we devised a strategy using CRISPR interference to selectively suppress the mutant gene while sparing the wild-type counterparts. Indeed, suppression of CALM2 expression produced a functional rescue in induced pluripotent stem cell-derived cardiomyocytes with D130G-CALM2, as shown by the normalization of action potential duration and Ca2+/CaM-dependent inactivation after treatment. Moreover, CRISPR interference can be designed to achieve selective knockdown of any of the 3 CALM genes, making it a generalizable therapeutic strategy for any calmodulinopathy. CONCLUSIONS: Overall, this therapeutic strategy holds great promise for calmodulinopathy patients as it represents a generalizable intervention capable of specifically altering CaM expression and potentially attenuating LQTS-triggered cardiac events, thus initiating a path toward precision medicine.

Liu, T., J. K. Shen, et al. "Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma." Cancer Lett. 2016 Apr 1;373(1):109-18. doi: 10.1016/j.canlet.2016.01.030. Epub 2016 Jan 21.

Sarcomas include some of the most aggressive tumors and typically respond poorly to chemotherapy. In recent years, specific gene fusion/mutations and gene over-expression/activation have been shown to drive sarcoma pathogenesis and development. These emerging genomic alterations may provide targets for novel therapeutic strategies and have the potential to transform sarcoma patient care. The RNA-guided nuclease CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein-9 nuclease) is a convenient and versatile platform for site-specific genome editing and epigenome targeted modulation. Given that sarcoma is believed to develop as a result of genetic alterations in mesenchymal progenitor/stem cells, CRISPR-Cas9 genome editing technologies hold extensive application potentials in sarcoma models and therapies. We review the development and mechanisms of the CRISPR-Cas9 system in genome editing and introduce its application in sarcoma research and potential therapy in clinic. Additionally, we propose future directions and discuss the challenges faced with these applications, providing concise and enlightening information for readers interested in this area.

Liu, Y. and W. Deng "Reverse engineering human neurodegenerative disease using pluripotent stem cell technology." Brain Res. 2016 May 1;1638(Pt A):30-41. doi: 10.1016/j.brainres.2015.09.023. Epub 2015 Sep 28.

With the technology of reprogramming somatic cells by introducing defined transcription factors that enables the generation of "induced pluripotent stem cells (iPSCs)" with pluripotency comparable to that of embryonic stem cells (ESCs), it has become possible to use this technology to produce various cells and tissues that have been difficult to obtain from living bodies. This advancement is bringing forth rapid progress in iPSC-based disease modeling, drug screening, and regenerative medicine. More and more studies have demonstrated that phenotypes of adult-onset neurodegenerative disorders could be rather faithfully recapitulated in iPSC-derived neural cell cultures. Moreover, despite the adult-onset nature of the diseases, pathogenic phenotypes and cellular abnormalities often exist in early developmental stages, providing new "windows of opportunity" for understanding mechanisms underlying neurodegenerative disorders and for discovering new medicines. The cell reprogramming technology enables a reverse engineering approach for modeling the cellular degenerative phenotypes of a

wide range of human disorders. An excellent example is the study of the human neurodegenerative disease amyotrophic lateral sclerosis (ALS) using iPSCs. ALS is a progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs), culminating in muscle wasting and death from respiratory failure. The iPSC approach provides innovative cell culture platforms to serve as ALS patient-derived model systems. Researchers have converted iPSCs derived from ALS patients into MNs and various types of glial cells, all of which are involved in ALS, to study the disease. The iPSC technology could be used to determine the role of specific genetic factors to track down what's wrong in the neurodegenerative disease process in the "disease-in-a-dish" model. Meanwhile, parallel experiments of targeting the same specific genes in human ESCs could also be performed to control and to complement the iPSC-based approach for ALS disease modeling studies. Much knowledge has been generated from the study of both ALS iPSCs and ESCs. As these methods have advantages and disadvantages that should be balanced on experimental design in order for them to complement one another, combining the diverse methods would help build an expanded knowledge of ALS pathophysiology. The goals are to reverse engineer the human disease using ESCs and iPSCs, generate lineage reporter lines and in vitro disease models, target disease related genes, in order to better understand the molecular and cellular mechanisms of differentiation regulation along neural (neuronal versus glial) lineages, to unravel the pathogenesis of the neurodegenerative disease, and to provide appropriate cell sources for replacement therapy. This article is part of a Special Issue entitled SI: PSC and the brain.

Liu, Y., J. Lin, et al. "PINK1 is required for timely cell-type specific mitochondrial clearance during Drosophila midgut metamorphosis." Dev Biol. 2016 Nov 15;419(2):357-372. doi: 10.1016/j.ydbio.2016.08.028. Epub 2016 Aug 26.

Mitophagy is the selective degradation of mitochondria by autophagy, which is an important mitochondrial quality and quantity control process. During Drosophila metamorphosis, the degradation of midgut involves a large change in length and organization, which is mediated by autophagy. Here we noticed a cell-type specific mitochondrial clearance process that occurs in enterocytes (ECs), while most mitochondria remain in intestinal stem cells (ISCs) during metamorphosis. Although PINK1/PARKIN represent the canonical pathway for the elimination of impaired mitochondria in varied pathological conditions, their roles in developmental processes or normal physiological conditions have been less studied. To examine the potential contribution of PINK1 in developmental processes, we monitored the dynamic expression pattern of PINK1 in the midgut development by taking advantage of a newly CRISPR/Cas9 generated knock-in fly strain expressing PINK1-mCherry fusion protein that presumably recapitulates the endogenous expression pattern of PINK1. We disclosed a spatiotemporal correlation between the expression pattern of PINK1 and the mitochondrial clearance or persistence in ECs or ISCs respectively. By mosaic genetic analysis, we then demonstrated that PINK1 and PARKIN function epistatically to mediate the specific timely removal of mitochondria, and are involved in global autophagy in ECs during Drosophila midgut metamorphosis, with kinase-dead PINK1 exerting dominant negative effects. Taken together, our studies concluded that the PINK1/PARKIN is crucial for timely cell-type specific mitophagy under physiological conditions and demonstrated again that Drosophila midgut metamorphosis might serve as an elegant in vivo model to study autophagy.

Liu, Z., Y. Hui, et al. "Efficient CRISPR/Cas9-Mediated Versatile, Predictable, and Donor-Free Gene Knockout in Human Pluripotent Stem Cells." Stem Cell Reports. 2016 Sep 13;7(3):496-507. doi: 10.1016/j.stemcr.2016.07.021. Epub 2016 Sep 1.

Loss-of-function studies in human pluripotent stem cells (hPSCs) require efficient methodologies for lesion of genes of interest. Here, we introduce a donor-free paired gRNA-guided CRISPR/Cas9 knockout strategy (paired-KO) for efficient and rapid gene ablation in hPSCs. Through paired-KO, we succeeded in targeting all genes of interest with high biallelic targeting efficiencies. More importantly, during paired-KO, the cleaved DNA was repaired mostly through direct end joining without insertions/deletions (precise ligation), and thus makes the lesion product predictable. The paired-KO remained highly efficient for one-step targeting of multiple genes and was also efficient for targeting of microRNA, while for long non-coding RNA over 8 kb, cleavage of a short fragment of the core promoter region was sufficient to eradicate downstream gene transcription. This work suggests that the paired-KO strategy is a simple and robust system for loss-of-function studies for both coding and non-coding genes in hPSCs.

Lu, C., Y. Yang, et al. "Role of circadian gene Clock during differentiation of mouse pluripotent stem cells." Protein Cell. 2016 Nov;7(11):820-832. Epub 2016 Sep 23.

Biological rhythms controlled by the circadian clock are absent in embryonic stem cells (ESCs). However, they start to develop during the

differentiation of pluripotent ESCs to downstream cells. Conversely, biological rhythms in adult somatic cells disappear when they are reprogrammed into induced pluripotent stem cells (iPSCs). These studies indicated that the development of biological rhythms in ESCs might be closely associated with the maintenance and differentiation of ESCs. The core circadian gene Clock is essential for regulation of biological rhythms. Its role in the development of biological rhythms of ESCs is totally unknown. Here, we used CRISPR/CAS9-mediated genetic editing techniques, to completely knock out the Clock expression in mouse ESCs. By AP, teratoma formation, quantitative real-time PCR and Immunofluorescent staining, we did not find any difference between Clock knockout mESCs and wild type mESCs in morphology and pluripotent capability under the pluripotent state. In brief, these data indicated Clock did not influence the maintaining of pluripotent state. However, they exhibited decreased proliferation and increased apoptosis. Furthermore, the biological rhythms failed to develop in Clock knockout mESCs after spontaneous differentiation, which indicated that there was no compensational factor in most peripheral tissues as described in mice models before (DeBruyne et al., 2007b). After spontaneous differentiation, loss of CLOCK protein due to Clock gene silencing induced spontaneous differentiation of mESCs, indicating an exit from the pluripotent state, or its differentiating ability. Our findings indicate that the core circadian gene Clock may be essential during normal mESCs differentiation by regulating mESCs proliferation, apoptosis and activity.

Mahony, C. B., R. J. Fish, et al. "tfec controls the hematopoietic stem cell vascular niche during zebrafish embryogenesis." Blood. 2016 Sep 8;128(10):1336-45. doi: 10.1182/blood-2016-04-710137. Epub 2016 Jul 11.

In mammals, embryonic hematopoiesis occurs in successive waves, culminating with the emergence of hematopoietic stem cells (HSCs) in the aorta. HSCs first migrate to the fetal liver (FL), where they expand, before they seed the bone marrow niche, where they will sustain hematopoiesis throughout adulthood. In zebrafish, HSCs emerge from the dorsal aorta and colonize the caudal hematopoietic tissue (CHT). Recent studies showed that they interact with endothelial cells (ECs), where they expand, before they reach their ultimate niche, the kidney marrow. We identified tfec, a transcription factor from the mitf family, which is highly enriched in caudal endothelial cells (cECs) at the time of HSC colonization in the CHT. Gain-of-function assays indicate that tfec is capable of expanding HSC-derived hematopoiesis in a non-cell-autonomous fashion. Furthermore, tfec mutants (generated by CRISPR/Cas9) showed reduced hematopoiesis in the CHT, leading to anemia. Tfec mediates these changes by increasing the expression of several cytokines in cECs from the CHT niche. Among these, we found kitlgb, which could rescue the loss of HSCs observed in tfec mutants. We conclude that tfec plays an important role in the niche to expand hematopoietic progenitors through the modulation of several cytokines. The full comprehension of the mechanisms induced by tfec will represent an important milestone toward the expansion of HSCs for regenerative purposes.

Mandegar, M. A., N. Huebsch, et al. "CRISPR Interference Efficiently Induces Specific and Reversible Gene Silencing in Human iPSCs." Cell Stem Cell. 2016 Apr 7;18(4):541-53. doi: 10.1016/j.stem.2016.01.022. Epub 2016 Mar 10.

Developing technologies for efficient and scalable disruption of gene expression will provide powerful tools for studying gene function, developmental pathways, and disease mechanisms. Here, we develop clustered regularly interspaced short palindromic repeat interference (CRISPRi) to repress gene expression in human induced pluripotent stem cells (iPSCs). CRISPRi, in which a doxycycline-inducible deactivated Cas9 is fused to a KRAB repression domain, can specifically and reversibly inhibit gene expression in iPSCs and iPSC-derived cardiac progenitors, cardiomyocytes, and T lymphocytes. This gene repression system is tunable and has the potential to silence single alleles. Compared with CRISPR nuclease (CRISPRn), CRISPRi gene repression is more efficient and homogenous across cell populations. The CRISPRi system in iPSCs provides a powerful platform to perform genome-scale screens in a wide range of iPSC-derived cell types, dissect developmental pathways, and model disease.

Mansouri, M., I. Bellon-Echeverria, et al. "Highly efficient baculovirus-mediated multigene delivery in primary cells." Nat Commun. 2016 May 4;7:11529. doi: 10.1038/ncomms11529.

Multigene delivery and subsequent cellular expression is emerging as a key technology required in diverse research fields including, synthetic and structural biology, cellular reprogramming and functional pharmaceutical screening. Current viral delivery systems such as retro- and adenoviruses suffer from limited DNA cargo capacity, thus impeding unrestricted multigene expression. We developed MultiPrime, a modular, non-cytotoxic, non-integrating, baculovirus-based vector system expediting highly efficient transient multigene expression from a variety of promoters. MultiPrime viruses efficiently transduce

a wide range of cell types, including non-dividing primary neurons and induced-pluripotent stem cells (iPS). We show that MultiPrime can be used for reprogramming, and for genome editing and engineering by CRISPR/Cas9. Moreover, we implemented dual-host-specific cassettes enabling multiprotein expression in insect and mammalian cells using a single reagent. Our experiments establish MultiPrime as a powerful and highly efficient tool, to deliver multiple genes for a wide range of applications in primary and established mammalian cells.

Mason, J. O. and D. J. Price "Building brains in a dish: Prospects for growing cerebral organoids from stem cells." Neuroscience. 2016 Oct 15;334:105-118. doi: 10.1016/j.neuroscience.2016.07.048. Epub 2016 Aug 6.

The recent development of organoid techniques, in which embryonic brain-like tissue can be grown from human or mouse stem cells in vitro offers the potential to transform the way in which brain development is studied. In this review, we summarize key aspects of the embryonic development of mammalian forebrains, focussing in particular on the cerebral cortex and highlight significant differences between mouse and primates, including human. We discuss recent work using cerebral organoids that has revealed key similarities and differences between their development and that of the brain in vivo. Finally, we outline the ways in which cerebral organoids can be used in combination with CRISPR/Cas9 genome editing to unravel genetic mechanisms that control embryonic development of the cerebral cortex, how this can help us understand the causes of neurodevelopmental disorders and some of the key challenges which will have to be resolved before organoids can become a mainstream tool to study brain development.

Matsa, E., P. W. Burridge, et al. "Transcriptome Profiling of Patient-Specific Human iPSC-Cardiomyocytes Predicts Individual Drug Safety and Efficacy Responses In Vitro." Cell Stem Cell. 2016 Sep 1;19(3):311-25. doi: 10.1016/j.stem.2016.07.006. Epub 2016 Aug 18.

Understanding individual susceptibility to drug-induced cardiotoxicity is key to improving patient safety and preventing drug attrition. Human induced pluripotent stem cells (hiPSCs) enable the study of pharmacological and toxicological responses in patient-specific cardiomyocytes (CMs) and may serve as preclinical platforms for precision medicine. Transcriptome profiling in hiPSC-CMs from seven individuals lacking known cardiovascular disease-associated mutations and in three isogenic human heart tissue and hiPSC-CM pairs showed greater inter-patient variation than intra-patient variation, verifying that reprogramming and differentiation preserve patient-specific gene expression, particularly in metabolic and stress-response genes. Transcriptome-based toxicology analysis predicted and risk-stratified patient-specific susceptibility to cardiotoxicity, and functional assays in hiPSC-CMs using tacrolimus and rosiglitazone, drugs targeting pathways predicted to produce cardiotoxicity, validated inter-patient differential responses. CRISPR/Cas9-mediated pathway correction prevented drug-induced cardiotoxicity. Our data suggest that hiPSC-CMs can be used in vitro to predict and validate patient-specific drug safety and efficacy, potentially enabling future clinical approaches to precision medicine.

Matsui, T., V. Nieto-Estevez, et al. "RB controls growth, survival, and neuronal migration in human cerebral organoids." Development. 2017 Jan 13. pii: dev.143636. doi: 10.1242/dev.143636.

Retinoblastoma (RB) is a tumor suppressor gene which regulates cell cycle entry to S phase via E2F transcription factors. Using knockout (KO) mice, it has been described that Rb plays a role in cell migration and differentiation in developing and adult brain as well as apoptosis. In addition, the RB family is required for the self-renewal and survival of human embryonic stem cells (ESCs). However, little is known about the role of this gene in human brain development. Here, we investigated the role of RB in cerebral organoids from human ESCs deficient for RB. We showed that RB is expressed abundantly in neural stem/progenitor cells in organoids at 15 and 28 days in culture. Our results revealed that the loss of RB promotes S phase entry of DCX+ cells and increases apoptosis of Sox2+ neural stem/progenitor cells, DCX+ and Tuj1+ neurons, which was associated with the upregulation of CYCLIN A2 and BAX genes. Moreover, we observed aberrant Tuj1+ neuronal migration in RB-KO organoids, and upregulation of the VLDLR gene, a receptor important in Reelin signaling. Interestingly, ectopically localized Tuj1+ cells were also found in teratomas from RB-KO human ESCs. These results suggest that RB gene has critical roles in human brain development.

McKenna, A., G. M. Findlay, et al. "Whole-organism lineage tracing by combinatorial and cumulative genome editing." Science. 2016 Jul 29;353(6298):aaf7907. doi: 10.1126/science.aaf7907. Epub 2016 May 26.

Multicellular systems develop from single cells through distinct lineages. However, current lineage-tracing approaches scale poorly to whole, complex organisms. Here, we use genome editing to progressively introduce and accumulate diverse mutations in a DNA barcode over multiple rounds of

cell division. The barcode, an array of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 target sites, marks cells and enables the elucidation of lineage relationships via the patterns of mutations shared between cells. In cell culture and zebrafish, we show that rates and patterns of editing are tunable and that thousands of lineage-informative barcode alleles can be generated. By sampling hundreds of thousands of cells from individual zebrafish, we find that most cells in adult organs derive from relatively few embryonic progenitors. In future analyses, genome editing of synthetic target arrays for lineage tracing (GESTALT) can be used to generate large-scale maps of cell lineage in multicellular systems for normal development and disease.

Mendoza-Parra, M. A., V. Malysheva, et al. "Reconstructed cell fate-regulatory programs in stem cells reveal hierarchies and key factors of neurogenesis." Genome Res. 2016 Nov;26(11):1505-1519. Epub 2016 Sep 20.

Cell lineages, which shape the body architecture and specify cell functions, derive from the integration of a plethora of cell intrinsic and extrinsic signals. These signals trigger a multiplicity of decisions at several levels to modulate the activity of dynamic gene regulatory networks (GRNs), which ensure both general and cell-specific functions within a given lineage, thereby establishing cell fates. Significant knowledge about these events and the involved key drivers comes from homogeneous cell differentiation models. Even a single chemical trigger, such as the morphogen all-trans retinoic acid (RA), can induce the complex network of gene-regulatory decisions that matures a stem/precursor cell to a particular step within a given lineage. Here we have dissected the GRNs involved in the RA-induced neuronal or endodermal cell fate specification by integrating dynamic RXRA binding, chromatin accessibility, epigenetic promoter epigenetic status, and the transcriptional activity inferred from RNA polymerase II mapping and transcription profiling. Our data reveal how RA induces a network of transcription factors (TFs), which direct the temporal organization of cognate GRNs, thereby driving neuronal/endodermal cell fate specification. Modeling signal transduction propagation using the reconstructed GRNs indicated critical TFs for neuronal cell fate specification, which were confirmed by CRISPR/Cas9-mediated genome editing. Overall, this study demonstrates that a systems view of cell fate specification combined with computational signal transduction models provides the necessary insight in cellular plasticity for cell fate engineering. The present integrated approach can be used to monitor the in vitro capacity of (engineered) cells/tissues to establish cell lineages for regenerative medicine.

Merkert, S. and U. Martin "Site-Specific Genome Engineering in Human Pluripotent Stem Cells." Int J Mol Sci. 2016 Jun 24;17(7). pii: E1000. doi: 10.3390/ijms17071000.

The possibility to generate patient-specific induced pluripotent stem cells (iPSCs) offers an unprecedented potential of applications in clinical therapy and medical research. Human iPSCs and their differentiated derivatives are tools for diseases modelling, drug discovery, safety pharmacology, and toxicology. Moreover, they allow for the engineering of bioartificial tissue and are promising candidates for cellular therapies. For many of these applications, the ability to genetically modify pluripotent stem cells (PSCs) is indispensable, but efficient site-specific and safe technologies for genetic engineering of PSCs were developed only recently. By now, customized engineered nucleases provide excellent tools for targeted genome editing, opening new perspectives for biomedical research and cellular therapies.

Merkert, S. and U. Martin "Targeted genome engineering using designer nucleases: State of the art and practical guidance for application in human pluripotent stem cells." Stem Cell Res. 2016 Mar;16(2):377-86. doi: 10.1016/j.scr.2016.02.027. Epub 2016 Feb 11.

Within the last years numerous publications successfully applied sequence specific designer nucleases for genome editing in human PSCs. However, despite this abundance of reports together with the rapid development and improvement accomplished with the technology, it is still difficult to choose the optimal methodology for a specific application of interest. With focus on the most suitable approach for specific applications, we present a practical guidance for successful gene editing in human PSCs using designer nucleases. We discuss experimental considerations, limitations and critical aspects which will guide the investigator for successful implementation of this technology.

Miyaoka, Y., J. R. Berman, et al. "Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing." Sci Rep. 2016 Mar 31;6:23549. doi: 10.1038/srep23549.

Precise genome-editing relies on the repair of sequence-specific nuclease-induced DNA nicking or double-strand breaks (DSBs) by homology-directed repair (HDR). However, nonhomologous end-joining (NHEJ), an error-prone repair, acts concurrently, reducing the rate of high-fidelity edits. The identification of genome-editing conditions that favor

HDR over NHEJ has been hindered by the lack of a simple method to measure HDR and NHEJ directly and simultaneously at endogenous loci. To overcome this challenge, we developed a novel, rapid, digital PCR-based assay that can simultaneously detect one HDR or NHEJ event out of 1,000 copies of the genome. Using this assay, we systematically monitored genome-editing outcomes of CRISPR-associated protein 9 (Cas9), Cas9 nickases, catalytically dead Cas9 fused to FokI, and transcription activator-like effector nuclease at three disease-associated endogenous gene loci in HEK293T cells, HeLa cells, and human induced pluripotent stem cells. Although it is widely thought that NHEJ generally occurs more often than HDR, we found that more HDR than NHEJ was induced under multiple conditions. Surprisingly, the HDR/NHEJ ratios were highly dependent on gene locus, nuclease platform, and cell type. The new assay system, and our findings based on it, will enable mechanistic studies of genome-editing and help improve genome-editing technology.

Moorthy, S. D., S. Davidson, et al. "Enhancers and super-enhancers have an equivalent regulatory role in embryonic stem cells through regulation of single or multiple genes." Genome Res. 2016 Nov 28. pii: gr.210930.116.

Transcriptional enhancers are critical for maintaining cell type-specific gene expression and driving cell fate changes during development. Highly transcribed genes are often associated with a cluster of individual enhancers such as those found in locus control regions. Recently these have been termed stretch enhancers or super-enhancers, which have been predicted to regulate critical cell identity genes. We employed a CRISPR/Cas9-mediated deletion approach to study the function of several enhancer clusters (ECs) and isolated enhancers in mouse embryonic stem (ES) cells. Our results reveal that the effect of deleting ECs, also classified as ES cell super-enhancers, is highly variable, resulting in target gene expression reductions ranging from 12% to as much as 92%. Partial deletions of these ECs which removed only one enhancer or a sub-cluster of enhancers revealed partially redundant control of the regulated gene by multiple enhancers within the larger cluster. Many highly transcribed genes in ES cells are not associated with a super-enhancer; furthermore, super-enhancer predictions ignore 81% of the potentially active regulatory elements predicted by co-binding of 5 or more pluripotency-associated transcription factors. Deletion of these additional enhancer regions revealed their robust regulatory role in gene transcription. In addition, select super-enhancers and enhancers were identified which regulated clusters of paralogous genes. We conclude that whereas robust transcriptional output can be achieved by an isolated enhancer, clusters of enhancers acting on a common target gene act in a partially redundant manner to fine tune transcriptional output of their target genes.

Moorthy, S. D. and J. A. Mitchell "Generating CRISPR/Cas9 Mediated Monoallelic Deletions to Study Enhancer Function in Mouse Embryonic Stem Cells." J Vis Exp. 2016 Apr 2;(110):e53552. doi: 10.3791/53552.

Enhancers control cell identity by regulating tissue-specific gene expression in a position and orientation independent manner. These enhancers are often located distally from the regulated gene in intergenic regions or even within the body of another gene. The position independent nature of enhancer activity makes it difficult to match enhancers with the genes they regulate. Deletion of an enhancer region provides direct evidence for enhancer activity and is the gold standard to reveal an enhancer's role in endogenous gene transcription. Conventional homologous recombination based deletion methods have been surpassed by recent advances in genome editing technology which enable rapid and precisely located changes to the genomes of numerous model organisms. CRISPR/Cas9 mediated genome editing can be used to manipulate the genome in many cell types and organisms rapidly and cost effectively, due to the ease with which Cas9 can be targeted to the genome by a guide RNA from a bespoke expression plasmid. Homozygous deletion of essential gene regulatory elements might lead to lethality or alter cellular phenotype whereas monoallelic deletion of transcriptional enhancers allows for the study of cis-regulation of gene expression without this confounding issue. Presented here is a protocol for CRISPR/Cas9 mediated deletion in F1 mouse embryonic stem (ES) cells (Mus musculus(129) x Mus castaneus). Monoallelic deletion, screening and expression analysis is facilitated by single nucleotide polymorphisms (SNP) between the two alleles which occur on average every 125 bp in these cells.

Muffat, J., Y. Li, et al. "CNS disease models with human pluripotent stem cells in the CRISPR age." Curr Opin Cell Biol. 2016 Dec;43:96-103. doi: 10.1016/j.ceb.2016.10.001. Epub 2016 Oct 19.

In vitro differentiation of human pluripotent stem cells provides a systematic platform to investigate the physiological development and function of the human nervous system, as well as the etiology and consequence when these processes go awry. Recent development in three-dimensional (3D) organotypic culture systems allows modeling of the complex structure formation of the human CNS, and

the intricate interactions between various resident neuronal and glial cell types. Combined with an ever-expanding genome editing and regulation toolkit such as CRISPR/Cas9, it is now a possibility to study human neurological disease in the relevant molecular, cellular and anatomical context. In this article, we review recent progress in 3D neural culture and the implications for disease modeling.

Mukherjee, N., Y. Lu, et al. "Use of a MCL-1 inhibitor alone to de-bulk melanoma and in combination to kill melanoma initiating cells." Oncotarget. 2016 Apr 12. doi: 10.18632/oncotarget.8695.

MCL-1 (BCL-2 family anti-apoptotic protein) is responsible for melanoma's resistance to therapy. Cancer initiating cells also contribute to resistance and relapse from treatments. Here we examined the effects of the MCL-1 inhibitor SC-2001 in killing non melanoma-initiating-cells (bulk of melanoma), and melanoma-initiating-cells (MICs). By itself, SC-2001 significantly kills melanoma cells under monolayer conditions in vitro and in a conventional mouse xenograft model. However, even at high doses (10muM), SC-2001 does not effectively eliminate MICs. In contrast, the combination of SC-2001 with ABT-737 (a BCL-2/BCL-XL/BCL-W inhibitor) significantly decreases ALDH+ cells, disrupts primary spheres, and inhibits the self-renewability of MICs. These results were observed in multiple melanomas, including short term cultures of relapsed tumors from current treatments, independent of the mutation status of BRAF or NRAS. Using a low-cell-number mouse xenograft model, we examined the effects of these treatments on the tumor initiating ability of MIC-enriched cultures. The combination therapy reduces tumor formation significantly compared to either drug alone. Mechanistic studies using shRNA and the CRISPR-Cas9 technology demonstrated that the upregulation of pro-apoptotic proteins NOXA and BIM contribute to the combination-induced cell death. These results indicate that the MCL-1 inhibitor SC-2001 combined with ABT-737 is a promising treatment strategy for targeting melanoma.

Murray, A., A. R. Sienerth, et al. "Plet1 is an epigenetically regulated cell surface protein that provides essential cues to direct trophoblast stem cell differentiation." Sci Rep. 2016 Apr 28;6:25112. doi: 10.1038/srep25112.

Gene loci that are hypermethylated and repressed in embryonic (ESCs) but hypomethylated and expressed in trophoblast (TSCs) stem cells are very rare and may have particularly important roles in early developmental cell fate decisions, as previously shown for Elf5. Here, we assessed another member of this small group of genes, Placenta Expressed Transcript 1 (Plet1), for its function in establishing trophoblast lineage identity and modulating trophoblast differentiation. We find that Plet1 is tightly repressed by DNA methylation in ESCs but expressed on the cell surface of TSCs and trophoblast giant cells. In hypomethylated ESCs that are prone to acquire some trophoblast characteristics, Plet1 is required to confer a trophoblast-specific gene expression pattern, including up-regulation of Elf5. Plet1 displays an unusual biphasic expression profile during TSC differentiation and thus may be pivotal in balancing trophoblast self-renewal and differentiation. Furthermore, overexpression and CRISPR/Cas9-mediated knockout in TSCs showed that high Plet1 levels favour differentiation towards the trophoblast giant cell lineage, whereas lack of Plet1 preferentially induces syncytiotrophoblast formation. Thus, the endogenous dynamics of Plet1 expression establish important patterning cues within the trophoblast compartment by promoting differentiation towards the syncytiotrophoblast or giant cell pathway in Plet1-low and Plet1-high cells, respectively.

Natsume, T., T. Kiyomitsu, et al. "Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors." Cell Rep. 2016 Apr 5;15(1):210-8. doi: 10.1016/j.celrep.2016.03.001. Epub 2016 Mar 24.

Studying the role of essential proteins is dependent upon a method for rapid inactivation, in order to study the immediate phenotypic consequences. Auxin-inducible degron (AID) technology allows rapid depletion of proteins in animal cells and fungi, but its application to human cells has been limited by the difficulties of tagging endogenous proteins. We have developed a simple and scalable CRISPR/Cas-based method to tag endogenous proteins in human HCT116 and mouse embryonic stem (ES) cells by using donor constructs that harbor synthetic short homology arms. Using a combination of AID tagging with CRISPR/Cas, we have generated conditional alleles of essential nuclear and cytoplasmic proteins in HCT116 cells, which can then be depleted very rapidly after the addition of auxin to the culture medium. This approach should greatly facilitate the functional analysis of essential proteins, particularly those of previously unknown function.

Nestor, M. W., A. W. Phillips, et al. "Human Inducible Pluripotent Stem Cells and Autism Spectrum Disorder: Emerging Technologies." Autism Res. 2016 May;9(5):513-35. doi: 10.1002/aur.1570. Epub 2015 Oct 1.

Autism Spectrum Disorder (ASD) is a behaviorally defined neurodevelopmental condition. Symptoms of ASD cover the spectrum from mild

qualitative differences in social interaction to severe communication and social and behavioral challenges that require lifelong support. Attempts at understanding the pathophysiology of ASD have been hampered by a multifactorial etiology that stretches the limits of current behavioral and cell based models. Recent progress has implicated numerous autism-risk genes but efforts to gain a better understanding of the underlying biological mechanisms have seen slow progress. This is in part due to lack of appropriate models for complete molecular and pharmacological studies. The advent of induced pluripotent stem cells (iPSC) has reinvigorated efforts to establish more complete model systems that more reliably identify molecular pathways and predict effective drug targets and candidates in ASD. iPSCs are particularly appealing because they can be derived from human patients and controls for research purposes and provide a technology for the development of a personalized treatment regimen for ASD patients. The pluripotency of iPSCs allow them to be reprogrammed into a number of CNS cell types and phenotypically screened across many patients. This quality is already being exploited in protocols to generate 2-dimensional (2-D) and three-dimensional (3-D) models of neurons and developing brain structures. iPSC models make powerful platforms that can be interrogated using electrophysiology, gene expression studies, and other cell-based quantitative assays. iPSC technology has limitations but when combined with other model systems has great potential for helping define the underlying pathophysiology of ASD. Autism Res 2016, 9: 513-535. (c) 2015 International Society for Autism Research, Wiley Periodicals, Inc.

Nettersheim, D., A. Heimsoeth, et al. "SOX2 is essential for in vivo reprogramming of seminoma-like TCam-2 cells to an embryonal carcinoma-like fate." Oncotarget. 2016 Jul 26;7(30):47095-47110. doi: 10.18632/oncotarget.9903.

Type II germ cell cancers (GCC) are divided into seminomas, which are highly similar to primordial germ cells and embryonal carcinomas (EC), often described as malignant counterparts to embryonic stem cells.Previously, we demonstrated that the development of GCCs is a highly plastic process and strongly influenced by the microenvironment. While orthotopic transplantation into the testis promotes seminomatous growth of the seminoma-like cell line TCam-2, ectopic xenotransplantation into the flank initiates reprogramming into an EC-like fate.During this reprogramming, BMP signaling is inhibited, leading to induction of NODAL signaling, upregulation of pluripotency factors and downregulation of seminoma markers, like SOX17. The pluripotency factor and EC-marker SOX2 is strongly induced.Here, we adressed the molecular role of SOX2 in this reprogramming. Using CRISPR/Cas9-mediated genome-editing, we established SOX2-deficient TCam-2 cells. Xenografting of SOX2-deficient cells into the flank of nude mice resulted in maintenance of a seminoma-like fate, indicated by the histology and expression of OCT3/4, SOX17, TFAP2C, PRDM1 and PRAME. In SOX2-deficient cells, BMP signaling is inhibited, but NODAL signaling is not activated. Thus, SOX2 appears to be downstream of BMP signaling but upstream of NODAL activation. So, SOX2 is an essential factor in acquiring an EC-like cell fate from seminomas.A small population of differentiated cells was identified resembling a mixed non-seminoma. Analyses of these cells revealed downregulation of the pluripotency and seminoma markers OCT3/4, SOX17, PRDM1 and TFAP2C. In contrast, the pioneer factor FOXA2 and its target genes were upregulated, suggesting that FOXA2 might play an important role in induction of non-seminomatous differentiation.

Niu, X., W. He, et al. "Combining Single Strand Oligodeoxynucleotides and CRISPR/Cas9 to Correct Gene Mutations in beta-Thalassemia-induced Pluripotent Stem Cells." J Biol Chem. 2016 Aug 5;291(32):16576-85. doi: 10.1074/jbc.M116.719237. Epub 2016 Jun 10.

beta-Thalassemia (beta-Thal) is one of the most common genetic diseases in the world. The generation of patient-specific beta-Thal-induced pluripotent stem cells (iPSCs), correction of the disease-causing mutations in those cells, and then differentiation into hematopoietic stem cells offers a new therapeutic strategy for this disease. Here, we designed a CRISPR/Cas9 to specifically target the Homo sapiens hemoglobin beta (HBB) gene CD41/42(-CTTT) mutation. We demonstrated that the combination of single strand oligodeoxynucleotides with CRISPR/Cas9 was capable of correcting the HBB gene CD41/42 mutation in beta-Thal iPSCs. After applying a correction-specific PCR assay to purify the corrected clones followed by sequencing to confirm mutation correction, we verified that the purified clones retained full pluripotency and exhibited normal karyotyping. Additionally, whole-exome sequencing showed that the mutation load to the exomes was minimal after CRISPR/Cas9 targeting. Furthermore, the corrected iPSCs were selected for erythroblast differentiation and restored the expression of HBB protein compared with the parental iPSCs. This method provides an efficient and safe strategy to correct the HBB gene mutation in beta-Thal iPSCs.

Oceguera-Yanez, F., S. I. Kim, et al. "Engineering the AAVS1 locus for consistent and scalable transgene

expression in human iPSCs and their differentiated derivatives." Methods. 2016 May 15;101:43-55. doi: 10.1016/j.ymeth.2015.12.012. Epub 2015 Dec 18.

The potential use of induced pluripotent stem cells (iPSCs) in personalized regenerative medicine applications may be augmented by transgenics, including the expression of constitutive cell labels, differentiation reporters, or modulators of disease phenotypes. Thus, there is precedence for reproducible transgene expression amongst iPSC sub-clones with isogenic or diverse genetic backgrounds. Using virus or transposon vectors, transgene integration sites and copy numbers are difficult to control, and nearly impossible to reproduce across multiple cell lines. Moreover, randomly integrated transgenes are often subject to pleiotropic position effects as a consequence of epigenetic changes inherent in differentiation, undermining applications in iPSCs. To address this, we have adapted popular TALEN and CRISPR/Cas9 nuclease technologies in order to introduce transgenes into pre-defined loci and overcome random position effects. AAVS1 is an exemplary locus within the PPP1R12C gene that permits robust expression of CAG promoter-driven transgenes. Gene targeting controls transgene copy number such that reporter expression patterns are reproducible and scalable by approximately 2-fold. Furthermore, gene expression is maintained during long-term human iPSC culture and in vitro differentiation along multiple lineages. Here, we outline our AAVS1 targeting protocol using standardized donor vectors and construction methods, as well as provide practical considerations for iPSC culture, drug selection, and genotyping.

O'Duibhir, E., N. O. Carragher, et al. "Accelerating glioblastoma drug discovery: Convergence of patient-derived models, genome editing and phenotypic screening." Mol Cell Neurosci. 2016 Nov 4. pii: S1044-7431(16)30215-9. doi: 10.1016/j.mcn.2016.11.001.

Patients diagnosed with glioblastoma (GBM) continue to face a bleak prognosis. It is critical that new effective therapeutic strategies are developed. GBM stem cells have molecular hallmarks of neural stem and progenitor cells and it is possible to propagate both non-transformed normal neural stem cells and GBM stem cells, in defined, feeder-free, adherent culture. These primary stem cell lines provide an experimental model that is ideally suited to cell-based drug discovery or genetic screens in order to identify tumour-specific vulnerabilities. For many solid tumours, including GBM, the genetic disruptions that drive tumour initiation and growth have now been catalogued. CRISPR/Cas-based genome editing technologies have recently emerged, transforming our ability to functionally annotate the human genome. Genome editing opens prospects for engineering precise genetic changes in normal and GBM-derived neural stem cells, which will provide more defined and reliable genetic models, with critical matched pairs of isogenic cell lines. Generation of more complex alleles such as knock in tags or fluorescent reporters is also now possible. These new cellular models can be deployed in cell-based phenotypic drug discovery (PDD). Here we discuss the convergence of these advanced technologies (iPS cells, neural stem cell culture, genome editing and high content phenotypic screening) and how they herald a new era in human cellular genetics that should have a major impact in accelerating glioblastoma drug discovery.

Oji, A., T. Noda, et al. "CRISPR/Cas9 mediated genome editing in ES cells and its application for chimeric analysis in mice." Sci Rep. 2016 Aug 17;6:31666. doi: 10.1038/srep31666.

Targeted gene disrupted mice can be efficiently generated by expressing a single guide RNA (sgRNA)/CAS9 complex in the zygote. However, the limited success of complicated genome editing, such as large deletions, point mutations, and knockins, remains to be improved. Further, the mosaicism in founder generations complicates the genotypic and phenotypic analyses in these animals. Here we show that large deletions with two sgRNAs as well as dsDNA-mediated point mutations are efficient in mouse embryonic stem cells (ESCs). The dsDNA-mediated gene knockins are also feasible in ESCs. Finally, we generated chimeric mice with biallelic mutant ESCs for a lethal gene, Dnajb13, and analyzed their phenotypes. Not only was the lethal phenotype of hydrocephalus suppressed, but we also found that Dnajb13 is required for sperm cilia formation. The combination of biallelic genome editing in ESCs and subsequent chimeric analysis provides a useful tool for rapid gene function analysis in the whole organism.

Onuma, A., W. Fujii, et al. "Efficient mutagenesis by CRISPR/Cas system during meiotic maturation of porcine oocytes." J Reprod Dev. 2016 Oct 21.

Genome editing using the CRISPR/Cas system can induce mutations with high efficiency, and allows easier production of genome-modified animals than that offered by the conventional method where embryonic stem cells are used. However, studies using CRISPR/Cas systems have been mostly limited to proliferating somatic cells and pronuclear-stage fertilized eggs. In contrast, the efficiency of a CRISPR/Cas system in immature and maturing oocytes progressing through meiosis has not yet been assessed. In the present study, we evaluated the genome-modification efficiency of the CRISPR/Cas system during meiotic maturation of porcine oocytes.

Additionally, the localization of the Cas9 protein in immature oocytes was analyzed in relation to nuclear transport and mutation induction. The results showed that CRISPR/Cas induced mutation with high efficiency even in maturing oocytes with condensed chromosomes, whereas mutations were not induced in GV-stage oocytes. The localization analysis of enhanced green fluorescent protein (EGFP)-tagged Cas9 (Cas9-EGFP) revealed that the nuclei contained lesser Cas9 than the cytoplasm in immature oocytes. Treatment with leptomycin B, a nuclear export inhibitor, increased the amount of nuclear Cas9 and enabled mutation induction in GV oocytes. Our results suggest that CRISPR/Cas systems can be applied to oocytes during meiotic maturation and be implemented in novel applications targeting female genomes.

Ordovas, L., R. Boon, et al. "Rapid and Efficient Generation of Recombinant Human Pluripotent Stem Cells by Recombinase-mediated Cassette Exchange in the AAVS1 Locus." J Vis Exp. 2016 Nov 20;(117). doi: 10.3791/54718.

Even with the revolution of gene-targeting technologies led by CRISPR-Cas9, genetic modification of human pluripotent stem cells (hPSCs) is still time consuming. Comparative studies that use recombinant lines with transgenes integrated into safe harbor loci could benefit from approaches that use site-specific targeted recombinases, like Cre or FLPe, which are more rapid and less prone to off-target effects. Such methods have been described, although they do not significantly outperform gene targeting in most aspects. Using Zinc-finger nucleases, we previously created a master cell line in the AAVS1 locus of hPSCs that contains a GFP-Hygromycin-tk expressing cassette, flanked by heterotypic FRT sequences. Here, we describe the procedures to perform FLPe recombinase-mediated cassette exchange (RMCE) using this line. The master cell line is transfected with a RMCE donor vector, which contains a promoterless Puromycin resistance, and with FLPe recombinase. Application of both a positive (Puromycin) and negative (FIAU) selection program leads to the selection of RMCE without random integrations. RMCE generates fully characterized pluripotent polyclonal transgenic lines in 15 d with 100% efficiency. Despite the recently described limitations of the AAVS1 locus, the ease of the system paves the way for hPSC transgenesis in isogenic settings, is necessary for comparative studies, and enables semi-high-throughput genetic screens for gain/loss of function analysis that would otherwise be highly time consuming.

Osborn, M. J., J. J. Belanto, et al. "Gene editing and its application for hematological diseases." Int J Hematol. 2016 Jul;104(1):18-28. doi: 10.1007/s12185-016-2017-z. Epub 2016 May 27.

The use of precise, rationally designed gene-editing nucleases allows for targeted genome and transcriptome modification, and at present, four major classes of nucleases are being employed: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases (MNs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9. Each reagent shares the ability to recognize and bind a target sequence of DNA. Depending on the properties of the reagent, the DNA can be cleaved on one or both strands, or epigenetic changes can be mediated. These novel properties can impact hematological disease by allowing for: (1) direct modification of hematopoietic stem/progenitor cells (HSPCs), (2) gene alteration of hematopoietic lineage committed terminal effectors, (3) genome engineering in non-hematopoietic cells with reprogramming to a hematopoietic phenotype, and (4) transcriptome modulation for gene regulation, modeling, and discovery.

Otonkoski, T. "New tools for experimental diabetes research: Cellular reprogramming and genome editing." Ups J Med Sci. 2016 May;121(2):146-50. doi: 10.3109/03009734.2016.1149529. Epub 2016 Mar 23.

Isolated human islets are a rare and precious material for diabetes research. However, their availability is limited, and it is impossible to obtain them from patients with specific genotypes. Human pluripotent stem cells provide an alternative. Induced pluripotent stem cells can be generated from any individual's somatic cells and differentiated into pancreatic cells. Currently, this approach is limited by the immaturity of the islet-like cells derived from stem cells. However, this approach can already be used to model developmental defects, and the possibilities for studying insulin secretion are continuously improving. In addition, genome editing using the CRISPR/Cas9 technology provides powerful possibilities to study the impact of specific genotypes. The same technology can also be used for transcriptional regulation in order to improve the functional maturation of stem cell-derived islets. These tools are today becoming available for tomorrow's translational diabetes research.

Ou, Z., X. Niu, et al. "The Combination of CRISPR/Cas9 and iPSC Technologies in the Gene Therapy of Human beta-thalassemia in Mice." Sci Rep. 2016 Sep 1;6:32463. doi: 10.1038/srep32463.

beta-thalassemia results from point mutations or small deletions in the beta-globin (HBB) gene that ultimately cause anemia. The generation of induced

pluripotent stem cells (iPSCs) from the somatic cells of patients in combination with subsequent homologous recombination-based gene correction provides new approaches to cure this disease. CRISPR/Cas9 is a genome editing tool that is creating a buzz in the scientific community for treating human diseases, especially genetic disorders. Here, we reported that correction of beta-thalassemia mutations in patient-specific iPSCs using the CRISPR/Cas9 tool promotes hematopoietic differentiation in vivo. CRISPR/Cas9-corrected iPSC-derived hematopoietic stem cells (HSCs) were injected into sublethally-irradiated NOD-scid-IL2Rg-/- (NSI) mice. HBB expression was observed in these HSCs after hematopoietic differentiation in the NSI mice. Importantly, no tumor was found in the livers, lungs, kidneys, or bone marrow at 10 weeks in the NSI mice after implantation with these HSCs. Collectively, our findings demonstrated that CRISPR/Cas9 successfully corrects beta-thalassemia mutations in patient-specific iPSCs. These CRISPR/Cas9-corrected iPSC-derived HSCs express normal HBB in mice without tumorigenic potential, suggesting a safe strategy for personalized treatment of beta-thalassemia.

Paksa, A., J. Bandemer, et al. "Repulsive cues combined with physical barriers and cell-cell adhesion determine progenitor cell positioning during organogenesis." Nat Commun. 2016 Apr 18;7:11288. doi: 10.1038/ncomms11288.

The precise positioning of organ progenitor cells constitutes an essential, yet poorly understood step during organogenesis. Using primordial germ cells that participate in gonad formation, we present the developmental mechanisms maintaining a motile progenitor cell population at the site where the organ develops. Employing high-resolution live-cell microscopy, we find that repulsive cues coupled with physical barriers confine the cells to the correct bilateral positions. This analysis revealed that cell polarity changes on interaction with the physical barrier and that the establishment of compact clusters involves increased cell-cell interaction time. Using particle-based simulations, we demonstrate the role of reflecting barriers, from which cells turn away on contact, and the importance of proper cell-cell adhesion level for maintaining the tight cell clusters and their correct positioning at the target region. The combination of these developmental and cellular mechanisms prevents organ fusion, controls organ positioning and is thus critical for its proper function.

Pankowicz, F. P., M. Barzi, et al. "Reprogramming metabolic pathways in vivo with CRISPR/Cas9 genome editing to treat hereditary tyrosinaemia." Nat Commun. 2016 Aug 30;7:12642. doi: 10.1038/ncomms12642.

Many metabolic liver disorders are refractory to drug therapy and require orthotopic liver transplantation. Here we demonstrate a new strategy, which we call metabolic pathway reprogramming, to treat hereditary tyrosinaemia type I in mice; rather than edit the disease-causing gene, we delete a gene in a disease-associated pathway to render the phenotype benign. Using CRISPR/Cas9 in vivo, we convert hepatocytes from tyrosinaemia type I into the benign tyrosinaemia type III by deleting Hpd (hydroxyphenylpyruvate dioxigenase). Edited hepatocytes (Fah(-/-)/Hpd(-/-)) display a growth advantage over non-edited hepatocytes (Fah(-/-)/Hpd(+/+)) and, in some mice, almost completely replace them within 8 weeks. Hpd excision successfully reroutes tyrosine catabolism, leaving treated mice healthy and asymptomatic. Metabolic pathway reprogramming sidesteps potential difficulties associated with editing a critical disease-causing gene and can be explored as an option for treating other diseases.

Paquet, D., D. Kwart, et al. "Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9." Nature. 2016 May 5;533(7601):125-9. doi: 10.1038/nature17664. Epub 2016 Apr 27.

The bacterial CRISPR/Cas9 system allows sequence-specific gene editing in many organisms and holds promise as a tool to generate models of human diseases, for example, in human pluripotent stem cells. CRISPR/Cas9 introduces targeted double-stranded breaks (DSBs) with high efficiency, which are typically repaired by non-homologous end-joining (NHEJ) resulting in nonspecific insertions, deletions or other mutations (indels). DSBs may also be repaired by homology-directed repair (HDR) using a DNA repair template, such as an introduced single-stranded oligo DNA nucleotide (ssODN), allowing knock-in of specific mutations. Although CRISPR/Cas9 is used extensively to engineer gene knockouts through NHEJ, editing by HDR remains inefficient and can be corrupted by additional indels, preventing its widespread use for modelling genetic disorders through introducing disease-associated mutations. Furthermore, targeted mutational knock-in at single alleles to model diseases caused by heterozygous mutations has not been reported. Here we describe a CRISPR/Cas9-based genome-editing framework that allows selective introduction of mono- and bi-allelic sequence changes with high efficiency and accuracy. We show that HDR accuracy is increased dramatically by incorporating silent CRISPR/Cas-blocking mutations along with pathogenic mutations, and establish a method termed 'CORRECT' for scarless

genome editing. By characterizing and exploiting a stereotyped inverse relationship between a mutation's incorporation rate and its distance to the DSB, we achieve predictable control of zygosity. Homozygous introduction requires a guide RNA targeting close to the intended mutation, whereas heterozygous introduction can be accomplished by distance-dependent suboptimal mutation incorporation or by use of mixed repair templates. Using this approach, we generated human induced pluripotent stem cells with heterozygous and homozygous dominant early onset Alzheimer's disease-causing mutations in amyloid precursor protein (APP(Swe)) and presenilin 1 (PSEN1(M146V)) and derived cortical neurons, which displayed genotype-dependent disease-associated phenotypes. Our findings enable efficient introduction of specific sequence changes with CRISPR/Cas9, facilitating study of human disease.

Park, A., P. Hong, et al. "Sendai virus, an RNA virus with no risk of genomic integration, delivers CRISPR/Cas9 for efficient gene editing." Mol Ther Methods Clin Dev. 2016 Aug 24;3:16057. doi: 10.1038/mtm.2016.57. eCollection 2016.

The advent of RNA-guided endonuclease (RGEN)-mediated gene editing, specifically via CRISPR/Cas9, has spurred intensive efforts to improve the efficiency of both RGEN delivery and targeted mutagenesis. The major viral vectors in use for delivery of Cas9 and its associated guide RNA, lentiviral and adeno-associated viral systems, have the potential for undesired random integration into the host genome. Here, we repurpose Sendai virus, an RNA virus with no viral DNA phase and that replicates solely in the cytoplasm, as a delivery system for efficient Cas9-mediated gene editing. The high efficiency of Sendai virus infection resulted in high rates of on-target mutagenesis in cell lines (75-98% at various endogenous and transgenic loci) and primary human monocytes (88% at the ccr5 locus) in the absence of any selection. In conjunction with extensive former work on Sendai virus as a promising gene therapy vector that can infect a wide range of cell types including hematopoietic stem cells, this proof-of-concept study opens the door to using Sendai virus as well as other related paramyxoviruses as versatile and efficient tools for gene editing.

Park, C. Y., J. J. Sung, et al. "Modeling and correction of structural variations in patient-derived iPSCs using CRISPR/Cas9." Nat Protoc. 2016 Nov;11(11):2154-2169. doi: 10.1038/nprot.2016.129. Epub 2016 Oct 6.

Genome engineering technology using engineered nucleases has been rapidly developing, enabling the efficient correction of simple mutations. However, the precise correction of structural variations (SVs) such as large inversions remains limited. Here we describe a detailed procedure for the modeling or correction of large chromosomal rearrangements and short nucleotide repeat expansions using engineered nucleases in human induced pluripotent stem cells (hiPSCs) from a healthy donor and patients with SVs. This protocol includes the delivery of engineered nucleases with no donor template to hiPSCs, and genotyping and derivation/characterization of gene-manipulated hiPSC clones. With engineered nucleases, genomic inversions, reversions, and deletions of short nucleotide expansions can be identified in 2 weeks, and desired clones can be generated in as little as 3-4 weeks. This protocol enables the correction of large inverted segments and short nucleotide repeat expansions in diseases such as hemophilia A, fragile X syndrome, Hunter syndrome, and Friedreich's ataxia.

Park, K. E., A. V. Kaucher, et al. "Generation of germline ablated male pigs by CRISPR/Cas9 editing of the NANOS2 gene." Sci Rep. 2017 Jan 10;7:40176. doi: 10.1038/srep40176.

Genome editing tools have revolutionized the generation of genetically modified animals including livestock. In particular, the domestic pig is a proven model of human physiology and an agriculturally important species. In this study, we utilized the CRISPR/Cas9 system to edit the NANOS2 gene in pig embryos to generate offspring with mono-allelic and bi-allelic mutations. We found that NANOS2 knockout pigs phenocopy knockout mice with male specific germline ablation but other aspects of testicular development are normal. Moreover, male pigs with one intact NANOS2 allele and female knockout pigs are fertile. From an agriculture perspective, NANOS2 knockout male pigs are expected to serve as an ideal surrogate for transplantation of donor spermatogonial stem cells to expand the availability of gametes from genetically desirable sires.

Parsi, K. M., E. Hennessy, et al. "Using an Inducible CRISPR-dCas9-KRAB Effector System to Dissect Transcriptional Regulation in Human Embryonic Stem Cells." Methods Mol Biol. 2017;1507:221-233.

CRISPR-Cas9 effector systems have wide applications for the stem cell and regenerative medicine field. The ability to dissect the functional gene regulatory networks in pluripotency and potentially in differentiation intermediates of all three germ layers makes this a valuable tool for the stem cell community. Catalytically inactive Cas9 fused to transcriptional/chromatin effector domains allows for silencing or activation of a genomic region of interest. Here, we describe the application of an inducible, RNA-guided, nuclease-deficient (d) Cas9-KRAB system (adapted from Streptococcus pyogenes) to

silence target gene expression in human embryonic stem cells, via KRAB repression at the promoter region. This chapter outlines a detailed protocol for generation of a stable human embryonic stem cell line containing both Sp-dCas9-KRAB and sgRNA, followed by inducible expression of Sp-dCas9-KRAB to analyze functional effects of dCas9-KRAB at target loci in human embryonic stem cells.

Partridge, E. C., T. A. Watkins, et al. "Every transcription factor deserves its map: Scaling up epitope tagging of proteins to bypass antibody problems." Bioessays. 2016 Aug;38(8):801-11. doi: 10.1002/bies.201600028. Epub 2016 Jun 17.

Genome-wide identification of transcription factor binding sites with the ChIP-seq method is an extremely important scientific endeavor - one that should ideally be performed for every transcription factor in as many cell types as possible. A major hurdle on the way to this goal is the necessity for a specific, ChIP-grade antibody for each transcription factor of interest, which is often not available. Here, we describe CETCh-seq, a recently published method utilizing genome engineering with the CRISPR/Cas9 system to circumvent the need for a specific antibody. Using the CETCh-seq method, targeted genomic editing results in an epitope-tagged transcription factor, which is recognized by a well-characterized, standard antibody, efficacious for ChIP-seq. We have used CETCh-seq in human cancer cell lines as well as mouse embryonic stem cells. We find that roughly 60% of transcription factors tagged using CETCh-seq produce a high quality ChIP-seq map, a significant improvement over traditional antibody-based methods.

Pastore, N., O. A. Brady, et al. "TFEB and TFE3 cooperate in the regulation of the innate immune response in activated macrophages." Autophagy. 2016 Aug 2;12(8):1240-58. doi: 10.1080/15548627.2016.1179405. Epub 2016 May 12.

The activation of transcription factors is critical to ensure an effective defense against pathogens. In this study we identify a critical and complementary role of the transcription factors TFEB and TFE3 in innate immune response. By using a combination of chromatin immunoprecipitation, CRISPR-Cas9-mediated genome-editing technology, and in vivo models, we determined that TFEB and TFE3 collaborate with each other in activated macrophages and microglia to promote efficient autophagy induction, increased lysosomal biogenesis, and transcriptional upregulation of numerous proinflammatory cytokines. Furthermore, secretion of key mediators of the inflammatory response (CSF2, IL1B, IL2, and IL27), macrophage differentiation (CSF1), and macrophage infiltration and migration to sites of inflammation (CCL2) was significantly reduced in TFEB and TFE3 deficient cells. These new insights provide us with a deeper understanding of the transcriptional regulation of the innate immune response.

Pernet, O., S. S. Yadav, et al. "Stem cell-based therapies for HIV/AIDS." Adv Drug Deliv Rev. 2016 Aug 1;103:187-201. doi: 10.1016/j.addr.2016.04.027. Epub 2016 May 2.

One of the current focuses in HIV/AIDS research is to develop a novel therapeutic strategy that can provide a life-long remission of HIV/AIDS without daily drug treatment and, ultimately, a cure for HIV/AIDS. Hematopoietic stem cell-based anti-HIV gene therapy aims to reconstitute the patient immune system by transplantation of genetically engineered hematopoietic stem cells with anti-HIV genes. Hematopoietic stem cells can self-renew, proliferate and differentiate into mature immune cells. In theory, anti-HIV gene-modified hematopoietic stem cells can continuously provide HIV-resistant immune cells throughout the life of a patient. Therefore, hematopoietic stem cell-based anti-HIV gene therapy has a great potential to provide a life-long remission of HIV/AIDS by a single treatment. Here, we provide a comprehensive review of the recent progress of developing anti-HIV genes, genetic modification of hematopoietic stem progenitor cells, engraftment and reconstitution of anti-HIV gene-modified immune cells, HIV inhibition in in vitro and in vivo animal models, and in human clinical trials.

Poon, A., B. Schmid, et al. "Generation of a gene-corrected isogenic control hiPSC line derived from a familial Alzheimer's disease patient carrying a L150P mutation in presenilin 1." Stem Cell Res. 2016 Sep 24;17(3):466-469. doi: 10.1016/j.scr.2016.09.018.

Mutations in the presenilin 1 (PSEN1) gene lead to the most aggressive form of familial Alzheimer's disease (AD). Human induced pluripotent stem cells (hiPSCs) derived from AD patients and subsequently differentiated can be used for disease modeling. We have previously generated a hiPSC line from a familial AD patient carrying a L150P point mutation in PSEN1. Here we used CRISPR/Cas9 gene editing to correct for the single base pair mutation. This gene-corrected line, L150P-GC-hiPSC, serves as an isogenic control to the mutant line for future investigation of mechanisms and cellular phenotypes altered by this specific PSEN1 mutation.

Qin, H., M. Hejna, et al. "YAP Induces Human Naive Pluripotency." Cell Rep. 2016 Mar 15;14(10):2301-12. doi: 10.1016/j.celrep.2016.02.036. Epub 2016 Mar 3.

The human naive pluripotent stem cell (PSC)

state, corresponding to a pre-implantation stage of development, has been difficult to capture and sustain in vitro. We report that the Hippo pathway effector YAP is nuclearly localized in the inner cell mass of human blastocysts. Overexpression of YAP in human embryonic stem cells (ESCs) and induced PSCs (iPSCs) promotes the generation of naive PSCs. Lysophosphatidic acid (LPA) can partially substitute for YAP to generate transgene-free human naive PSCs. YAP- or LPA-induced naive PSCs have a rapid clonal growth rate, a normal karyotype, the ability to form teratomas, transcriptional similarities to human pre-implantation embryos, reduced heterochromatin levels, and other hallmarks of the naive state. YAP/LPA act in part by suppressing differentiation-inducing effects of GSK3 inhibition. CRISPR/Cas9-generated YAP(-/-) cells have an impaired ability to form colonies in naive but not primed conditions. These results uncover an unexpected role for YAP in the human naive state, with implications for early human embryology.

Rajagopal, N., S. Srinivasan, et al. "High-throughput mapping of regulatory DNA." Nat Biotechnol. 2016 Feb;34(2):167-74. doi: 10.1038/nbt.3468. Epub 2016 Jan 25.

Quantifying the effects of cis-regulatory DNA on gene expression is a major challenge. Here, we present the multiplexed editing regulatory assay (MERA), a high-throughput CRISPR-Cas9-based approach that analyzes the functional impact of the regulatory genome in its native context. MERA tiles thousands of mutations across approximately 40 kb of cis-regulatory genomic space and uses knock-in green fluorescent protein (GFP) reporters to read out gene activity. Using this approach, we obtain quantitative information on the contribution of cis-regulatory regions to gene expression. We identify proximal and distal regulatory elements necessary for expression of four embryonic stem cell-specific genes. We show a consistent contribution of neighboring gene promoters to gene expression and identify unmarked regulatory elements (UREs) that control gene expression but do not have typical enhancer epigenetic or chromatin features. We compare thousands of functional and nonfunctional genotypes at a genomic location and identify the base pair-resolution functional motifs of regulatory elements.

Rhee, H. S., M. Closser, et al. "Expression of Terminal Effector Genes in Mammalian Neurons Is Maintained by a Dynamic Relay of Transient Enhancers." Neuron. 2016 Dec 21;92(6):1252-1265. doi: 10.1016/j.neuron.2016.11.037. Epub 2016 Dec 8.

Generic spinal motor neuron identity is established by cooperative binding of programming transcription factors (TFs), Isl1 and Lhx3, to motor-neuron-specific enhancers. How expression of effector genes is maintained following downregulation of programming TFs in maturing neurons remains unknown. High-resolution exonuclease (ChIP-exo) mapping revealed that the majority of enhancers established by programming TFs are rapidly deactivated following Lhx3 downregulation in stem-cell-derived hypaxial motor neurons. Isl1 is released from nascent motor neuron enhancers and recruited to new enhancers bound by clusters of Onecut1 in maturing neurons. Synthetic enhancer reporter assays revealed that Isl1 operates as an integrator factor, translating the density of Lhx3 or Onecut1 binding sites into transient enhancer activity. Importantly, independent Isl1/Lhx3- and Isl1/Onecut1-bound enhancers contribute to sustained expression of motor neuron effector genes, demonstrating that outwardly stable expression of terminal effector genes in postmitotic neurons is controlled by a dynamic relay of stage-specific enhancers.

Rubio, A., M. Luoni, et al. "Rapid and efficient CRISPR/Cas9 gene inactivation in human neurons during human pluripotent stem cell differentiation and direct reprogramming." Sci Rep. 2016 Nov 18;6:37540. doi: 10.1038/srep37540.

The CRISPR/Cas9 system is a rapid and customizable tool for gene editing in mammalian cells. In particular, this approach has widely opened new opportunities for genetic studies in neurological disease. Human neurons can be differentiated in vitro from hPSC (human Pluripotent Stem Cells), hNPCs (human Neural Precursor Cells) or even directly reprogrammed from fibroblasts. Here, we described a new platform which enables, rapid and efficient CRISPR/Cas9-mediated genome targeting simultaneously with three different paradigms for in vitro generation of neurons. This system was employed to inactivate two genes associated with neurological disorder (TSC2 and KCNQ2) and achieved up to 85% efficiency of gene targeting in the differentiated cells. In particular, we devised a protocol that, combining the expression of the CRISPR components with neurogenic factors, generated functional human neurons highly enriched for the desired genome modification in only 5 weeks. This new approach is easy, fast and that does not require the generation of stable isogenic clones, practice that is time consuming and for some genes not feasible.

Rutkowski, T. P., J. P. Schroeder, et al. "Unraveling the genetic architecture of copy number variants associated with schizophrenia and other neuropsychiatric disorders." J Neurosci Res. 2016 Nov 8. doi: 10.1002/jnr.23970.

Recent studies show that the complex genetic

architecture of schizophrenia (SZ) is driven in part by polygenic components, or the cumulative effect of variants of small effect in many genes, as well as rare single-locus variants with large effect sizes. Here we discuss genetic aberrations known as copy number variants (CNVs), which fall in the latter category and are associated with a high risk for SZ and other neuropsychiatric disorders. We briefly review recurrent CNVs associated with SZ, and then highlight one CNV in particular, a recurrent 1.6-Mb deletion on chromosome 3q29, which is estimated to confer a 40-fold increased risk for SZ. Additionally, we describe the use of genetic mouse models, behavioral tools, and patient-derived induced pluripotent stem cells as a means to study CNVs in the hope of gaining mechanistic insight into their respective disorders. Taken together, the genomic data connecting CNVs with a multitude of human neuropsychiatric disease, our current technical ability to model such chromosomal anomalies in mouse, and the existence of precise behavioral measures of endophenotypes argue that the time is ripe for systematic dissection of the genetic mechanisms underlying such disease. (c) 2016 Wiley Periodicals, Inc.

Samarut, E. "Zebrafish embryos as in vivo test tubes to unravel cell-specific mechanisms of neurogenesis during neurodevelopment and in diseases." Neurogenesis (Austin). 2016 Oct 7;3(1):e1232678. eCollection 2016.

Zebrafish has become a model of choice for developmental studies in particular for studying neural development and related mechanisms involved in diseases. Indeed, zebrafish provides a fast, handy and accurate model to perform functional genomics on a gene or network of genes of interest. Recently, we successfully purified neural stem cells (NSCs) by fluorescence-activated cell sorting (FACS) from whole embryos in order to analyze cell-specific transcriptomic effects by RNA sequencing. As a result, our work sheds light on signaling pathways that are more likely to be involved in our morpholino-induced neurogenesis phenotype. This cell purification strategy brings zebrafish to a higher level since it now allows one to investigate cell-specific effects of a genetic condition of interest (knockout, knock-down, gain-of-function etc.) at the genomic, transcriptomic and proteomic levels in a genuine in vivo context. With this new potential, there is no doubt that zebrafish will be of a major model with which to unravel complex underlying molecular mechanisms of neurological disorders such as epilepsy, autism spectrum disorders and schizophrenia.

Shinkuma, S., Z. Guo, et al. "Site-specific genome editing for correction of induced pluripotent stem cells derived from dominant dystrophic epidermolysis bullosa." Proc Natl Acad Sci U S A. 2016 May 17;113(20):5676-81. doi: 10.1073/pnas.1512028113. Epub 2016 May 3.

Genome editing with engineered site-specific endonucleases involves nonhomologous end-joining, leading to reading frame disruption. The approach is applicable to dominant negative disorders, which can be treated simply by knocking out the mutant allele, while leaving the normal allele intact. We applied this strategy to dominant dystrophic epidermolysis bullosa (DDEB), which is caused by a dominant negative mutation in the COL7A1 gene encoding type VII collagen (COL7). We performed genome editing with TALENs and CRISPR/Cas9 targeting the mutation, c.8068_8084delinsGA. We then cotransfected Cas9 and guide RNA expression vectors expressed with GFP and DsRed, respectively, into induced pluripotent stem cells (iPSCs) generated from DDEB fibroblasts. After sorting, 90% of the iPSCs were edited, and we selected four gene-edited iPSC lines for further study. These iPSCs were differentiated into keratinocytes and fibroblasts secreting COL7. RT-PCR and Western blot analyses revealed gene-edited COL7 with frameshift mutations degraded at the protein level. In addition, we confirmed that the gene-edited truncated COL7 could neither associate with normal COL7 nor undergo triple helix formation. Our data establish the feasibility of mutation site-specific genome editing in dominant negative disorders.

Shy, B. R., M. S. MacDougall, et al. "Co-incident insertion enables high efficiency genome engineering in mouse embryonic stem cells." Nucleic Acids Res. 2016 Sep 19;44(16):7997-8010. doi: 10.1093/nar/gkw685. Epub 2016 Aug 2.

CRISPR/Cas9 nucleases have enabled powerful, new genome editing capabilities; however, the preponderance of non-homologous end joining (NHEJ) mediated repair events over homology directed repair (HDR) in most cell types limits the ability to engineer precise changes in mammalian genomes. Here, we increase the efficiency of isolating precise HDR-mediated events in mouse embryonic stem (ES) cells by more than 20-fold through the use of co-incidental insertion (COIN) of independent donor DNA sequences. Analysis of on:off-target frequencies at the Lef1 gene revealed that bi-allelic insertion of a PGK-Neo cassette occurred more frequently than expected. Using various selection cassettes targeting multiple loci, we show that the insertion of a selectable marker at one control site frequently coincided with an insertion at an unlinked, independently targeted site, suggesting enrichment of a sub-population of HDR-proficient cells. When

individual cell events were tracked using flow cytometry and fluorescent protein markers, individual cells frequently performed either a homology-dependent insertion event or a homology-independent event, but rarely both types of insertions in a single cell. Thus, when HDR-dependent selection donors are used, COIN enriches for HDR-proficient cells among heterogeneous cell populations. When combined with a self-excising selection cassette, COIN provides highly efficient and scarless genome editing.

Smith, C., Z. Ye, et al. "Genome Editing in Human Pluripotent Stem Cells." Cold Spring Harb Protoc. 2016 Apr 1;2016(4):pdb.top086819. doi: 10.1101/pdb.top086819.

Pluripotent stem cells (PSCs), defined by their capacity for self-renewal and differentiation into all cell types, are an integral tool for basic biological research and disease modeling. However, full use of PSCs for research and regenerative medicine requires the ability to precisely edit their DNA to correct disease-causing mutations and for functional analysis of genetic variations. Recent advances in DNA editing of human stem cells (including PSCs) have benefited from the use of designer nucleases capable of making double-strand breaks (DSBs) at specific sequences that stimulate endogenous DNA repair. The clustered, regularly interspaced short palindromic repeats (CRISPR)-Cas9 system has become the preferred designer nuclease for genome editing in human PSCs and other cell types. Here we describe the principles for designing a single guide RNA to uniquely target a gene of interest and describe strategies for disrupting, inserting, or replacing a specific DNA sequence in human PSCs. The improvements in efficiency and ease provided by these techniques allow individuals to precisely engineer PSCs in a way previously limited to large institutes and core facilities.

Smith, C., Z. Ye, et al. "A Method for Genome Editing in Human Pluripotent Stem Cells." Cold Spring Harb Protoc. 2016 Apr 1;2016(4):pdb.prot090217. doi: 10.1101/pdb.prot090217.

Human pluripotent stem cells (PSCs) hold great potential for regenerative medicine and currently are being used as a research tool for basic discovery and disease modeling. To evaluate the role of a single genetic variant, a system of genome editing is needed to precisely mutate any desired DNA sequence in isolation and measure its effect on phenotype when compared to the isogenic parental PSC from which it was derived. This protocol describes the general targeting schemes used by researchers to edit PSCs to knock out, knock-in, or precisely alter a single nucleotide, covering conditions for electroporation, clonal isolation, and screening of edited PSCs for the targeted mutation. These recent advances simplify the procedure for genome editing, allowing individual researchers to induce nearly any desired mutation to further study its function or to reverse a disease-causing variant for future applications in regenerative medicine.

Soh, C. L. and D. Huangfu "CRISPR/Cas9-Mediated Mutagenesis of Human Pluripotent Stem Cells in Defined Xeno-Free E8 Medium." Methods Mol Biol. 2017;1498:57-78.

The recent advent of engineered nucleases including the CRISPR/Cas9 system has greatly facilitated genome manipulation in human pluripotent stem cells (hPSCs). In addition to facilitating hPSC-based disease studies, the application of genome engineering in hPSCs has also opened up new avenues for cell replacement therapy. To improve consistency and reproducibility of hPSC-based studies, and to meet the safety and regulatory requirements for clinical translation, it is necessary to use a defined, xeno-free cell culture system. This chapter describes protocols for CRISPR/Cas9 genome editing in an inducible Cas9 hPSC-based system, using cells cultured in chemically defined, xeno-free E8 Medium on a recombinant human vitronectin substrate. We detail procedures for the design and transfection of CRISPR guide RNAs, colony selection, and the expansion and validation of clonal mutant lines, all within this fully defined culture condition. These methods may be applied to a wide range of genome-engineering applications in hPSCs, including those that utilize different types of site-specific nucleases such as zinc finger nucleases (ZFNs) and TALENs, and form a closer step towards clinical utility of these cells.

Soldner, F., Y. Stelzer, et al. "Parkinson-associated risk variant in distal enhancer of alpha-synuclein modulates target gene expression." Nature. 2016 May 5;533(7601):95-9. doi: 10.1038/nature17939. Epub 2016 Apr 20.

Genome-wide association studies (GWAS) have identified numerous genetic variants associated with complex diseases, but mechanistic insights are impeded by a lack of understanding of how specific risk variants functionally contribute to the underlying pathogenesis. It has been proposed that cis-acting effects of non-coding risk variants on gene expression are a major factor for phenotypic variation of complex traits and disease susceptibility. Recent genome-scale epigenetic studies have highlighted the enrichment of GWAS-identified variants in regulatory DNA elements of disease-relevant cell types. Furthermore, single nucleotide polymorphism (SNP)-specific changes in transcription factor binding are correlated with heritable alterations in chromatin state and considered

a major mediator of sequence-dependent regulation of gene expression. Here we describe a novel strategy to functionally dissect the cis-acting effect of genetic risk variants in regulatory elements on gene expression by combining genome-wide epigenetic information with clustered regularly-interspaced short palindromic repeats (CRISPR)/Cas9 genome editing in human pluripotent stem cells. By generating a genetically precisely controlled experimental system, we identify a common Parkinson's disease associated risk variant in a non-coding distal enhancer element that regulates the expression of alpha-synuclein (SNCA), a key gene implicated in the pathogenesis of Parkinson's disease. Our data suggest that the transcriptional deregulation of SNCA is associated with sequence-dependent binding of the brain-specific transcription factors EMX2 and NKX6-1. This work establishes an experimental paradigm to functionally connect genetic variation with disease-relevant phenotypes.

Song, J., D. Yang, et al. "RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency." Nat Commun. 2016 Jan 28;7:10548. doi: 10.1038/ncomms10548.

Zinc-finger nuclease, transcription activator-like effector nuclease and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) are becoming major tools for genome editing. Importantly, knock-in in several non-rodent species has been finally achieved thanks to these customizable nucleases; yet the rates remain to be further improved. We hypothesize that inhibiting non-homologous end joining (NHEJ) or enhancing homology-directed repair (HDR) will improve the nuclease-mediated knock-in efficiency. Here we show that the in vitro application of an HDR enhancer, RS-1, increases the knock-in efficiency by two- to five-fold at different loci, whereas NHEJ inhibitor SCR7 has minimal effects. We then apply RS-1 for animal production and have achieved multifold improvement on the knock-in rates as well. Our work presents tools to nuclease-mediated knock-in animal production, and sheds light on improving gene-targeting efficiencies on pluripotent stem cells.

Sontag, S., M. Forster, et al. "Modelling IRF8 Deficient Human Hematopoiesis and Dendritic Cell Development with Engineered iPS Cells." Stem Cells. 2017 Jan 16. doi: 10.1002/stem.2565.

Human induced pluripotent stem (iPS) cells can differentiate into cells of all three germ layers, including hematopoietic stem cells and their progeny. Interferon regulatory factor 8 (IRF8) is a transcription factor, which acts in hematopoiesis as lineage determining factor for myeloid cells, including dendritic cells (DC). Autosomal recessive or dominant IRF8 mutations occurring in patients cause severe monocytic and DC immunodeficiency. To study IRF8 in human hematopoiesis we generated human IRF8-/- iPS cells and IRF8-/- embryonic stem (ES) cells using RNA guided CRISPR/Cas9n genome editing. Upon induction of hematopoietic differentiation, we demonstrate that IRF8 is dispensable for iPS cell and ES cell differentiation into hemogenic endothelium and for endothelial-to-hematopoietic transition, and thus development of hematopoietic progenitors. We differentiated iPS cell and ES cell derived progenitors into CD141+ cross-presenting cDC1 and CD1c+ classical cDC2 and CD303+ plasmacytoid DC (pDC). We found that IRF8 deficiency compromised cDC1 and pDC development while cDC2 development was largely unaffected. Additionally, in an unrestricted differentiation regimen, IRF8-/- iPS cells and ES cells exhibited a clear bias towards granulocytes at the expense of monocytes. IRF8-/- DC showed reduced MHC class II expression and were impaired in cytokine responses, migration and antigen presentation. Taken together, we engineered a human IRF8 knockout model that allows studying molecular mechanisms of human immunodeficiencies in vitro, including the pathophysiology of IRF8 deficient DC. This article is protected by copyright. All rights reserved.

Sun, J. J. and R. Ray "Generation of Two Noradrenergic-Specific Dopamine-Beta-Hydroxylase-FLPo Knock-In Mice Using CRISPR/Cas9-Mediated Targeting in Embryonic Stem Cells." PLoS One. 2016 Jul 21;11(7):e0159474. doi: 10.1371/journal.pone.0159474. eCollection 2016.

CRISPR/Cas9 mediated DNA double strand cutting is emerging as a powerful approach to increase rates of homologous recombination of large targeting vectors, but the optimization of parameters, equipment and expertise required remain barriers to successful mouse generation by single-step zygote injection. Here, we sought to apply CRISPR/Cas9 methods to traditional embryonic stem (ES) cell targeting followed by blastocyst injection to overcome the common issues of difficult vector construction and low targeting efficiency. To facilitate the study of noradrenergic function, which is implicated in myriad behavioral and physiological processes, we generated two different mouse lines that express FLPo recombinase under control of the noradrenergic-specific Dopamine-Beta-Hydroxylase (DBH) gene. We found that by co-electroporating a circular vector expressing Cas9 and a locus-specific sgRNA, we could target FLPo to the DBH locus in ES cells with shortened 1 kb homology arms. Two different sites in the DBH gene were targeted; the translational start codon with 6-8% targeting efficiency, and the

translational stop codon with 75% targeting efficiency. Using this approach, we established two mouse lines with DBH-specific expression of FLPo in brainstem catecholaminergic populations that are publically available on MMRRC (MMRRC_041575-UCD and MMRRC_041577-UCD). Altogether, this study supports simplified, high-efficiency Cas9/CRISPR-mediated targeting in embryonic stem cells for production of knock-in mouse lines in a wider variety of contexts than zygote injection alone.

Tabebordbar, M., K. Zhu, et al. "In vivo gene editing in dystrophic mouse muscle and muscle stem cells." Science. 2016 Jan 22;351(6271):407-11. doi: 10.1126/science.aad5177. Epub 2015 Dec 31.

Frame-disrupting mutations in the DMD gene, encoding dystrophin, compromise myofiber integrity and drive muscle deterioration in Duchenne muscular dystrophy (DMD). Removing one or more exons from the mutated transcript can produce an in-frame mRNA and a truncated, but still functional, protein. In this study, we developed and tested a direct gene-editing approach to induce exon deletion and recover dystrophin expression in the mdx mouse model of DMD. Delivery by adeno-associated virus (AAV) of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 endonucleases coupled with paired guide RNAs flanking the mutated Dmd exon23 resulted in excision of intervening DNA and restored the Dmd reading frame in myofibers, cardiomyocytes, and muscle stem cells after local or systemic delivery. AAV-Dmd CRISPR treatment partially recovered muscle functional deficiencies and generated a pool of endogenously corrected myogenic precursors in mdx mouse muscle.

Tagliafierro, L. and O. Chiba-Falek "Up-regulation of SNCA gene expression: implications to synucleinopathies." Neurogenetics. 2016 Jul;17(3):145-57. doi: 10.1007/s10048-016-0478-0. Epub 2016 Mar 7.

Synucleinopathies are a group of neurodegenerative diseases that share a common pathological lesion of intracellular protein inclusions largely composed by aggregates of alpha-synuclein protein. Accumulating evidence, including genome wide association studies, has implicated alpha-synuclein (SNCA) gene in the etiology of synucleinopathies. However, the precise variants within SNCA gene that contribute to the sporadic forms of Parkinson's disease (PD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), and other synucleinopathies and their molecular mechanisms of action remain elusive. It has been suggested that SNCA expression levels are critical for the development of these diseases. Here, we review several model systems that have been developed to advance the understanding of the role of SNCA expression levels in the etiology of synucleinopathies. We also describe different molecular mechanisms that regulate SNCA gene expression and discuss possible strategies for SNCA down-regulation as means for therapeutic approaches. Finally, we highlight some examples that underscore the relationships between the genetic association findings and the regulatory mechanisms of SNCA expression, which suggest that genetic variability in SNCA locus is directly responsible, at least in part, to the changes in gene expression and explain the reported associations of SNCA with synucleinopathies. Future studies utilizing induced pluripotent stem cells (iPSCs)-derived neuronal lines and genome editing by CRISPR/Cas9, will allow us to validate, characterize, and manipulate the effects of particular cis-genetic variants on SNCA expression. Moreover, this model system will enable us to compare different neuronal and glial lineages involved in synucleinopathies representing an attractive strategy to elucidate-common and specific-SNCA-genetic variants, regulatory mechanisms, and vulnerable expression levels underlying synucleinopathy spectrum disorders. This forthcoming knowledge will support the development of precision medicine for synucleinopathies.

Tang, Z. H., J. R. Chen, et al. "Genetic Correction of Induced Pluripotent Stem Cells From a Deaf Patient With MYO7A Mutation Results in Morphologic and Functional Recovery of the Derived Hair Cell-Like Cells." Stem Cells Transl Med. 2016 May;5(5):561-71. doi: 10.5966/sctm.2015-0252. Epub 2016 Mar 24.

The genetic correction of induced pluripotent stem cells (iPSCs) induced from somatic cells of patients with sensorineural hearing loss (caused by hereditary factors) is a promising method for its treatment. The correction of gene mutations in iPSCs could restore the normal function of cells and provide a rich source of cells for transplantation. In the present study, iPSCs were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T; P-iPSCs), the asymptomatic father of the patient (MYO7A c.1184G>A mutation; CF-iPSCs), and a normal donor (MYO7A(WT/WT); C-iPSCs). One of MYO7A mutation sites (c.4118C>T) in the P-iPSCs was corrected using CRISPR/Cas9. The corrected iPSCs (CP-iPSCs) retained cell pluripotency and normal karyotypes. Hair cell-like cells induced from CP-iPSCs showed restored organization of stereocilia-like protrusions; moreover, the electrophysiological function of these cells was similar to that of cells induced from C-iPSCs and CF-iPSCs. These results might facilitate the development of iPSC-based gene

therapy for genetic disorders. SIGNIFICANCE: Induced pluripotent stem cells (iPSCs) were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T). One of the MYO7A mutation sites (c.4118C>T) in the iPSCs was corrected using CRISPR/Cas9. The genetic correction of MYO7A mutation resulted in morphologic and functional recovery of hair cell-like cells derived from iPSCs. These findings confirm the hypothesis that MYO7A plays an important role in the assembly of stereocilia into stereociliary bundles. Thus, the present study might provide further insight into the pathogenesis of sensorineural hearing loss and facilitate the development of therapeutic strategies against monogenic disease through the genetic repair of patient-specific iPSCs.

Termglinchan, V., T. Seeger, et al. "Efficient Genome Editing in Induced Pluripotent Stem Cells with Engineered Nucleases In Vitro." Methods Mol Biol. 2017;1521:55-68.

Precision genome engineering is rapidly advancing the application of the induced pluripotent stem cells (iPSCs) technology for in vitro disease modeling of cardiovascular diseases. Targeted genome editing using engineered nucleases is a powerful tool that allows for reverse genetics, genome engineering, and targeted transgene integration experiments to be performed in a precise and predictable manner. However, nuclease-mediated homologous recombination is an inefficient process. Herein, we describe the development of an optimized method combining site-specific nucleases and the piggyBac transposon system for "seamless" genome editing in pluripotent stem cells with high efficiency and fidelity in vitro.

Tian, L., A. Deshmukh, et al. "Efficient and Controlled Generation of 2D and 3D Bile Duct Tissue from Human Pluripotent Stem Cell-Derived Spheroids." Stem Cell Rev. 2016 Aug;12(4):500-8. doi: 10.1007/s12015-016-9657-5.

While in vitro liver tissue engineering has been increasingly studied during the last several years, presently engineered liver tissues lack the bile duct system. The lack of bile drainage not only hinders essential digestive functions of the liver, but also leads to accumulation of bile that is toxic to hepatocytes and known to cause liver cirrhosis. Clearly, generation of bile duct tissue is essential for engineering functional and healthy liver. Differentiation of human induced pluripotent stem cells (iPSCs) to bile duct tissue requires long and/or complex culture conditions, and has been inefficient so far. Towards generating a fully functional liver containing biliary system, we have developed defined and controlled conditions for efficient 2D and 3D bile duct epithelial tissue generation. A marker for multipotent liver progenitor in both adult human liver and ductal plate in human fetal liver, EpCAM, is highly expressed in hepatic spheroids generated from human iPSCs. The EpCAM high hepatic spheroids can, not only efficiently generate a monolayer of biliary epithelial cells (cholangiocytes), in a 2D differentiation condition, but also form functional ductal structures in a 3D condition. Importantly, this EpCAM high spheroid based biliary tissue generation is significantly faster than other existing methods and does not require cell sorting. In addition, we show that a knock-in CK7 reporter human iPSC line generated by CRISPR/Cas9 genome editing technology greatly facilitates the analysis of biliary differentiation. This new ductal differentiation method will provide a more efficient method of obtaining bile duct cells and tissues, which may facilitate engineering of complete and functional liver tissue in the future.

Tsai, H. J. and C. P. Tseng "The adaptor protein Disabled-2: new insights into platelet biology and integrin signaling." Thromb J. 2016 Oct 4;14(Suppl 1):28. eCollection 2016.

Multiple functions of platelets in various physiological and pathological conditions have prompted considerable attention on understanding how platelets are generated and activated. Of the adaptor proteins that are expressed in megakaryocytes and platelets, Disabled-2 (Dab2) has been demonstrated in the past decades as a key regulator of platelet signaling. Dab2 has two alternative splicing isoforms p82 and p59. However, the mode of Dab2's action remains to be clearly defined. In this review, we highlight the current understanding of Dab2 expression and function in megakaryocytic differentiation, platelet activation and integrin signaling. Accordingly, Dab2 is upregulated when the human K562 cells, human CD34+ hematopoietic stem cells, and murine embryonic stem cells were undergone megakaryocytic differentiation. Appropriate level of Dab2 expression is essential for fate determination of mesodermal and megakaryocytic differentiation. Dab2 is also shown to regulate cell-cell and cell-fibrinogen adhesion, integrin alphaIIbbeta3 activation, fibrinogen uptake, and intracellular signaling of the megakaryocytic cells. In human platelets, p82 is the sole Dab2 isoform present in the cytoplasm and alpha-granules. Dab2 is released from the alpha-granules and forms two pools of Dab2 on the outer surface of the platelet plasma membrane, one at the sulfatide-bound and the other at integrin alphaIIbbeta3-bound forms. The balance between these two pools of Dab2 controls the extent of clotting reaction, platelet-fibrinogen interactions and outside-in signaling. In murine platelets, p59 is the only Dab2

isoform and is required for platelet aggregation, fibrinogen uptake, RhoA-ROCK activation, adenosine diphosphate release and integrin alphaIIbbeta3 activation stimulated by low concentration of thrombin. As a result, the bleeding time is prolonged and thrombus formation is impaired for the megakaryocyte lineage-restricted Dab2 deficient mouse. Although discrepancies of Dab2 function and isoform expression are noted between human and murine platelets, the studies up-to-date define Dab2 playing a pivotal role in integrin signaling and platelet activation. With the new tools such as CRISPR and TALEN in the generation of genetically modified animals, the progress in gaining new insights into the functions of Dab2 in megakaryocyte and platelet biology is expected to accelerate.

Tsuchiya, Y., Y. Umemura, et al. "Effect of Multiple Clock Gene Ablations on the Circadian Period Length and Temperature Compensation in Mammalian Cells." J Biol Rhythms. 2016 Feb;31(1):48-56. doi: 10.1177/0748730415613888. Epub 2015 Oct 28.

Most organisms have cell-autonomous circadian clocks to coordinate their activity and physiology according to 24-h environmental changes. Despite recent progress in circadian studies, it is not fully understood how the period length and the robustness of mammalian circadian rhythms are determined. In this study, we established a series of mouse embryonic stem cell (ESC) lines with single or multiplex clock gene ablations using the CRISPR/Cas9-based genome editing method. ESC-based in vitro circadian clock formation assay shows that the CRISPR-mediated clock gene disruption not only reproduces the intrinsic circadian molecular rhythms of previously reported mice tissues and cells lacking clock genes but also reveals that complexed mutations, such as CKIdelta(m/m):CKIepsilon(+/m):Cry2(m/m) mutants, exhibit an additively lengthened circadian period. By using these mutant cells, we also investigated the relation between period length alteration and temperature compensation. Although CKIdelta-deficient cells slightly affected the temperature insensitivity of period length, we demonstrated that the temperature compensation property is largely maintained in all mutants. These results show that the ESC-based assay system could offer a more systematic and comprehensive approach to the genotype-chronotype analysis of the intracellular circadian clockwork in mammals.

Turan, S., A. P. Farruggio, et al. "Precise Correction of Disease Mutations in Induced Pluripotent Stem Cells Derived From Patients With Limb Girdle Muscular Dystrophy." Mol Ther. 2016 Apr;24(4):685-96. doi: 10.1038/mt.2016.40. Epub 2016 Feb 26.

Limb girdle muscular dystrophies types 2B (LGMD2B) and 2D (LGMD2D) are degenerative muscle diseases caused by mutations in the dysferlin and alpha-sarcoglycan genes, respectively. Using patient-derived induced pluripotent stem cells (iPSC), we corrected the dysferlin nonsense mutation c.5713C>T; p.R1905X and the most common alpha-sarcoglycan mutation, missense c.229C>T; p.R77C, by single-stranded oligonucleotide-mediated gene editing, using the CRISPR/Cas9 gene-editing system to enhance the frequency of homology-directed repair. We demonstrated seamless, allele-specific correction at efficiencies of 0.7-1.5%. As an alternative, we also carried out precise gene addition strategies for correction of the LGMD2B iPSC by integration of wild-type dysferlin cDNA into the H11 safe harbor locus on chromosome 22, using dual integrase cassette exchange (DICE) or TALEN-assisted homologous recombination for insertion precise (THRIP). These methods employed TALENs and homologous recombination, and DICE also utilized site-specific recombinases. With DICE and THRIP, we obtained targeting efficiencies after selection of ~20%. We purified iPSC corrected by all methods and verified rescue of appropriate levels of dysferlin and alpha-sarcoglycan protein expression and correct localization, as shown by immunoblot and immunocytochemistry. In summary, we demonstrate for the first time precise correction of LGMD iPSC and validation of expression, opening the possibility of cell therapy utilizing these corrected iPSC.

Vassena, R., B. Heindryckx, et al. "Genome engineering through CRISPR/Cas9 technology in the human germline and pluripotent stem cells." Hum Reprod Update. 2016 Jun;22(4):411-9. doi: 10.1093/humupd/dmw005. Epub 2016 Feb 29.

BACKGROUND: With the recent development of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 genome editing technology, the possibility to genetically manipulate the human germline (gametes and embryos) has become a distinct technical possibility. Although many technical challenges still need to be overcome in order to achieve adequate efficiency and precision of the technology in human embryos, the path leading to genome editing has never been simpler, more affordable, and widespread. OBJECTIVE AND RATIONALE: In this narrative review we seek to understand the possible impact of CRISR/Cas9 technology on human reproduction from the technical and ethical point of view, and suggest a course of action for the scientific community. SEARCH METHODS: This non-systematic review was carried out using Medline articles in English, as well as

technical documents from the Human Fertilisation and Embryology Authority and reports in the media. The technical possibilities of the CRISPR/Cas9 technology with regard to human reproduction are analysed based on results obtained in model systems such as large animals and laboratory rodents. Further, the possibility of CRISPR/Cas9 use in the context of human reproduction, to modify embryos, germline cells, and pluripotent stem cells is reviewed based on the authors' expert opinion. Finally, the possible uses and consequences of CRISPR/cas9 gene editing in reproduction are analysed from the ethical point of view. OUTCOMES: We identify critical technical and ethical issues that should deter from employing CRISPR/Cas9 based technologies in human reproduction until they are clarified. WIDER IMPLICATIONS: Overcoming the numerous technical limitations currently associated with CRISPR/Cas9 mediated editing of the human germline will depend on intensive research that needs to be transparent and widely disseminated. Rather than a call to a generalized moratorium, or banning, of this type of research, efforts should be placed on establishing an open, international, collaborative and regulated research framework. Equally important, a societal discussion on the risks, benefits, and preferred applications of the new technology, including all relevant stakeholders, is urgently needed and should be promoted, and ultimately guide research priorities in this area.

Verissimo, C. S., R. M. Overmeer, et al. "Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening." Elife. 2016 Nov 15;5. pii: e18489. doi: 10.7554/eLife.18489.

Colorectal cancer (CRC) organoids can be derived from almost all CRC patients and therefore capture the genetic diversity of this disease. We assembled a panel of CRC organoids carrying either wild-type or mutant RAS, as well as normal organoids and tumor organoids with a CRISPR-introduced oncogenic KRAS mutation. Using this panel, we evaluated RAS pathway inhibitors and drug combinations that are currently in clinical trial for RAS mutant cancers. Presence of mutant RAS correlated strongly with resistance to these targeted therapies. This was observed in tumorigenic as well as in normal organoids. Moreover, dual inhibition of the EGFR-MEK-ERK pathway in RAS mutant organoids induced a transient cell-cycle arrest rather than cell death. In vivo drug response of xenotransplanted RAS mutant organoids confirmed this growth arrest upon pan-HER/MEK combination therapy. Altogether, our studies demonstrate the potential of patient-derived CRC organoid libraries in evaluating inhibitors and drug combinations in a preclinical setting.

Verma, N., Z. Zhu, et al. "CRISPR/Cas-Mediated Knockin in Human Pluripotent Stem Cells." Methods Mol Biol. 2017;1513:119-140.

Fluorescent reporter and epitope-tagged human pluripotent stem cells (hPSCs) greatly facilitate studies on the pluripotency and differentiation characteristics of these cells. Unfortunately traditional procedures to generate such lines are hampered by a low targeting efficiency that necessitates a lengthy process of selection followed by the removal of the selection cassette. Here we describe a procedure to generate fluorescent reporter and epitope tagged hPSCs in an efficient one-step process using the CRISPR/Cas technology. Although the method described uses our recently developed iCRISPR platform, the protocols can be adapted for general use with CRISPR/Cas or other engineered nucleases. The transfection procedures described could also be used for additional applications, such as overexpression or lineage tracing studies.

West, J. and W. W. Gill "Genome Editing in Large Animals." J Equine Vet Sci. 2016 Jun;41:1-6. Epub 2016 Mar 25.

Genome editing in large animals has tremendous practical applications, from more accurate models for medical research through improved animal welfare and production efficiency. Although genetic modification in large animals has a 30 year history, until recently technical issues limited its utility. The original methods - pronuclear injection and integrating viruses - were plagued with problems associated with low efficiency, silencing, poor regulation of gene expression, and variability associated with random integration. With the advent of site specific nucleases such as TALEN and CRISPR/Cas9, precision editing became possible. When used on their own, these can be used to truncate or knockout genes through non-homologous end joining (NHEJ) with relatively high efficiency. When used with a template containing desired gene edits, these can be used to allow insertion of any desired changes to the genome through homologous recombination (HR) with substantially lower efficiency. Consideration must be given to the issues of marker sets and off-target effects. Somatic cell nuclear transfer is most commonly used to create animals from gene edited cells, but direct zygote injection and use of spermatogonial stem cells are alternatives under development. In developing gene editing projects, priority must be given to understanding the potential for off-target or unexpected effects of planned edits, which have been common in the past. Because of the increasing technical sophistication with which it can be accomplished, genome editing is poised to

revolutionize large animal genetics, but attention must be paid to the underlying biology in order to maximize benefit.

Williams, C. A., R. Fernandez-Alonso, et al. "Erk5 Is a Key Regulator of Naive-Primed Transition and Embryonic Stem Cell Identity." Cell Rep. 2016 Aug 16;16(7):1820-8. doi: 10.1016/j.celrep.2016.07.033. Epub 2016 Aug 4.

Embryonic stem cells (ESCs) can self-renew or differentiate into any cell type, a phenomenon known as pluripotency. Distinct pluripotent states, termed naive and primed pluripotency, have been described. However, the mechanisms that control naive-primed pluripotent transition are poorly understood. Here, we perform a targeted screen for kinase inhibitors, which modulate the naive-primed pluripotent transition. We find that XMD compounds, which selectively inhibit Erk5 kinase and BET bromodomain family proteins, drive ESCs toward primed pluripotency. Using compound selectivity engineering and CRISPR/Cas9 genome editing, we reveal distinct functions for Erk5 and Brd4 in pluripotency regulation. We show that Erk5 signaling maintains ESCs in the naive state and suppresses progression toward primed pluripotency and neuroectoderm differentiation. Additionally, we identify a specialized role for Erk5 in defining ESC lineage selection, whereby Erk5 inhibits a cardiomyocyte-specific differentiation program. Our data therefore reveal multiple critical functions for Erk5 in controlling ESC identity.

Wissel, S., A. Kieser, et al. "A Combination of CRISPR/Cas9 and Standardized RNAi as a Versatile Platform for the Characterization of Gene Function." G3 (Bethesda). 2016 Aug 9;6(8):2467-78. doi: 10.1534/g3.116.028571.

Traditional loss-of-function studies in Drosophila suffer from a number of shortcomings, including off-target effects in the case of RNA interference (RNAi) or the stochastic nature of mosaic clonal analysis. Here, we describe minimal in vivo GFP interference (miGFPi) as a versatile strategy to characterize gene function and to conduct highly stringent, cell type-specific loss-of-function experiments in Drosophila miGFPi combines CRISPR/Cas9-mediated tagging of genes at their endogenous locus with an immunotag and an exogenous 21 nucleotide RNAi effector sequence with the use of a single reagent, highly validated RNAi line targeting this sequence. We demonstrate the utility and time effectiveness of this method by characterizing the function of the Polymerase I (Pol I)-associated transcription factor Tif-1a, and the previously uncharacterized gene MESR4, in the Drosophila female germline stem cell lineage. In addition, we show that miGFPi serves as a powerful technique to functionally characterize individual isoforms of a gene. We exemplify this aspect of miGFPi by studying isoform-specific loss-of-function phenotypes of the longitudinals lacking (lola) gene in neural stem cells. Altogether, the miGFPi strategy constitutes a generalized loss-of-function approach that is amenable to the study of the function of all genes in the genome in a stringent and highly time effective manner.

Woo, D. H., Q. Chen, et al. "Enhancing a Wnt-Telomere Feedback Loop Restores Intestinal Stem Cell Function in a Human Organotypic Model of Dyskeratosis Congenita." Cell Stem Cell. 2016 Sep 1;19(3):397-405. doi: 10.1016/j.stem.2016.05.024. Epub 2016 Aug 18.

Patients with dyskeratosis congenita (DC) suffer from stem cell failure in highly proliferative tissues, including the intestinal epithelium. Few therapeutic options exist for this disorder, and patients are treated primarily with bone marrow transplantation to restore hematopoietic function. Here, we generate isogenic DC patient and disease allele-corrected intestinal tissue using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated gene correction in induced pluripotent stem cells and directed differentiation. We show that DC tissue has suboptimal Wnt pathway activity causing intestinal stem cell failure and that enhanced expression of the telomere-capping protein TRF2, a Wnt target gene, can alleviate DC phenotypes. Treatment with the clinically relevant Wnt agonists LiCl or CHIR99021 restored TRF2 expression and reversed gastrointestinal DC phenotypes, including organoid formation in vitro, and maturation of intestinal tissue and xenografted organoids in vivo. Thus, the isogenic DC cell model provides a platform for therapeutic discovery and identifies Wnt modulation as a potential strategy for treatment of DC patients.

Wu, J., S. D. Hunt, et al. "Generation and validation of PAX7 reporter lines from human iPS cells using CRISPR/Cas9 technology." Stem Cell Res. 2016 Mar;16(2):220-8. doi: 10.1016/j.scr.2016.01.003. Epub 2016 Jan 13.

Directed differentiation of iPS cells toward various tissue progenitors has been the focus of recent research. Therefore, generation of tissue-specific reporter iPS cell lines provides better understanding of developmental stages in iPS cells. This technical report describes an efficient strategy for generation and validation of knock-in reporter lines in human iPS cells using the Cas9-nickase system. Here, we have generated a knock-in human iPS cell line for the early myogenic lineage specification gene of PAX7. By

introduction of site-specific double-stranded breaks (DSB) in the genomic locus of PAX7 using CRISPR/Cas9 nickase pairs, a 2A-GFP reporter with selection markers has been incorporated before the stop codon of the PAX7 gene at the last exon. After positive and negative selection, single cell-derived human iPS clones have been isolated and sequenced for in-frame positioning of the reporter construct. Finally, by using a nuclease-dead Cas9 activator (dCas9-VP160) system, the promoter region of PAX7 has been targeted for transient gene induction to validate the GFP reporter activity. This was confirmed by flow cytometry analysis and immunostaining for PAX7 and GFP. This technical report provides a practical guideline for generation and validation of knock-in reporters using CRISPR/Cas9 system.

Xiong, K., Y. Zhou, et al. "Generation of induced pluripotent stem cells (iPSCs) stably expressing CRISPR-based synergistic activation mediator (SAM)." Stem Cell Res. 2016 Nov 17;17(3):665-669. doi: 10.1016/j.scr.2016.10.011.

Human fibroblasts were engineered to express the CRISPR-based synergistic activation mediator (SAM) complex: dCas9-VP64 and MS2-P65-HSF1. Two induced pluripotent stem cells (iPSCs) clones expressing SAM were established by transducing these fibroblasts with lentivirus expressing OCT4, SOX2, KLF4 and C-MYC. We have validated that the reprogramming cassette is silenced in the SAM iPSC clones. Expression of pluripotency genes (OCT4, SOX2, LIN28A, NANOG, GDF3, SSEA4, and TRA-1-60), differentiation potential to all three germ layers, and normal karyotypes are validated. These SAM-iPSCs provide a novel, useful tool to investigate genetic regulation of stem cell proliferation and differentiation through CRISPR-mediated activation of endogenous genes.

Xu, K., X. Chen, et al. "Maternal Sall4 Is Indispensable for Epigenetic Maturation of Mouse Oocytes." J Biol Chem. 2016 Dec 28. pii: jbc.M116.767061. doi: 10.1074/jbc.M116.767061.

Splat-like 4 (Sall4) plays important roles in maintaining pluripotency of embryonic stem cells and in various developmental processes. Here, we find that Sall4 is highly expressed in oocytes and early embryos. To investigate the roles of SALL4 in oogenesis, we generated Sall4 maternal specific knockout mice by using CRISPR/Cas9 system. And we find that the maternal deletion of Sall4 causes developmental arrest of oocytes at germinal vesicle stage with non-surrounded nucleus and the subsequent meiosis resumption is prohibited. We further discover that the loss of maternal Sall4 causes failure in establishment of DNA methylation in oocytes. Furthermore, we find that Sall4 modulates H3K4me3 and H3K27me3 modifications by regulating the expression of key histone demethylases coding genes Kdm5b, Kdm6a and Kdm6b in oocytes. Moreover, we demonstrate that the aberrant H3K4me3 and H3K27me3 cause mis-expression of genes that are critical for oocytes maturation and meiosis resumption. Taken together, our study explores a pivotal role of Sall4 in regulating epigenetic maturation of mouse oocytes.

Xue, Z., S. Hennelly, et al. "A G-Rich Motif in the lncRNA Braveheart Interacts with a Zinc-Finger Transcription Factor to Specify the Cardiovascular Lineage." Mol Cell. 2016 Oct 6;64(1):37-50. doi: 10.1016/j.molcel.2016.08.010. Epub 2016 Sep 8.

Long non-coding RNAs (lncRNAs) are an emerging class of transcripts that can modulate gene expression; however, their mechanisms of action remain poorly understood. Here, we experimentally determine the secondary structure of Braveheart (Bvht) using chemical probing methods and show that this approximately 590 nt transcript has a modular fold. Using CRISPR/Cas9-mediated editing of mouse embryonic stem cells, we find that deletion of 11 nt in a 5' asymmetric G-rich internal loop (AGIL) of Bvht (bvhtdAGIL) dramatically impairs cardiomyocyte differentiation. We demonstrate a specific interaction between AGIL and cellular nucleic acid binding protein (CNBP/ZNF9), a zinc-finger protein known to bind single-stranded G-rich sequences. We further show that CNBP deletion partially rescues the bvhtdAGIL mutant phenotype by restoring differentiation capacity. Together, our work shows that Bvht functions with CNBP through a well-defined RNA motif to regulate cardiovascular lineage commitment, opening the door for exploring broader roles of RNA structure in development and disease.

Zhang, J. P., X. L. Li, et al. "Different Effects of sgRNA Length on CRISPR-mediated Gene Knockout Efficiency." Sci Rep. 2016 Jun 24;6:28566. doi: 10.1038/srep28566.

CRISPR-Cas9 is a powerful genome editing technology, yet with off-target effects. Truncated sgRNAs (17nt) have been found to decrease off-target cleavage without affecting on-target disruption in 293T cells. However, the potency of 17nt sgRNAs relative to the full-length 20nt sgRNAs in stem cells, such as human mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs), has not been assessed. Using a GFP reporter system, we found that both 17nt and 20nt sgRNAs expressed by lentiviral vectors induce ~95% knockout (KO) in 293T cells, whereas the KO efficiencies are significantly lower in iPSCs (60-70%) and MSCs (65-75%). Furthermore, we observed a decrease of 10-20 percentage points in

KO efficiency with 17nt sgRNAs compared to full-length sgRNAs in both iPSCs and MSCs. Off-target cleavage was observed in 17nt sgRNAs with 1-2nt but not 3-4nt mismatches; whereas 20nt sgRNAs with up to 5nt mismatches can still induce off-target mutations. Of interest, we occasionally observed off-target effects induced by the 17nt but not the 20nt sgRNAs. These results indicate the importance of balancing on-target gene cleavage potency with off-target effects: when efficacy is a major concern such as genome editing in stem cells, the use of 20nt sgRNAs is preferable.

Zhang, N., H. Zhi, et al. "CRISPR/Cas9-mediated conversion of human platelet alloantigen allotypes." Blood. 2016 Feb 11;127(6):675-80. doi: 10.1182/blood-2015-10-675751. Epub 2015 Dec 3.

Human platelet alloantigens (HPAs) reside on functionally important platelet membrane glycoproteins and are caused by single nucleotide polymorphisms in the genes that encode them. Antibodies that form against HPAs are responsible for several clinically important alloimmune bleeding disorders, including fetal and neonatal alloimmune thrombocytopenia and posttransfusion purpura. The HPA-1a/HPA-1b alloantigen system, also known as the Pl(A1)/Pl(A2) polymorphism, is the most frequently implicated HPA among whites, and a single Leu33Pro amino acid polymorphism within the integrin beta3 subunit is responsible for generating the HPA-1a/HPA-1b alloantigenic epitopes. HPA-1b/b platelets, like those bearing other low-frequency platelet-specific alloantigens, are relatively rare in the population and difficult to obtain for purposes of transfusion therapy and diagnostic testing. We used CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) gene-editing technology to transform Leu33 (+) megakaryocytelike DAMI cells and induced pluripotent stem cells (iPSCs) to the Pro33 allotype. CD41(+) megakaryocyte progenitors derived from these cells expressed the HPA-1b (Pl(A2)) alloantigenic epitope, as reported by diagnostic NciI restriction enzyme digestion, DNA sequencing, and western blot analysis using HPA-1b-specific human maternal alloantisera. Application of CRISPR/Cas9 technology to genetically edit this and other clinically-important HPAs holds great potential for production of designer platelets for diagnostic, investigative, and, ultimately, therapeutic use.

Zhou, G., S. Meng, et al. "Optimal ROS Signaling Is Critical for Nuclear Reprogramming." Cell Rep. 2016 May 3;15(5):919-25. doi: 10.1016/j.celrep.2016.03.084. Epub 2016 Apr 21.

Efficient nuclear reprogramming of somatic cells to pluripotency requires activation of innate immunity. Because innate immune activation triggers reactive oxygen species (ROS) signaling, we sought to determine whether there was a role of ROS signaling in nuclear reprogramming. We examined ROS production during the reprogramming of doxycycline (dox)-inducible mouse embryonic fibroblasts (MEFs) carrying the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc [OSKM]) into induced pluripotent stem cells (iPSCs). ROS generation was substantially increased with the onset of reprogramming. Depletion of ROS via antioxidants or Nox inhibitors substantially decreased reprogramming efficiency. Similarly, both knockdown and knockout of p22(phox)-a critical subunit of the Nox (1-4) complex-decreased reprogramming efficiency. However, excessive ROS generation using genetic and pharmacological approaches also impaired reprogramming. Overall, our data indicate that ROS signaling is activated early with nuclear reprogramming, and optimal levels of ROS signaling are essential to induce pluripotency.

Zhou, Y., R. A. Al-Saaidi, et al. "Mitochondrial Spare Respiratory Capacity Is Negatively Correlated with Nuclear Reprogramming Efficiency." Stem Cells Dev. 2016 Nov 28.

Nuclear reprogramming efficiency has been shown to be highly variable among different types of somatic cells and different individuals, yet the underlying mechanism remains largely unknown. Several studies have shown that reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs) requires remodeling of mitochondria and a metabolic shift from an oxidative state to a more glycolytic state. In this study, we evaluated the nuclear reprogramming efficiency in relation to mitochondrial bioenergetic parameters of fibroblasts from seven different human individuals. Using the Seahorse extracellular energy flux analyzer, we measured oxygen consumption rate (OCR) profiles of the cells, along with their nuclear reprogramming efficiency into iPSCs. Our results showed that fibroblasts with the lowest mitochondrial spare respiratory capacity (SRC) had the highest nuclear reprogramming efficiency, opposed to fibroblasts with the highest mitochondrial SRC, which showed lowest reprogramming efficiency. Furthermore, we found that targeted fluorescent tagging of endogenous genes (MYH6 and COL2A1) by CRISPR/Cas9-mediated homologous recombination was accompanied by an increase in the SRC level of the modified fibroblasts and impaired reprogramming efficiency. Our findings indicate a negative correlation between high mitochondrial SRC in somatic cells and low reprogramming efficiencies. This type of analysis potentially allows screening and predicting reprogramming efficiency before reprogramming, and further suggests that nuclear reprogramming might be

improved by approaches that modulate the SRC.

Zhu, Z., Q. V. Li, et al. "Genome Editing of Lineage Determinants in Human Pluripotent Stem Cells Reveals Mechanisms of Pancreatic Development and Diabetes." Cell Stem Cell. 2016 Jun 2;18(6):755-68. doi: 10.1016/j.stem.2016.03.015. Epub 2016 Apr 28.

Directed differentiation of human pluripotent stem cells (hPSCs) into somatic counterparts is a valuable tool for studying disease. However, examination of developmental mechanisms in hPSCs remains challenging given complex multi-factorial actions at different stages. Here, we used TALEN and CRISPR/Cas-mediated gene editing and hPSC-directed differentiation for a systematic analysis of the roles of eight pancreatic transcription factors (PDX1, RFX6, PTF1A, GLIS3, MNX1, NGN3, HES1, and ARX). Our analysis not only verified conserved gene requirements between mice and humans but also revealed a number of previously unsuspected developmental mechanisms with implications for type 2 diabetes. These include a role of RFX6 in regulating the number of pancreatic progenitors, a haploinsufficient requirement for PDX1 in pancreatic beta cell differentiation, and a potentially divergent role of NGN3 in humans and mice. Our findings support use of systematic genome editing in hPSCs as a strategy for understanding mechanisms underlying congenital disorders.

Zuo, Q., Y. Wang, et al. "Site-Directed Genome Knockout in Chicken Cell Line and Embryos Can Use CRISPR/Cas Gene Editing Technology." G3 (Bethesda). 2016 Jun 1;6(6):1787-92. doi: 10.1534/g3.116.028803.

The present study established an efficient genome editing approach for the construction of stable transgenic cell lines of the domestic chicken (Gallus gallus domesticus). Our objectives were to facilitate the breeding of high-yield, high-quality chicken strains, and to investigate gene function in chicken stem cells. Three guide RNA (gRNAs) were designed to knockout the C2EIP gene, and knockout efficiency was evaluated in DF-1 chicken fibroblasts and chicken ESCs using the luciferase single-strand annealing (SSA) recombination assay, T7 endonuclease I (T7EI) assay, and TA clone sequencing. In addition, the polyethylenimine-encapsulated Cas9/gRNA plasmid was injected into fresh fertilized eggs. At 4.5 d later, frozen sections of the embryos were prepared, and knockout efficiency was evaluated by the T7EI assay. SSA assay results showed that luciferase activity of the vector expressing gRNA-3 was double that of the control. Results of the T7EI assay and TA clone sequencing indicated that Cas9/gRNA vector-mediated gene knockdown efficiency was approximately 27% in both DF-1 cells and ESCs. The CRISPR/Cas9 vector was also expressed in chicken embryos, resulting in gene knockdown in three of the 20 embryos (gene knockdown efficiency 15%). Taken together, our results indicate that the CRISPR/Cas9 system can mediate stable gene knockdown at the cell and embryo levels in domestic chickens.

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