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Stem Cell

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Genome Editing 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. Genome editing, or genome engineering, or gene editing, is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly inserts genetic material into a host genome, genome editing targets the insertions to site specific locations. This article introduces recent research reports as references in the related studies.
[Mark H. **Genome Editing and Stem Cell Research Literatures.** *Stem Cell* 2020;11(1):61-101]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 5. doi:10.7537/marsscj110120.05.

Key words: stem cell; genome editing; 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. Genome editing, or genome engineering, or gene editing, is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly inserts genetic material into a host genome, genome editing targets the insertions to site specific locations. This article introduces recent research reports as references in the related studies.

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

Abulaiti, X., et al. (2017). "Phosphorylation of Threonine (343) Is Crucial for OCT4 Interaction with SOX2 in the Maintenance of Mouse Embryonic Stem Cell Pluripotency." Stem Cell Reports **9**(5): 1630-1641.

OCT4 is required to maintain the pluripotency of embryonic stem cells (ESCs); yet, overdose-expression of OCT4 induces ESC differentiation toward primitive endoderm. The molecular mechanism underlying this differentiation switch is not fully understood. Here, we found that substitution of threonine (343) by alanine (T343A), but not aspartic acid (T343D), caused a significant loss of OCT4-phosphorylation signal in ESCs. Loss of such OCT4-phosphorylation compromises its interaction with SOX2 but promotes interaction with SOX17. We therefore propose that threonine (343)-based OCT4-phosphorylation is crucial for the maintenance of ESC pluripotency. This OCT4-phosphorylation-based mechanism may provide insight into the regulation of lineage specification during early embryonic development.

Alexeeva, V., et al. (2016). "A human MIXL1 green fluorescent protein reporter embryonic stem cell line engineered using TALEN-based genome editing." Stem Cell Res **17**(1): 93-96.

We have generated a MIXL1-eGFP reporter human embryonic stem cell (hESC) line using TALEN-based genome engineering. This line accurately traces endogenous MIXL1 expression via an eGFP reporter to mesendodermal precursor cells. The utility of the MIXL1-eGFP reporter hESC line lies in the prospective isolation, lineage tracing, and

developmental and mechanistic studies of MIXL1(+) cell populations.

Alzubi, J., et al. (2017). "Targeted genome editing restores T cell differentiation in a humanized X-SCID pluripotent stem cell disease model." Sci Rep **7**(1): 12475.

The generation of T cells from pluripotent stem cells (PSCs) is attractive for investigating T cell development and validating genome editing strategies in vitro. X-linked severe combined immunodeficiency (X-SCID) is an immune disorder caused by mutations in the IL2RG gene and characterised by the absence of T and NK cells in patients. IL2RG encodes the common gamma chain, which is part of several interleukin receptors, including IL-2 and IL-7 receptors. To model X-SCID in vitro, we generated a mouse embryonic stem cell (ESC) line in which a disease-causing human IL2RG gene variant replaces the endogenous Il2rg locus. We developed a stage-specific T cell differentiation protocol to validate genetic correction of the common G691A mutation with transcription activator-like effector nucleases. While all ESC clones could be differentiated to hematopoietic precursor cells, stage-specific analysis of T cell maturation confirmed early arrest of T cell differentiation at the T cell progenitor stage in X-SCID cells. In contrast, genetically corrected ESCs differentiated to CD4 + or CD8 + single-positive T cells, confirming correction of the cellular X-SCID phenotype. This study emphasises the value of PSCs for disease modelling and underlines the significance of in vitro models as tools to validate genome editing strategies before clinical application.

Anderson, R. H. and K. R. Francis (2018). "Modeling rare diseases with induced pluripotent stem cell technology." Mol Cell Probes **40**: 52-59.

Rare diseases, in totality, affect a significant proportion of the population and represent an unmet medical need facing the scientific community. However, the treatment of individuals affected by rare diseases is hampered by poorly understood mechanisms preventing the development of viable therapeutics. The discovery and application of cellular reprogramming to create novel induced pluripotent stem cell models of rare diseases has revolutionized the rare disease community. Through developmental and functional analysis of differentiated cell types, these stem cell models carrying patient-specific mutations have become an invaluable tool for rare disease research. In this review article, we discuss the reprogramming of samples from individuals affected with rare diseases to induced pluripotent stem cells, current and future applications for this technology, and how integration of genome editing to rare disease research will help to improve our understanding of disease pathogenesis and lead to patient therapies.

Apati, A., et al. (2018). "Application of human pluripotent stem cells and pluripotent stem cell-derived cellular models for assessing drug toxicity." Expert Opin Drug Metab Toxicol.

INTRODUCTION: Human pluripotent stem cells (hPSCs) are capable of differentiating into all types of cells in the body and so provide suitable toxicology screening systems even for hard-to-obtain human tissues. Since hPSCs can also be generated from differentiated cells and current gene editing technologies allow targeted genome modifications, hPSCs can be applied for drug toxicity screening both in normal and disease-specific models. Targeted hPSC differentiation is still a challenge but cardiac, neuronal or liver cells, and complex cellular models are already available for practical applications. Areas covered: The authors review new gene-editing and cell-biology technologies to generate sensitive toxicity screening systems based on hPSCs. Then the authors present the use of undifferentiated hPSCs for examining embryonic toxicity and discuss drug screening possibilities in hPSC-derived models. The authors focus on the application of human cardiomyocytes, hepatocytes, and neural cultures in toxicity testing, and discuss the recent possibilities for drug screening in a "body-on-a-chip" model system. Expert opinion: hPSCs and their genetically engineered derivatives provide new possibilities to investigate drug toxicity in human tissues. The key issues in this regard are still the selection and generation of proper model systems, and the interpretation of the results in understanding in vivo drug effects.

Argani, H. (2019). "Genome Engineering for Stem Cell Transplantation." Exp Clin Transplant **17**(Suppl 1): 31-37.

To avoid the ethical issues of embryonic stem cells, genome engineering has focused on inducible pluripotent stem cells, which can develop into all 3 germ layers. The ability to detect methylation patterns in these cells allows research into pluripotency markers. The recently developed CRISPR system has allowed widespread application of genome engineering techniques. The CRISPR-Cas9 system, a potent system for genome editing, can be used for gene knockout or knock-in genome manipulations through substitution of a target genetic sequence with a desired donor sequence. Two types of genome engineering can be initiated: homologous or nonhomologous DNA repair by the Cas9 nuclease. Delivery of the CRISPR-Cas9 and target donor vectors in human pluripotent stem cells can be accomplished via viral and nonviral delivery methods. Nonviral

delivery includes lipid-mediated transfection and electroporation. It has become the most common and efficient in vitro delivery method for human pluripotent stem cells. The CRISPR-Cas9 system can be combined with inducible pluripotent stem cells to generate single or multiple gene knockouts, correct mutations, or insert reporter transgenes. Knockouts can also be utilized to investigate epigenetic roles and targets, such as investigation of DNA methylation. CRISPR could be combined with human pluripotent stem cells to explore genetic determinants of lineage choice, differentiation, and stem cell fate, allowing investigators to study how various genes or noncoding elements contribute to specific processes and pathways. The CRISPR-Cas9 system can also be used to create null or nucleasedead Cas9, which has no enzymatic activity but has been utilized through fusion with other functional protein domains. In conclusion, RNA-guided genome targeting will have broad implications for synthetic biology, direct perturbation of gene networks, and targeted ex vivo and in vivo gene therapy.

Artero Castro, A., et al. (2018). "Concise Review: Human Induced Pluripotent Stem Cell Models of Retinitis Pigmentosa." Stem Cells **36**(4): 474-481.

Hereditary retinal dystrophies, specifically retinitis pigmentosa (RP) are clinically and genetically heterogeneous diseases affecting primarily retinal cells and retinal pigment epithelial cells with blindness as a final outcome. Understanding the pathogenicity behind these diseases has been largely precluded by the unavailability of affected tissue from patients, large genetic heterogeneity and animal models that do not faithfully represent some human diseases. A landmark discovery of human induced pluripotent stem cells (hiPSCs) permitted the derivation of patient-specific cells. These cells have unlimited self-renewing capacity and the ability to differentiate into RP-affected cell types, allowing the studies of disease mechanism, drug discovery, and cell replacement therapies, both as individual cell types and organoid cultures. Together with precise genome editing, the patient specific hiPSC technology offers novel strategies for targeting the pathogenic mutations and design therapies toward retinal dystrophies. This study summarizes current hiPSC-based RP models and highlights key achievements and challenges of these cellular models, as well as questions that still remain unanswered. Stem Cells 2018;36:474-481.

Baden, P., et al. (2019). "Insights into GBA Parkinson's disease pathology and therapy with induced pluripotent stem cell model systems." Neurobiol Dis **127**: 1-12.

While the link between GBA and Parkinson's disease (PD) was initially unexpected, it is now well established that GBA mutations are the most frequent genetic risk for PD. GBA has also been linked to sporadic PD, dementia with Lewy bodies, and ageing. Thus, GBA represents a promising target to counteract brain disease and the age-related decline of lysosomal function. The exact mechanisms involved in the risk of developing PD in GBA mutation carriers are still unclear and research in this field has faced the major challenge of a lack of proper modeling systems. Induced pluripotent stem cells (iPSCs) as well as advances in disease modeling and genome editing have facilitated studies of human brain disease. With regard to GBA-PD, iPSCs offer several advantages including the possibility of investigating sphingolipid (SPL) biology in relevant cells, the role of dopamine metabolism as well as non-cell autonomous mechanisms that are likely involved in the disease process. This review will summarize findings that emerged from iPSC-based studies in the context of GBA-PD pathology and therapy. We also highlight current advantages and challenges of stem cell models for neurological disease modeling and drug discovery.

Balboa, D. and T. Otonkoski (2015). "Human pluripotent stem cell based islet models for diabetes research." Best Pract Res Clin Endocrinol Metab **29**(6): 899-909.

Although similar, mouse and human pancreatic development and beta cell physiology have significant differences. For this reason, mouse models present shortcomings that can obscure the understanding of human diabetes pathology. Progress in the field of human pluripotent stem cell (hPSC) differentiation now makes it possible to derive unlimited numbers of human beta cells in vitro. This constitutes an invaluable approach to gain insight into human beta cell development and physiology and to generate improved disease models. Here we summarize the main differences in terms of development and physiology of the pancreatic endocrine cells between mouse and human, and describe the recent progress in modeling diabetes using hPSC. We highlight the need of developing more physiological hPSC-derived beta cell models and anticipate the future prospects of these approaches.

Balboa, D., et al. (2019). "Concise Review: Human Pluripotent Stem Cells for the Modeling of Pancreatic beta-Cell Pathology." Stem Cells **37**(1): 33-41.

Pancreatic beta-cells are the only source of insulin. Disturbances in beta-cell development or function may thus result in insulin deficiency or excess, presenting as hyper- or hypoglycemia. It is

increasingly evident that common forms of diabetes (types 1 and 2) are pathogenically heterogeneous. Development of efficient therapies is dependent on reliable disease models. Although animal models are remarkably useful research tools, they present limitations because of species differences. As an alternative, human pluripotent stem cell technologies offer multiple possibilities for the study of human diseases in vitro. In the last decade, advances in the derivation of induced pluripotent stem cells from diabetic patients, combined with beta-cell differentiation protocols, have resulted in the generation of useful disease models for diabetes. First disease models have been focusing on monogenic diabetes. The development of genome editing technologies, more advanced differentiation protocols and humanized mouse models based on transplanted cells have opened new horizons for the modeling of more complex forms of beta-cell dysfunction. We present here the incremental progress made in the modeling of diabetes using pluripotent stem cells. We discuss the current challenges and opportunities of these approaches to dissect beta-cell pathology and devise new pharmacological and cell replacement therapies. Stem Cells 2019;37:33-41.

Bao, X., et al. (2017). "Human pluripotent stem cell-derived epicardial progenitors can differentiate to endocardial-like endothelial cells." Bioeng Transl Med **2**(2): 191-201.

During heart development, epicardial progenitors contribute various cardiac lineages including smooth muscle cells, cardiac fibroblasts, and endothelial cells. However, their specific contribution to the human endothelium has not yet been resolved, at least in part due to the inability to expand and maintain human primary or pluripotent stem cell (hPSC)-derived epicardial cells. Here we first generated CDH5-2A-eGFP knock-in hPSC lines and differentiated them into self-renewing WT1+ epicardial cells, which gave rise to endothelial cells upon VEGF treatment in vitro. In addition, we found that the percentage of endothelial cells correlated with WT1 expression in a WT1-2A-eGFP reporter line. The resulting endothelial cells displayed many endocardium-like endothelial cell properties, including high expression levels of endocardial-specific markers, nutrient transporters and well-organized tight junctions. These findings suggest that human epicardial progenitors may have the capacity to form endocardial endothelium during development and have implications for heart regeneration and cardiac tissue engineering.

Beer, N. L. and A. L. Gloyn (2016). "Genome-edited human stem cell-derived beta cells: a powerful tool for drilling down on type 2 diabetes GWAS biology." F1000Res **5**.

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.

Bhalerao, R. P. and U. Fischer (2017). "Environmental and hormonal control of cambial stem cell dynamics." J Exp Bot **68**(1): 79-87.

Perennial trees have the amazing ability to adjust their growth rate to both adverse and favorable seasonally reoccurring environmental conditions over hundreds of years. In trunks and stems, the basis for the tuning of seasonal growth rate is the regulation of cambial stem cell activity. Cambial stem cell quiescence and dormancy protect the tree from potential physiological and genomic damage caused by adverse growing conditions and may permit a long lifespan. Cambial dormancy and longevity are both aspects of a tree's life for which the study of cambial stem cell behavior in the annual model plant Arabidopsis is inadequate. Recent functional analyses of hormone perception and catabolism mutants in Populus indicate that shoot-derived long-range signals,

as well as local cues, steer cambial activity. Auxin is central to the regulation of cambial activity and probably also maintenance. Emerging genome editing and phenotyping technologies will enable the identification of down-stream targets of hormonal action and facilitate the genetic dissection of complex traits of cambial biology.

Blair, J. D., et al. (2016). "Establishment of Genome-edited Human Pluripotent Stem Cell Lines: From Targeting to Isolation." J Vis Exp (108): e53583.

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.

Blomberg, L. A. and B. P. Telugu (2012). "Twenty years of embryonic stem cell research in farm animals." Reprod Domest Anim **47 Suppl 4**: 80-85.

Notable distinctions between an embryonic stem cell (ESC) and somatic cell are that an ESC can maintain an undifferentiated state indefinitely, self-renew, and is pluripotent, meaning that the ESC can potentially generate cells representing all the three primordial germ layers and contribute to the terminally differentiated cells of a conceptus. These attributes make the ESC an ideal source for genome editing for both agricultural and biomedical applications. Although, ESC lines have been successfully established from rodents and primates, authentic ungulate stem cell lines on the contrary are still not available. Outstanding issues including but not limited to differences in pluripotency characteristics among the existing ESC lines, pre-implantation embryo development, pluripotency pathways, and culture conditions plague our efforts to establish authentic ESC lines from farm animals. In this review, we highlight some of these issues and discuss how the recent derivation of induced pluripotent stem cells (iPSCs) might augur the establishment of robust authentic ESC lines from farm animals.

Buikema, J. W. and S. M. Wu (2017). "Untangling the Biology of Genetic Cardiomyopathies with Pluripotent Stem Cell Disease Models." Curr Cardiol Rep **19**(4): 30.

PURPOSE OF REVIEW: Recently, the discovery of strategies to reprogram somatic cells into induced pluripotent stem (iPS) cells has led to a major paradigm change in developmental and stem cell biology. The application of iPS cells and their cardiac progeny has opened novel directions to study cardiomyopathies at a cellular and molecular level. This review discusses approaches currently undertaken to unravel known inherited cardiomyopathies in a dish. RECENT FINDINGS: With improved efficiency for mutation correction by genome editing, human iPS cells have now provided a platform to untangle the biology of cardiomyopathies. Multiple studies have derived pluripotent stem cells lines from patients with genetic heart diseases. The generation of cardiomyocytes from these cells lines has, for the first time, enable the study of cardiomyopathies using cardiomyocytes harboring patient-specific mutations and their corrected isogenic counterpart. The molecular analyses, functional assays, and drug tests of these lines have led to new molecular insights in the early pathophysiology of left ventricular non-compaction cardiomyopathy (LVNC), hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and others. The advent of iPS cells offers an exceptional opportunity for creating disease-specific cellular models to investigate their underlying mechanisms and to optimize future therapy through drug and toxicity screening. Thus far, the iPS cell model has improved our understanding of the genetic and molecular pathophysiology of patients with various genetic cardiomyopathies. It is hoped that the new discoveries arising from using these novel

platforms for cardiomyopathy research will lead to new diagnostic and therapeutic approaches to prevent and treat these diseases.

Cai, L., et al. (2018). "A Universal Approach to Correct Various HBB Gene Mutations in Human Stem Cells for Gene Therapy of Beta-Thalassemia and Sickle Cell Disease." Stem Cells Transl Med **7**(1): 87-97.

Beta-thalassemia is one of the most common recessive genetic diseases, caused by mutations in the HBB gene. Over 200 different types of mutations in the HBB gene containing three exons have been identified in patients with beta-thalassemia (beta-thal) whereas a homozygous mutation in exon 1 causes sickle cell disease (SCD). Novel therapeutic strategies to permanently correct the HBB mutation in stem cells that are able to expand and differentiate into erythrocytes producing corrected HBB proteins are highly desirable. Genome editing aided by CRISPR/Cas9 and other site-specific engineered nucleases offers promise to precisely correct a genetic mutation in the native genome without alterations in other parts of the human genome. Although making a sequence-specific nuclease to enhance correction of a specific HBB mutation by homology-directed repair (HDR) is becoming straightforward, targeting various HBB mutations of beta-thal is still challenging because individual guide RNA as well as a donor DNA template for HDR of each type of HBB gene mutation have to be selected and validated. Using human induced pluripotent stem cells (iPSCs) from two beta-thal patients with different HBB gene mutations, we devised and tested a universal strategy to achieve targeted insertion of the HBB cDNA in exon 1 of HBB gene using Cas9 and two validated guide RNAs. We observed that HBB protein production was restored in erythrocytes derived from iPSCs of two patients. This strategy of restoring functional HBB gene expression will be able to correct most types of HBB gene mutations in beta-thal and SCD. Stem Cells Translational Medicine 2018;7:87-97.

Cerbini, T., et al. (2015). "Transcription activator-like effector nuclease (TALEN)-mediated CLYBL targeting enables enhanced transgene expression and one-step generation of dual reporter human induced pluripotent stem cell (iPSC) and neural stem cell (NSC) lines." PLoS One **10**(1): e0116032.

Targeted genome engineering to robustly express transgenes is an essential methodology for stem cell-based research and therapy. Although designer nucleases have been used to drastically enhance gene editing efficiency, targeted addition and stable expression of transgenes to date is limited at single gene/locus and mostly PPP1R12C/AAVS1 in human stem cells. Here we constructed transcription activator-like effector nucleases (TALENs) targeting the safe-harbor like gene CLYBL to mediate reporter gene integration at 38%-58% efficiency, and used both AAVS1-TALENs and CLYBL-TALENs to simultaneously knock-in multiple reporter genes at dual safe-harbor loci in human induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs). The CLYBL-TALEN engineered cell lines maintained robust reporter expression during self-renewal and differentiation, and revealed that CLYBL targeting resulted in stronger transgene expression and less perturbation on local gene expression than PPP1R12C/AAVS1. TALEN-mediated CLYBL engineering provides improved transgene expression and options for multiple genetic modification in human stem cells.

Chao, M. P. and R. Majeti (2019). "Induced pluripotent stem cell modeling of malignant hematopoiesis." Exp Hematol **71**: 68-76.

The ability to epigenetically reprogram differentiated somatic cells to pluripotency resulting in the discovery of induced pluripotent stem cells (iPSCs), has unlocked fundamental biologic insights into numerous genetic diseases. These insights have resulted from the key property of iPSCs to differentiate into all cell lineages in an unlimited manner while maintaining the genetic identity of the originating cell. iPSCs have been utilized to investigate both monogenic and complex genetic disorders spanning hereditary and acquired diseases. Recently, iPSCs have been utilized to model human cancer, with a specific focus on modeling conditions of malignant hematopoiesis. In addition to serving as a genetic disease model in cancer, iPSCs can also be used as a tool to address a key question in interrogating the interaction between the cancer epigenome-genome. Specifically, how does reprogramming the epigenome affect cancer phenotype and specifically malignant hematopoiesis? This review will address this question and highlight the state of the field in generating iPSCs from hematologic malignancies, key biologic insights that can be uniquely generated from cancer-derived iPSCs, and their clinical applications. Last, challenges to expanding the use of iPSC modeling in blood cancers will be discussed.

Chaterji, S., et al. (2017). "CRISPR Genome Engineering for Human Pluripotent Stem Cell Research." Theranostics **7**(18): 4445-4469.

The emergence of targeted and efficient genome editing technologies, such as repurposed bacterial programmable nucleases (e.g., CRISPR-Cas systems), has abetted the development of cell engineering

approaches. Lessons learned from the development of RNA-interference (RNA-i) therapies can spur the translation of genome editing, such as those enabling the translation of human pluripotent stem cell engineering. In this review, we discuss the opportunities and the challenges of repurposing bacterial nucleases for genome editing, while appreciating their roles, primarily at the epigenomic granularity. First, we discuss the evolution of high-precision, genome editing technologies, highlighting CRISPR-Cas9. They exist in the form of programmable nucleases, engineered with sequence-specific localizing domains, and with the ability to revolutionize human stem cell technologies through precision targeting with greater on-target activities. Next, we highlight the major challenges that need to be met prior to bench-to-bedside translation, often learning from the path-to-clinic of complementary technologies, such as RNA-i. Finally, we suggest potential bioinformatics developments and CRISPR delivery vehicles that can be deployed to circumvent some of the challenges confronting genome editing technologies en route to the clinic.

Chattong, S., et al. (2018). "Efficient ZFN-Mediated Stop Codon Integration into the CCR5 Locus in Hematopoietic Stem Cells: A Possible Source for Intrabone Marrow Cell Transplantation." AIDS Res Hum Retroviruses **34**(7): 575-579.

We reported a simple genome editing approach that can generate human immunodeficiency virus-1 (HIV) coreceptor defective cells, which may be useful for latent viral eradication treatment. Samples of bone marrow leftover after diagnostic procedures and crude bone marrow from aviremic HIV patients were subjected to zinc finger nuclease-mediated stop codon insertion into chemokine receptor 5 (CCR5) loci. Locked nucleic acid-based polymerase chain reaction was used to estimate the amount of insertion in the expandable CD34(+) cells. The results showed that about 0.5% of CD34(+) cells carried stop codon insertions in CCR5 loci. Cells edited using this simple protocol have the potential to be infused back into the bone marrow.

Chavali, N. V., et al. (2019). "Patient-independent human induced pluripotent stem cell model: A new tool for rapid determination of genetic variant pathogenicity in long QT syndrome." Heart Rhythm.

BACKGROUND: Commercial genetic testing for long QT syndrome (LQTS) has rapidly expanded, but the inability to accurately predict whether a rare variant is pathogenic has limited its clinical benefit. Novel missense variants are routinely reported as variant of unknown significance (VUS) and cannot be used to screen family members at risk for sudden cardiac death. Better approaches to determine the pathogenicity of VUS are needed. OBJECTIVE: The purpose of this study was to rapidly determine the pathogenicity of a CACNA1C variant reported by commercial genetic testing as a VUS using a patient-independent human induced pluripotent stem cell (hiPSC) model. METHODS: Using CRISPR/Cas9 genome editing, CACNA1C-p.N639T was introduced into a previously established hiPSC from an unrelated healthy volunteer, thereby generating a patient-independent hiPSC model. Three independent heterozygous N639T hiPSC lines were generated and differentiated into cardiomyocytes (CM). Electrophysiological properties of N639T hiPSC-CM were compared to those of isogenic and population control hiPSC-CM by measuring the extracellular field potential (EFP) of 96-well hiPSC-CM monolayers and by patch clamp. RESULTS: Significant EFP prolongation was observed only in optically stimulated but not in spontaneously beating N639T hiPSC-CM. Patch-clamp studies revealed that N639T prolonged the ventricular action potential by slowing voltage-dependent inactivation of CaV1.2 currents. Heterologous expression studies confirmed the effect of N639T on CaV1.2 inactivation. CONCLUSION: The patient-independent hiPSC model enabled rapid generation of functional data to support reclassification of a CACNA1C VUS to likely pathogenic, thereby establishing a novel LQTS type 8 mutation. Furthermore, our results indicate the importance of controlling beating rates to evaluate the functional significance of LQTS VUS in high-throughput hiPSC-CM assays.

Chen, Y., et al. (2015). "Engineering Human Stem Cell Lines with Inducible Gene Knockout using CRISPR/Cas9." Cell Stem Cell **17**(2): 233-244.

Precise temporal control of gene expression or deletion is critical for elucidating gene function in biological systems. However, the establishment of human pluripotent stem cell (hPSC) lines with inducible gene knockout (iKO) remains challenging. We explored building iKO hPSC lines by combining CRISPR/Cas9-mediated genome editing with the Flp/FRT and Cre/LoxP system. We found that "dual-sgRNA targeting" is essential for biallelic knockin of FRT sequences to flank the exon. We further developed a strategy to simultaneously insert an activity-controllable recombinase-expressing cassette and remove the drug-resistance gene, thus speeding up the generation of iKO hPSC lines. This two-step strategy was used to establish human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) lines with iKO of SOX2, PAX6, OTX2, and AGO2, genes that exhibit diverse structural layout and

temporal expression patterns. The availability of iKO hPSC lines will substantially transform the way we examine gene function in human cells.

Chen, Y. H. and S. M. Pruett-Miller (2018). "Improving single-cell cloning workflow for gene editing in human pluripotent stem cells." Stem Cell Res **31**: 186-192.

The availability of human pluripotent stem cells (hPSCs) and progress in genome engineering technology have altered the way we approach scientific research and drug development screens. Unfortunately, the procedures for genome editing of hPSCs often subject cells to harsh conditions that compromise viability: a major problem that is compounded by the innate challenge of single-cell culture. Here we describe a generally applicable workflow that supports single-cell cloning and expansion of hPSCs after genome editing and single-cell sorting. Stem-Flex and RevitaCell supplement, in combination with Geltrex or Vitronectin (VN), promote reliable single-cell growth in a feeder-free and defined environment. Characterization of final genome-edited clones reveals that pluripotency and normal karyotype are retained following this single-cell culture protocol. This time-efficient and simplified culture method paves the way for high-throughput hPSC culture and will be valuable for both basic research and clinical applications.

Christidi, E., et al. (2018). "CRISPR/Cas9-mediated genome editing in human stem cell-derived cardiomyocytes: Applications for cardiovascular disease modelling and cardiotoxicity screening." Drug Discov Today Technol **28**: 13-21.

Cardiovascular diseases (CVDs) are leading causes of death worldwide, and drug-induced cardiotoxicity is among the most common cause of drug withdrawal from the market. Improved models of cardiac tissue are needed to study the mechanisms of CVDs and drug-induced cardiotoxicity. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) have provided a major advance to our ability to study these conditions. Combined with efficient genome editing technologies, such as CRISPR/Cas9, we now have the ability to study with greater resolution the genetic causes and underlying mechanisms of inherited and drug-induced cardiotoxicity, and to investigate new treatments. Here, we review recent advances in the use of hPSC-CMs and CRISPR/Cas9-mediated genome editing to study cardiotoxicity and model CVD.

Chuah, J. K. C. and D. Zink (2017). "Stem cell-derived kidney cells and organoids: Recent breakthroughs and emerging applications." Biotechnol Adv **35**(2): 150-167.

The global rise in the numbers of kidney patients and the shortage in transplantable organs have led to an increasing interest in kidney-specific regenerative therapies, renal disease modelling and bioartificial kidneys. Sources for large quantities of high-quality renal cells and tissues would be required, also for applications in in vitro platforms for compound safety and efficacy screening. Stem cell-based approaches for the generation of renal-like cells and tissues would be most attractive, but such methods were not available until recently. This situation has drastically changed since 2013, and various protocols for the generation of renal-like cells and precursors from pluripotent stem cells (PSC) have been established. The most recent breakthroughs were related to the establishment of various protocols for the generation of PSC-derived kidney organoids. In combination with recent advances in genome editing, bioprinting and the establishment of predictive renal screening platforms this results in exciting new possibilities. This review will give a comprehensive overview over current PSC-based protocols for the generation of renal-like cells, precursors and organoids, and their current and potential applications in regenerative medicine, compound screening, disease modelling and bioartificial organs.

Chun, Y. W., et al. (2018). "Genome Editing and Induced Pluripotent Stem Cell Technologies for Personalized Study of Cardiovascular Diseases." Curr Cardiol Rep **20**(6): 38.

PURPOSE OF REVIEW: The goal of this review is to highlight the potential of induced pluripotent stem cell (iPSC)-based modeling as a tool for studying human cardiovascular diseases. We present some of the current cardiovascular disease models utilizing genome editing and patient-derived iPSCs. RECENT FINDINGS: The incorporation of genome-editing and iPSC technologies provides an innovative research platform, providing novel insight into human cardiovascular disease at molecular, cellular, and functional level. In addition, genome editing in diseased iPSC lines holds potential for personalized regenerative therapies. The study of human cardiovascular disease has been revolutionized by cellular reprogramming and genome editing discoveries. These exceptional technologies provide an opportunity to generate human cell cardiovascular disease models and enable therapeutic strategy development in a dish. We anticipate these technologies to improve our understanding of cardiovascular disease pathophysiology leading to optimal treatment for heart diseases in the future.

Ding, Q., et al. (2013). "A TALEN genome-editing system for generating human stem cell-based disease models." Cell Stem Cell **12**(2): 238-251.

Transcription activator-like effector nucleases (TALENs) are a new class of engineered nucleases that are easier to design to cleave at desired sites in a genome than previous types of nucleases. We report here the use of TALENs to rapidly and efficiently generate mutant alleles of 15 genes in cultured somatic cells or human pluripotent stem cells, the latter for which we differentiated both the targeted lines and isogenic control lines into various metabolic cell types. We demonstrate cell-autonomous phenotypes directly linked to disease-dyslipidemia, insulin resistance, hypoglycemia, lipodystrophy, motor-neuron death, and hepatitis C infection. We found little evidence of TALEN off-target effects, but each clonal line nevertheless harbors a significant number of unique mutations. Given the speed and ease with which we were able to derive and characterize these cell lines, we anticipate TALEN-mediated genome editing of human cells becoming a mainstay for the investigation of human biology and disease.

Dolatshad, H., et al. (2019). "Application of induced pluripotent stem cell technology for the investigation of hematological disorders." Adv Biol Regul **71**: 19-33.

Induced pluripotent stem cells (iPSCs) were first described over a decade ago and are currently used in various basic biology and clinical research fields. Recent advances in the field of human iPSCs have opened the way to a better understanding of the biology of human diseases. Disease-specific iPSCs provide an unparalleled opportunity to establish novel human cell-based disease models, with the potential to enhance our understanding of the molecular mechanisms underlying human malignancies, and to accelerate the identification of effective new drugs. When combined with genome editing technologies, iPSCs represent a new approach to study single or multiple disease-causing mutations and model specific diseases in vitro. In addition, genetically corrected patient-specific iPSCs could potentially be used for stem cell based therapy. Furthermore, the reprogrammed cells share patient-specific genetic background, offering a new platform to develop personalized therapy/medicine for patients. In this review we discuss the recent advances in iPSC research technology and their potential applications in hematological diseases. Somatic cell reprogramming has presented new routes for generating patient-derived iPSCs, which can be differentiated to hematopoietic stem cells and the various downstream hematopoietic lineages. iPSC technology shows promise in the modeling of both inherited and acquired hematological disorders. A direct reprogramming and differentiation strategy is able to recapitulate hematological disorder progression and capture the earliest molecular alterations that underlie the initiation of hematological malignancies.

Droz-Georget Lathion, S., et al. (2015). "A single epidermal stem cell strategy for safe ex vivo gene therapy." EMBO Mol Med **7**(4): 380-393.

There is a widespread agreement from patient and professional organisations alike that the safety of stem cell therapeutics is of paramount importance, particularly for ex vivo autologous gene therapy. Yet current technology makes it difficult to thoroughly evaluate the behaviour of genetically corrected stem cells before they are transplanted. To address this, we have developed a strategy that permits transplantation of a clonal population of genetically corrected autologous stem cells that meet stringent selection criteria and the principle of precaution. As a proof of concept, we have stably transduced epidermal stem cells (holoclones) obtained from a patient suffering from recessive dystrophic epidermolysis bullosa. Holoclones were infected with self-inactivating retroviruses bearing a COL7A1 cDNA and cloned before the progeny of individual stem cells were characterised using a number of criteria. Clonal analysis revealed a great deal of heterogeneity among transduced stem cells in their capacity to produce functional type VII collagen (COLVII). Selected transduced stem cells transplanted onto immunodeficient mice regenerated a non-blistering epidermis for months and produced a functional COLVII. Safety was assessed by determining the sites of proviral integration, rearrangements and hit genes and by whole-genome sequencing. The progeny of the selected stem cells also had a diploid karyotype, was not tumorigenic and did not disseminate after long-term transplantation onto immunodeficient mice. In conclusion, a clonal strategy is a powerful and efficient means of by-passing the heterogeneity of a transduced stem cell population. It guarantees a safe and homogenous medicinal product, fulfilling the principle of precaution and the requirements of regulatory affairs. Furthermore, a clonal strategy makes it possible to envision exciting gene-editing technologies like zinc finger nucleases, TALENs and homologous recombination for next-generation gene therapy.

Elert-Dobkowska, E., et al. (2015). "Multiplex ligation-dependent probe amplification for identification of correctly targeted murine embryonic stem cell clones." Anal Biochem **474**: 35-37.

Following locus-specific genome editing of mouse embryonic stem cells (ESCs), the identification

of correctly targeted clones remains a challenge. We applied multiplex ligation-dependent probe amplification (MLPA) to screen for homologous recombination-based genomic integration of a knockout construct in which part of a gene is deleted. All candidate ESCs thereby identified were subsequently validated by conventional methods. Thus, MLPA represents a highly reliable as well as cost- and time-efficient alternative to currently applied methods such as Southern blotting and polymerase chain reaction (PCR)-based approaches. It is also applicable to knockin recombination strategies and compatible with the CRISPR/Cas9 system and other genome editing strategies.

Eshraghi, A. A., et al. (2019). "Recent Advancements in Gene and Stem Cell-Based Treatment Modalities: Potential Implications in Noise-Induced Hearing Loss." Anat Rec (Hoboken).

Noise-induced hearing loss (NIHL) poses a significant burden on not only the economics of health care but also the quality of life of an individual, as we approach an unprecedented age of longevity. In this article, we will delineate the current landscape of management of NIHL. We discuss the most recent results from in vitro and in vivo studies that determine the effectiveness of established pharmacotherapy such as corticosteroid and potential emerging therapies like N-acetyl cysteine and neurotrophins (NTs), as well as highlight ongoing clinical trials for these therapeutic agents. We present an overview of how the recent advancements in the field of gene-based and stem cell-based therapies can help in developing effective therapeutic strategies for NIHL. Gene-based therapies have shown exciting results demonstrating cochlear cellular regeneration using Atoh1, NRF2 as well as NT gene therapy employing viral vectors. In addition, we will discuss the recent advancements in genome-editing technologies, such as CRISPR/Cas9, and its potential role in NIHL therapy. We will further discuss the current state of stem cell therapy as it pertains to treating neurodegenerative conditions including NIHL. Embryonic stem cells, adult-derived stem cells, and induced pluripotent stem cells all represent an enticing reservoir of replacing damaged cells as a result of NIHL. Finally, we will discuss the barriers that need to be overcome to translate these promising treatment modalities to the clinical practice in pursuit of improving quality of life of patients having NIHL. Anat Rec, 2019. (c) 2019 Wiley Periodicals, Inc.

Fletcher, J. C. (2018). "The CLV-WUS Stem Cell Signaling Pathway: A Roadmap to Crop Yield Optimization." Plants (Basel) **7**(4).

The shoot apical meristem at the growing shoot tip acts a stem cell reservoir that provides cells to generate the entire above-ground architecture of higher plants. Many agronomic plant yield traits such as tiller number, flower number, fruit number, and kernel row number are therefore defined by the activity of the shoot apical meristem and its derivatives, the floral meristems. Studies in the model plant Arabidopsis thaliana demonstrated that a molecular negative feedback loop called the CLAVATA (CLV)-WUSCHEL (WUS) pathway regulates stem cell maintenance in shoot and floral meristems. CLV-WUS pathway components are associated with quantitative trait loci (QTL) for yield traits in crop plants such as oilseed, tomato, rice, and maize, and may have played a role in crop domestication. The conservation of these pathway components across the plant kingdom provides an opportunity to use cutting edge techniques such as genome editing to enhance yield traits in a wide variety of agricultural plant species.

Gao, Y., et al. (2018). "Generation of RAB39B knockout isogenic human embryonic stem cell lines to model RAB39B-mediated Parkinson's disease." Stem Cell Res **28**: 161-164.

Mutations in RAB39B are a known cause of X-linked early onset Parkinson's disease. Isogenic human embryonic stem cell lines carrying two independent deletions of RAB39B were generated using CRISPR/Cas9 genome editing tool. The deletions were confirmed by PCR and direct sequence analysis in two edited stem cell lines. Both cell lines showed pluripotency and displayed a normal karyotype. Further, they were able to form embryoid bodies in vitro, and express markers indicative of differentiation to the three germ layers.

Gerace, D., et al. (2017). "CRISPR-targeted genome editing of mesenchymal stem cell-derived therapies for type 1 diabetes: a path to clinical success?" Stem Cell Res Ther **8**(1): 62.

Due to their ease of isolation, differentiation capabilities, and immunomodulatory properties, the therapeutic potential of mesenchymal stem cells (MSCs) has been assessed in numerous pre-clinical and clinical settings. Currently, whole pancreas or islet transplantation is the only cure for people with type 1 diabetes (T1D) and, due to the autoimmune nature of the disease, MSCs have been utilised either natively or transdifferentiated into insulin-producing cells (IPCs) as an alternative treatment. However, the initial success in pre-clinical animal models has not translated into successful clinical outcomes. Thus, this review will summarise the current state of MSC-derived therapies for the treatment of T1D in both the pre-clinical and clinical setting, in particular their use as an immunomodulatory therapy and targets for the generation of IPCs via gene modification. In this

review, we highlight the limitations of current clinical trials of MSCs for the treatment of T1D, and suggest the novel clustered regularly interspaced short palindromic repeat (CRISPR) gene-editing technology and improved clinical trial design as strategies to translate pre-clinical success to the clinical setting.

Giani, F. C., et al. (2016). "Targeted Application of Human Genetic Variation Can Improve Red Blood Cell Production from Stem Cells." Cell Stem Cell **18**(1): 73-78.

Multipotent and pluripotent stem cells are potential sources for cell and tissue replacement therapies. For example, stem cell-derived red blood cells (RBCs) are a potential alternative to donated blood, but yield and quality remain a challenge. Here, we show that application of insight from human population genetic studies can enhance RBC production from stem cells. The SH2B3 gene encodes a negative regulator of cytokine signaling and naturally occurring loss-of-function variants in this gene increase RBC counts in vivo. Targeted suppression of SH2B3 in primary human hematopoietic stem and progenitor cells enhanced the maturation and overall yield of in-vitro-derived RBCs. Moreover, inactivation of SH2B3 by CRISPR/Cas9 genome editing in human pluripotent stem cells allowed enhanced erythroid cell expansion with preserved differentiation. Our findings therefore highlight the potential for combining human genome variation studies with genome editing approaches to improve cell and tissue production for regenerative medicine.

Giri, S., et al. (2019). "Generation of a FMR1 homozygous knockout human embryonic stem cell line (WAe009-A-16) by CRISPR/Cas9 editing." Stem Cell Res **39**: 101494.

Mutations in FMR1 gene is the cause of Fragile X Syndrome (FXS) leading inherited cause of intellectual disability and autism spectrum disorders. FMR1 gene encodes Fragile X Mental Retardation Protein (FMRP) which is a RNA binding protein and play important role in synaptic plasticity and translational regulation in neurons. We have generated a homozygous FMR1 knockout (FMR1-KO) hESC line using CRISPR/Cas9 based genome editing. It created a homozygous 280 nucleotide deletion at exon1, removing the start codon. This FMR1-KO cell line maintains stem cell like morphology, pluripotency, normal karyotype and ability to in-vitro differentiation.

Grabole, N., et al. (2016). "Genomic analysis of the molecular neuropathology of tuberous sclerosis using a human stem cell model." Genome Med **8**(1): 94.

BACKGROUND: Tuberous sclerosis complex (TSC) is a genetic disease characterized by benign tumor growths in multiple organs and neurological symptoms induced by mTOR hyperfunction. Because the molecular pathology is highly complex and the etiology poorly understood, we employed a defined human neuronal model with a single mTOR activating mutation to dissect the disease-relevant molecular responses driving the neuropathology and suggest new targets for treatment. METHODS: We investigate the disease phenotype of TSC by neural differentiation of a human stem cell model that had been deleted for TSC2 by genome editing. Comprehensive genomic analysis was performed by RNA sequencing and ribosome profiling to obtain a detailed genome-wide description of alterations on both the transcriptional and translational level. The molecular effect of mTOR inhibitors used in the clinic was monitored and comparison to published data from patient biopsies and mouse models highlights key pathogenic processes. RESULTS: TSC2-deficient neural stem cells showed severely reduced neuronal maturation and characteristics of astrogliosis instead. Transcriptome analysis indicated an active inflammatory response and increased metabolic activity, whereas at the level of translation ribosomal transcripts showed a 5'UTR motif-mediated increase in ribosome occupancy. Further, we observed enhanced protein synthesis rates of angiogenic growth factors. Treatment with mTOR inhibitors corrected translational alterations but transcriptional dysfunction persisted. CONCLUSIONS: Our results extend the understanding of the molecular pathophysiology of TSC brain lesions, and suggest phenotype-tailored pharmacological treatment strategies.

Guilak, F., et al. (2019). "Designer Stem Cells: Genome Engineering and the Next Generation of Cell-Based Therapies." J Orthop Res **37**(6): 1287-1293.

Stem cells provide tremendous promise for the development of new therapeutic approaches for musculoskeletal conditions. In addition to their multipotency, certain types of stem cells exhibit immunomodulatory effects that can mitigate inflammation and enhance tissue repair. However, the translation of stem cell therapies to clinical practice has proven difficult due to challenges in intradonor and interdonor variability, engraftment, variability in recipient microenvironment and patient indications, and limited therapeutic biological activity. In this regard, the success of stem cell-based therapies may benefit from cellular engineering approaches to enhance factors such as purification, homing and cell survival, trophic effects, or immunomodulatory signaling. By combining recent advances in gene editing, synthetic biology, and tissue engineering, the

potential exists to create new classes of "designer" cells that have prescribed cell-surface molecules and receptors as well as synthetic gene circuits that provide for autoregulated drug delivery or enhanced tissue repair. Published by Wiley Periodicals, Inc. J Orthop Res 37:1287-1293, 2019.

Guo, F., et al. (2019). "Patient-Specific and Gene-Corrected Induced Pluripotent Stem Cell-Derived Cardiomyocytes Elucidate Single-Cell Phenotype of Short QT Syndrome." Circ Res **124**(1): 66-78.

RATIONALE: Short QT syndrome (SQT) is a rare but arrhythmogenic disorder featured by shortened ventricular repolarization and a propensity toward life-threatening ventricular arrhythmias and sudden cardiac death. OBJECTIVE: This study aimed to investigate the single-cell mechanism of SQT using patient-specific and gene-corrected induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). METHODS AND RESULTS: One SQT patient carrying missense mutation T618I in potassium voltage-gated channel subfamily H member 2 (KCNH2) was recruited as well as 2 healthy control subjects in this study. Control and SQT patient-specific iPSCs were generated from skin fibroblasts using nonintegrated Sendai virus. The KCNH2 T618I mutation was corrected by genome editing in SQT iPSC lines to generate isogenic controls. All iPSCs were differentiated into iPSC-CMs using monolayer-based differentiation protocol. SQT iPSC-CMs exhibited abnormal action potential phenotype featured by shortened action potential duration and increased beat-beat interval variability, when compared with control and gene-corrected iPSC-CMs. Furthermore, SQT iPSC-CMs showed KCNH2 gain-of-function with increased rapid delayed rectifying potassium current (IKr) density and enhanced membrane expression. Gene expression profiling of iPSC-CMs exhibited a differential cardiac ion-channel gene expression profile of SQT. Moreover, QTc of SQT patient and action potential durations of SQT iPSC-CMs were both normalized by quinidine, indicating that quinidine is beneficial to KCNH2 T618I of SQT. Importantly, shortened action potential duration phenotype observed in SQT iPSC-CMs was effectively rescued by a short-peptide scorpion toxin BmKKx2 with a mechanism of targeting KCNH2. CONCLUSIONS: We demonstrate that patient-specific and gene-corrected iPSC-CMs are able to recapitulate single-cell phenotype of SQT, which is caused by the gain-of-function mutation KCNH2 T618I. These findings will help elucidate the mechanisms underlying SQT and discover therapeutic drugs for treating the disease by using peptide toxins as lead compounds.

Gupta, N., et al. (2017). "Regenerative Medicine, Disease Modeling, and Drug Discovery in Human Pluripotent Stem Cell-derived Kidney Tissue." Eur Med J Reprod Health **3**(1): 57-67.

The multitude of research clarifying critical factors in embryonic organ development has been instrumental in human stem cell research. Mammalian organogenesis serves as the archetype for directed differentiation protocols, subdividing the process into a series of distinct intermediate stages that can be chemically induced and monitored for the expression of stage-specific markers. Significant advances over the past few years include established directed differentiation protocols of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) into human kidney organoids in vitro. Human kidney tissue in vitro simulate the in vivo response when subject to nephrotoxins, providing a novel screening platform during drug discovery to facilitate identification of lead candidates, reduce developmental expenditures, and reduce future rates of drug-induced acute kidney injury. Patient-derived hiPSCs, which bear naturally occurring DNA mutations, may allow for modeling of human genetic diseases to determine pathologic mechanisms and screen for novel therapeutics. In addition, recent advances in genome editing with CRISPR/Cas9 enable to generate specific mutations to study genetic disease with non-mutated lines serving as an ideal isogenic control. The growing population of patients with end-stage kidney disease (ESKD) is a world-wide healthcare problem with higher morbidity and mortality that warrants the discovery of novel forms of renal replacement therapy. Coupling the outlined advances in hiPSC research with innovative bioengineering techniques, such as decellularized kidney and 3D printed scaffolds, may contribute to the development of bioengineered transplantable human kidney tissue as a means of renal replacement therapy.

Gupta, R. M., et al. (2016). "Genome-Edited Human Pluripotent Stem Cell-Derived Macrophages as a Model of Reverse Cholesterol Transport--Brief Report." Arterioscler Thromb Vasc Biol **36**(1): 15-18.

OBJECTIVE: To create isogenic human pluripotent stem cell-derived macrophages with and without ABCA1 expression as a model for reverse cholesterol transport. APPROACH AND RESULTS: The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) genome-editing system was used to introduce frameshift mutations into the coding sequence of ATP-binding cassette, subfamily A, member 1. Individual human pluripotent stem cell clones with deleterious mutations were identified, expanded, and

differentiated into mature macrophages with a cytokine-based, feeder-free differentiation protocol. Wild-type cells demonstrated effective cholesterol efflux to apoAI acceptor, whereas ABCA1(-/-) cells displayed significantly reduced efflux ability and increased expression of proinflammatory cytokines. CONCLUSIONS: Human pluripotent stem cell-derived macrophages capable of reverse cholesterol transport can be rapidly generated and genetically edited with CRISPR/Cas9. Introduction of homozygous frameshift mutations results in loss of ABCA1 expression in differentiated macrophages and subsequent reduction of cholesterol efflux capability. This facile genome-editing approach and differentiation protocol pave the way for future studies of the molecular determinants of reverse cholesterol transport and other macrophage properties.

Ha, S., et al. (2017). "Phosphorylation of p62 by AMP-activated protein kinase mediates autophagic cell death in adult hippocampal neural stem cells." J Biol Chem **292**(33): 13795-13808.

In the adult brain, programmed death of neural stem cells is considered to be critical for tissue homeostasis and cognitive function and is dysregulated in neurodegeneration. Previously, we have reported that adult rat hippocampal neural (HCN) stem cells undergo autophagic cell death (ACD) following insulin withdrawal. Because the apoptotic capability of the HCN cells was intact, our findings suggested activation of unique molecular mechanisms linking insulin withdrawal to ACD rather than apoptosis. Here, we report that phosphorylation of autophagy-associated protein p62 by AMP-activated protein kinase (AMPK) drives ACD and mitophagy in HCN cells. Pharmacological inhibition of AMPK or genetic ablation of the AMPK alpha2 subunit by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing suppressed ACD, whereas AMPK activation promoted ACD in insulin-deprived HCN cells. We found that following insulin withdrawal AMPK phosphorylated p62 at a novel site, Ser-293/Ser-294 (in rat and human p62, respectively). Phosphorylated p62 translocated to mitochondria and induced mitophagy and ACD. Interestingly, p62 phosphorylation at Ser-293 was not required for staurosporine-induced apoptosis in HCN cells. To the best of our knowledge, this is the first report on the direct phosphorylation of p62 by AMPK. Our data suggest that AMPK-mediated p62 phosphorylation is an ACD-specific signaling event and provide novel mechanistic insight into the molecular mechanisms in ACD.

Haggarty, S. J., et al. (2016). "Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models." Mol Cell Neurosci **73**: 104-115.

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.

Hallmann, A. L., et al. (2017). "Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein." Sci Rep **7**: 42991.

Astroglial pathology is seen in various neurodegenerative diseases including frontotemporal dementia (FTD), which can be caused by mutations in the gene encoding the microtubule-associated protein TAU (MAPT). Here, we applied a stem cell model of FTD to examine if FTD astrocytes carry an intrinsic propensity to degeneration and to determine if they can induce non-cell-autonomous effects in neighboring neurons. We utilized CRISPR/Cas9 genome editing in human induced pluripotent stem (iPS) cell-derived neural progenitor cells (NPCs) to repair the FTD-associated N279K MAPT mutation. While astrocytic differentiation was not impaired in FTD NPCs derived from one patient carrying the N279K MAPT mutation, FTD astrocytes appeared larger, expressed increased levels of 4R-TAU isoforms, demonstrated increased vulnerability to oxidative stress and elevated protein ubiquitination and exhibited disease-associated changes in transcriptome profiles when compared to astrocytes derived from one control individual and to the isogenic control. Interestingly, co-culture experiments with FTD astrocytes revealed increased oxidative stress and robust changes in whole genome expression in previously healthy neurons. Our study highlights the utility of iPS cell-derived NPCs to elucidate the role of astrocytes in the pathogenesis of FTD.

Hamazaki, T., et al. (2017). "Concise Review: Induced Pluripotent Stem Cell Research in the Era of Precision Medicine." Stem Cells **35**(3): 545-550.

Recent advances in DNA sequencing technologies are revealing how human genetic variations associate with differential health risks, disease susceptibilities, and drug responses. Such information is now expected to help evaluate individual health risks, design personalized health plans and treat patients with precision. It is still challenging, however, to understand how such genetic variations cause the phenotypic alterations in pathobiologies and treatment response. Human induced pluripotent stem cell (iPSC) technologies are emerging as a promising strategy to fill the knowledge gaps between genetic association studies and underlying molecular mechanisms. Breakthroughs in genome editing technologies and continuous improvement in iPSC differentiation techniques are particularly making this research direction more realistic and practical. Pioneering studies have shown that iPSCs derived from a variety of monogenic diseases can faithfully recapitulate disease phenotypes in vitro when differentiated into disease-relevant cell types. It has been shown possible to partially recapitulate disease phenotypes, even with late onset and polygenic diseases. More recently, iPSCs have been shown to validate effects of disease and treatment-related single nucleotide polymorphisms identified through genome wide association analysis. In this review, we will discuss how iPSC research will further contribute to human health in the coming era of precision medicine. Stem Cells 2017;35:545-550.

Hanenberg, H., et al. (2017). "Stem Cell Genetic Therapy for Fanconi Anemia - A New Hope." Curr Gene Ther **16**(5): 309-320.

Fanconi anemia (FA) is a rare inherited DNA disorder clinically characterized by congenital malformations, progressive bone marrow failure, and cancer susceptibility. Due to a strong survival advantage of spontaneously corrected 'normal' hematopoietic stem cells (HSCs) in a few patients, FA is considered a model disorder for genetic correction of autologous stem cells, where genetically corrected stem cells and their progeny have a strong in vivo selective advantage, ultimately leading to normal hematopoiesis. Despite these apparently ideal circumstances, three HSC gene therapy trials with gammaretroviral vectors (stage I) designed to cure the hematological manifestation of FA completely failed to provide long-term clinical benefits for patients, predominantly due to the combination of insufficient gene transfer technologies and incompletely understood FA HSC pathobiology. Currently, FA gene therapy is in stage II where, based on an improved understanding of the cellular defects in FA HSCs, consequently adapted transduction protocols are being used in two phase I/II trials for in vitro genetic correction of FANCA-deficient hematopoietic stem cells. These results are eagerly awaited. Independent from the outcome of these studies, technologies are already available that seem highly attractive for testing in FA. In stage III, this would ultimately include targeted in vivo correction of autologous HSCs by overexpression of nonintegrating lentiviral vectors with scaffold/matrix attachment region elements using specific envelopes as pseudotypes. Although currently still challenging, in a few years in vivo genome editing approaches will be readily available in stage IV, in which the delivery of the editing machinery/ complex is targeted to the autologous FA HSCs by the nonintegrating lentiviral vectors established in stage III. Even low levels of corrected stem cells will then quickly repopulate the entire hematopoiesis of the patient. We therefore are sanguine that in the future, genetic therapy can be used clinically for the correction of FA HSCs in the standard care of FA patients.

Hewitt, K. J., et al. (2015). "Hematopoietic Signaling Mechanism Revealed from a Stem/Progenitor Cell Cistrome." Mol Cell **59**(1): 62-74.

Thousands of cis-elements in genomes are predicted to have vital functions. Although conservation, activity in surrogate assays, polymorphisms, and disease mutations provide functional clues, deletion from endogenous loci constitutes the gold-standard test. A GATA-2-binding, Gata2 intronic cis-element (+9.5) required for hematopoietic stem cell genesis in mice is mutated in a human immunodeficiency syndrome. Because +9.5 is the only cis-element known to mediate stem cell genesis, we devised a strategy to identify functionally comparable enhancers ("+9.5-like") genome-wide. Gene editing revealed +9.5-like activity to mediate GATA-2 occupancy, chromatin opening, and transcriptional activation. A +9.5-like element resided in Samd14, which encodes a protein of unknown function. Samd14 increased hematopoietic progenitor levels/activity and promoted signaling by a pathway vital for hematopoietic stem/progenitor cell regulation (stem cell factor/c-Kit), and c-Kit rescued Samd14 loss-of-function phenotypes. Thus, the hematopoietic stem/progenitor cell cistrome revealed a mediator of a signaling pathway that has broad importance for stem/progenitor cell biology.

Hirabayashi, M., et al. (2019). "Pluripotent stem cell-derived organogenesis in the rat model system." Transgenic Res.

Rats make an excellent model system for studying xenotransplantation since, like mice pluripotent stem cell lines, such as embryonic stem cells and induced pluripotent stem cells as well as gene knock-outs are also available for rats, besides rats have larger organs. The emergence of new genome-editing tools combined with stem cell technology, has revolutionized biomedical research including the field of regenerative medicine. The aim of this manuscript is to provide an overview of the recent progresses in stem cell-derived organ regeneration involving "gene knock-out" and "blastocyst complementation" in the rat model system. Knocking-out Pdx1, Foxn1, and Sall1 genes have successfully generated rat models lacking the pancreas, thymus, and kidney, respectively. When allogeneic (rat) or xenogeneic (mouse) pluripotent stem cells were microinjected into blastocyst-stage rat embryos that had been designed to carry a suitable organogenetic niche, devoid of specific organs, the complemented blastocysts were able to develop to full-term chimeric rat offspring containing stem cell-derived functional organs in their respective niches. Thus, organs with a tridimensional structure can be generated with pluripotent stem cells in vivo, accelerating regenerative medical research, which is crucial for organ-based transplantation therapies. However, to address ethical concerns, public consent after informed discussions is essential before production of human organs within domestic animals.

Hu, X., et al. (2017). "Nanodiamonds Mediate Oral Delivery of Proteins for Stem Cell Activation and Intestinal Remodeling in Drosophila." ACS Appl Mater Interfaces **9**(22): 18575-18583.

Introduction of exogenous biomacromolecules into living systems is of great interest in genome editing, cancer immunotherapy, and stem cell reprogramming. Whereas current strategies generally depend on nucleic acids transfection, direct delivery of functional proteins that provides enhanced specificity, increased safety, and fast and temporal regulation is highly desirable. Nevertheless, intracellular delivery of intact and bioactive proteins, especially in vivo, remains poorly explored. In this study, we developed a nanodiamonds (NDs)-based protein delivery system in cultured cells and in Drosophila that showed high adsorption, high efficiency, and effective cytosolic release of fully functional proteins. Through live-cell imaging, we observed a novel phenomenon wherein a substantial amount of internalized NDs-protein complex rejected fusion with the early endosome, thereby evading protein degradation in the lysosome. More significantly, we demonstrated that dietary NDs-RNase induced apoptosis in enterocytes, stimulating regenerative divisions in intestinal stem cells and increasing the number of stem cells and precursor cells in Drosophila intestine. As stem cells are poorly accessible by exogenous agents in vivo, NDs-mediated oral delivery of proteins provides a new approach to modulate the stem cell microenvironment for intestinal remodeling, which has important implications for colorectal cancer therapy and regenerative medicine.

Huo, Z., et al. (2019). "Generation of a heterozygous p53 R249S mutant human embryonic stem cell line by TALEN-mediated genome editing." Stem Cell Res **34**: 101360.

As one of the most essential genome guardians, p53 and its mutants have been suggested associated with many types of cancers. Many p53 mutants function induce unique phenotypes, including carcinogenesis, metastasis, and drug resistance. The p53(R249S) mutation is the most prevalent and specific mutation associated with liver cancer development. Here, we demonstrate the generation of a heterozygous p53(R249S) mutation in the H9 human embryonic stem cell line using TALEN-mediated genome editing. The generated cell line maintains a normal karyotype, a pluripotent state and the in vivo capacity to develop a teratoma containing all three germ layer tissues.

Hutter, G. (2016). "Stem cell transplantation in strategies for curing HIV/AIDS." AIDS Res Ther **13**(1): 31.

HIV-1 can persist in a latent form in resting memory CD4+ cells and macrophages carrying an integrated copy of the HIV genome. Because of the presence of these stable reservoir cells, eradication by antiretroviral therapy is unlikely and in order to achieve eradication, alternative treatment options are required. Stem cell transplantation has been considered previously to effect the clinical course of HIV-infection but in practice eradication or virus control was not achievable. However, modifications of stem cell transplantation using natural or artificial resistant cell sources, combination with new techniques of gene editing or generating cytotoxic anti HIV effector cells have stimulated this field of HIV cell therapy substantially. Here, we look back on 30 years of stem cell therapy in HIV patients and discuss most recent developments in this direction.

Jayavaradhan, R., et al. (2019). "A Versatile Tool for the Quantification of CRISPR/Cas9-Induced Genome Editing Events in Human Hematopoietic Cell Lines and Hematopoietic Stem/Progenitor Cells." J Mol Biol **431**(1): 102-110.

The efficient site-specific DNA double-strand breaks (DSB) created by CRISPR/Cas9 has revolutionized genome engineering and has great potential for editing hematopoietic stem/progenitor cells (HSPCs). However, detailed understanding of the variables that influence choice of DNA-DSB repair (DDR) pathways by HSPC is required for therapeutic levels of editing in these clinically relevant cells. We developed a hematopoietic-reporter system that rapidly quantifies the three major DDR pathways utilized at the individual DSB created by CRISPR/Cas9-NHEJ, MMEJ, and HDR-and show its applicability in evaluating the different DDR outcomes utilized by human hematopoietic cell lines and primary human HSPC.

Jiang, W., et al. (2019). "Rapid identification of genome-edited mesenchymal stem cell colonies via Cas9." Biotechniques **66**(5): 231-234.

Mesenchymal stem cells (MSCs) have been intensively investigated and widely applied in regenerative medicine and immune modulation. However, their efficacy declines during the aging or disease process. Thus, genome-edited MSCs with over-expression or inhibition of specific genes hold a great deal of promise in terms of their therapeutic application. Here we optimized the direct PCR approach for rapid identification of genome-edited MSCs with only ten cells required, which reduces the time and labor to expand the MSC colonies. Combined with our previously optimized guide RNA structure and plasmid construction strategy for Cas9, we successfully identified MSC colonies over-expressing IL-10 in the AAVS1 locus.

Johnson, J. Z. and D. Hockemeyer (2015). "Human stem cell-based disease modeling: prospects and challenges." Curr Opin Cell Biol **37**: 84-90.

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

Kalra, S., et al. (2016). "Can Human Pluripotent Stem Cell-Derived Cardiomyocytes Advance Understanding of Muscular Dystrophies?" J Neuromuscul Dis **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.

Kamiya, A. and H. Chikada (2015). "Human pluripotent stem cell-derived cholangiocytes: current status and future applications." Curr Opin Gastroenterol **31**(3): 233-238.

PURPOSE OF REVIEW: Pluripotent stem cells, such as embryonic stem cells and inducible pluripotent stem (iPS) cells, have high proliferative multipotency for differentiation into mature functional cells that are useful for treatment and basic research on several diseases. Cholangiocytes are differentiated from fetal hepatic progenitor cells (hepatoblasts) and are important for transport of bile acids that are synthesized by mature hepatocytes in the liver. However, the molecular mechanisms of development and function of human cholangiocytes remain unknown. This review mentions the potential of human cholangiocytic culture from pluripotent stem cells to contribute to the analyses of the human bile duct system and diseases. RECENT FINDINGS: Recent studies found that human hepatic cholangiocytic cells can be differentiated from human embryonic stem and iPS cells in a suitable culture condition. Cholangiocytic cysts have epithelial cell polarity formed in a three-dimensional cell culture system using extracellular matrices. SUMMARY: Disease pathogenesis was elucidated in vitro using differentiated cells from disease-related iPS cells. Using genome-editing enzymes, iPS cells with disease-specific gene mutations can be easily and rapidly established. These disease-related iPS cells and cholangiocytic culture system may be useful for analyses and drug screening of human bile duct diseases.

Karakikes, I., et al. (2017). "A Comprehensive TALEN-Based Knockout Library for Generating Human-Induced Pluripotent Stem Cell-Based Models for Cardiovascular Diseases." Circ Res **120**(10): 1561-1571.

RATIONALE: Targeted genetic engineering using programmable nucleases such as transcription activator-like effector nucleases (TALENs) is a valuable tool for precise, site-specific genetic modification in the human genome. OBJECTIVE: The emergence of novel technologies such as human induced pluripotent stem cells (iPSCs) and nuclease-mediated genome editing represent a unique opportunity for studying cardiovascular diseases in vitro. METHODS AND RESULTS: By incorporating extensive literature and database searches, we designed a collection of TALEN constructs to knockout 88 human genes that are associated with cardiomyopathies and congenital heart diseases. The TALEN pairs were designed to induce double-strand DNA break near the starting codon of each gene that either disrupted the start codon or introduced a frameshift mutation in the early coding region, ensuring faithful gene knockout. We observed that all the constructs were active and disrupted the target locus at high frequencies. To illustrate the utility of the TALEN-mediated knockout technique, 6 individual genes (TNNT2, LMNA/C, TBX5, MYH7, ANKRD1, and NKX2.5) were knocked out with high efficiency and specificity in human iPSCs. By selectively targeting a pathogenic mutation (TNNT2 p.R173W) in patient-specific iPSC-derived cardiac myocytes, we demonstrated that the knockout strategy ameliorates the dilated cardiomyopathy phenotype in vitro. In addition, we modeled the Holt-Oram syndrome in iPSC-cardiac myocytes in vitro and uncovered novel pathways regulated by TBX5 in human cardiac myocyte development. CONCLUSIONS: Collectively, our study illustrates the powerful combination of iPSCs and genome editing technologies for understanding the biological function of genes, and the pathological significance of genetic variants in human cardiovascular diseases. The methods, strategies, constructs, and iPSC lines developed in this study provide a validated, readily available resource for cardiovascular research.

Khateb, M., et al. (2019). "The Third Intron of IRF8 Is a Cell-Type-Specific Chromatin Priming Element during Mouse Embryonal Stem Cell Differentiation." J Mol Biol **431**(2): 210-222.

Interferon regulatory factor 8 (IRF8) is a nuclear transcription factor that plays a key role in the hierarchical differentiation of hematopoietic stem cells toward monocyte/dendritic cell lineages. Therefore, its expression is mainly limited to bone marrow-derived cells. The molecular mechanisms governing this cell-type-restricted expression have been described. However, the molecular mechanisms that are responsible for its silencing in non-hematopoietic cells are elusive. Recently, we demonstrated a role for IRF8 third intron in restricting its expression in non-hematopoietic cells. Furthermore, we showed that this intron alone is sufficient to promote repressed chromatin a cell-type-specific manner. Here we demonstrate the effect of the IRF8 third intron on chromatin conformation during murine embryonal stem cell differentiation. Using genome editing, we provide data showing that the third intron plays a key

role in priming the chromatin state of the IRF8 locus during cell differentiation. It mediates dual regulatory effects in a cell-type-specific mode. It acts as a repressor element governing chromatin state of the IRF8 locus during embryonal stem cell differentiation to cardiomyocytes that are expression-restrictive cells. Conversely, it functions as an activator element that is essential for open chromatin structure during the differentiation of these cells to dendritic cells that are expression-permissive cells. Together, these results point to the role of IRF8 third intron as a cell-type-specific chromatin priming element during embryonal stem cell differentiation. These data add another layer to our understanding of the molecular mechanisms governing misexpression of a cell-type-specific gene such as IRF8.

Khurana, V., et al. (2015). "Toward stem cell-based phenotypic screens for neurodegenerative diseases." Nat Rev Neurol **11**(6): 339-350.

In the absence of a single preventive or disease-modifying strategy, neurodegenerative diseases are becoming increasingly prevalent in our ageing population. The mechanisms underlying neurodegeneration are poorly understood, making the target-based drug screening strategies that are employed by the pharmaceutical industry fraught with difficulty. However, phenotypic screening in neurons and glia derived from patients is now conceivable through unprecedented developments in reprogramming, transdifferentiation, and genome editing. We outline progress in this nascent field, but also consider the formidable hurdles to identifying robust, disease-relevant and screenable cellular phenotypes in patient-derived cells. We illustrate how analysis in the simple baker's yeast cell Saccharaomyces cerevisiae is driving discovery in patient-derived neurons, and how approaches in this model organism can establish a paradigm to guide the development of stem cell-based phenotypic screens.

Kim, C. Y., et al. (2018). "Improved Transfection Efficiency and Metabolic Activity in Human Embryonic Stem Cell Using Non-Enzymatic Method." Int J Stem Cells **11**(2): 149-156.

Human embryonic stem cells (hESCs) are pluripotent cells widely used in conventional and regenerative medicine due to their ability to self-renew, proliferate and differentiate. Recently, genetic modification of stem cells using genome editing is the most advanced technique for treating hereditary diseases. Nevertheless, the low transfection efficiency of hESCs using enzymatic methods is still limited in in vitro preclinical research. To overcome these limitations, we have developed transfection methods using non-enzymatic treatments on hESCs. In this study, hESCs were transfected following enzymatic (TrypLE and trypsin) and non-enzymatic treatment ethylenediaminetetraacetic acid (EDTA) to increase transfection efficiency. Flow cytometric analysis using an enhanced green fluorescent protein vector showed a significantly increased transfection efficiency of EDTA method compared to standard enzyme method. In addition, the EDTA approach maintained stable cell viability and recovery rate of hESCs after transfection. Also, metabolic activity by using Extracellular Flux Analyzer revealed that EDTA method maintained as similar levels of cell functionality as normal group comparing with enzymatic groups. These results suggest that transfection using EDTA is a more efficient and safe substitute for transfection than the use of standard enzymatic methods.

Kim, J., et al. (2019). "Modeling Host-Virus Interactions in Viral Infectious Diseases Using Stem-Cell-Derived Systems and CRISPR/Cas9 Technology." Viruses **11**(2).

Pathologies induced by viral infections have undergone extensive study, with traditional model systems such as two-dimensional (2D) cell cultures and in vivo mouse models contributing greatly to our understanding of host-virus interactions. However, the technical limitations inherent in these systems have constrained efforts to more fully understand such interactions, leading to a search for alternative in vitro systems that accurately recreate in vivo physiology in order to advance the study of viral pathogenesis. Over the last decade, there have been significant technological advances that have allowed researchers to more accurately model the host environment when modeling viral pathogenesis in vitro, including induced pluripotent stem cells (iPSCs), adult stem-cell-derived organoid culture systems and CRISPR/Cas9-mediated genome editing. Such technological breakthroughs have ushered in a new era in the field of viral pathogenesis, where previously challenging questions have begun to be tackled. These include genome-wide analysis of host-virus crosstalk, identification of host factors critical for viral pathogenesis, and the study of viral pathogens that previously lacked a suitable platform, e.g., noroviruses, rotaviruses, enteroviruses, adenoviruses, and Zika virus. In this review, we will discuss recent advances in the study of viral pathogenesis and host-virus crosstalk arising from the use of iPSC, organoid, and CRISPR/Cas9 technologies.

Kim, S. J., et al. (2017). "Generation of a Nrf2 homozygous knockout human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **19**: 46-48.

Nuclear factor erythroid 2-related factor 2 (NFE2L2 or Nrf2) is a well-known transcription factor

that regulates the expression of a large number of anti-oxidant genes in mammalian cells (J.H. Kim et al., 2014). Here, we generated a homozygous Nrf2 knockout human embryonic stem cell (hESC) line, H9Nrf2KO-A13, using the CRISPR/Cas9 genome editing method. The Nrf2 homozygous knockout H9 cell line maintains pluripotency, differentiation potential into three germ layers, and a normal karyotype.

Kim, S. J., et al. (2017). "A homozygous Keap1-knockout human embryonic stem cell line generated using CRISPR/Cas9 mediates gene targeting." Stem Cell Res **19**: 52-54.

Kelch-like ECH-associated protein 1 (keap1) is a cysteine-rich protein that interacts with transcription factor Nrf2 in a redox-sensitive manner, leading to the degradation of Nrf2 (Kim et al., 2014a). Disruption of Keap1 results in the induction of Nrf2-related signaling pathways involving the expression of a set of anti-oxidant and anti-inflammatory genes. We generated biallelic mutants of the Keap1 gene using a CRISPR-Cas9 genome editing method in the H9 human embryonic stem cell (hESC). The Keap1 homozygous-knockout H9 cell line retained normal morphology, gene expression, and in vivo differentiation potential.

Kim, T., et al. (2017). "A novel system for correcting large-scale chromosomal aberrations: ring chromosome correction via reprogramming into induced pluripotent stem cell (iPSC)." Chromosoma **126**(4): 457-463.

Approximately 1 in 500 newborns are born with chromosomal abnormalities that include trisomies, translocations, large deletions, and duplications. There is currently no therapeutic approach for correcting such chromosomal aberrations in vivo or in vitro. When we attempted to produce induced pluripotent stem cell (iPSC) models from patient-derived fibroblasts that contained ring chromosomes, we found that the ring chromosomes were eliminated and replaced by duplicated normal copies of chromosomes through a mechanism of uniparental isodisomy (Bershteyn et al. 2014, Nature 507:99). The discovery of this previously unforeseen system for aberrant chromosome correction during reprogramming enables us for the first time to model and understand this process of cell-autonomous correction of ring chromosomes during human patient somatic cell reprograming to iPSCs. This knowledge could lead to a potential therapeutic strategy to correct common large-scale chromosomal aberrations, termed "chromosome therapy".

Kokubu, C. and J. Takeda (2014). "When half is better than the whole: advances in haploid embryonic stem cell technology." Cell Stem Cell **14**(3): 265-267.

Recent advances in embryonic stem cell (ESC) derivation and genome editing offer efficient platforms for genetic screening. In this issue, Li et al. and Leeb et al., respectively, expand such applications by generating haploid rat ESCs for screening, mutagenesis, and CRISPR-Cas-mediated gene targeting and by developing a forward genetic screen for interrogating haploid mESCs.

Lagresle-Peyrou, C., et al. (2018). "Plerixafor enables safe, rapid, efficient mobilization of hematopoietic stem cells in sickle cell disease patients after exchange transfusion." Haematologica **103**(5): 778-786.

Sickle cell disease is characterized by chronic anemia and vaso-occlusive crises, which eventually lead to multi-organ damage and premature death. Hematopoietic stem cell transplantation is the only curative treatment but it is limited by toxicity and poor availability of HLA-compatible donors. A gene therapy approach based on the autologous transplantation of lentiviral-corrected hematopoietic stem and progenitor cells was shown to be efficacious in one patient. However, alterations of the bone marrow environment and properties of the red blood cells hamper the harvesting and immunoselection of patients' stem cells from bone marrow. The use of Filgrastim to mobilize large numbers of hematopoietic stem and progenitor cells into the circulation has been associated with severe adverse events in sickle cell patients. Thus, broader application of the gene therapy approach requires the development of alternative mobilization methods. We set up a phase I/II clinical trial whose primary objective was to assess the safety of a single injection of Plerixafor in sickle cell patients undergoing red blood cell exchange to decrease the hemoglobin S level to below 30%. The secondary objective was to measure the efficiency of mobilization and isolation of hematopoietic stem and progenitor cells. No adverse events were observed. Large numbers of CD34(+) cells were mobilized extremely quickly. Importantly, the mobilized cells contained high numbers of hematopoietic stem cells, expressed high levels of stemness genes, and engrafted very efficiently in immunodeficient mice. Thus, Plerixafor can be safely used to mobilize hematopoietic stem cells in sickle cell patients; this finding opens up new avenues for treatment approaches based on gene addition and genome editing. Clinicaltrials.gov identifier: NCT02212535.

Lepperhof, V., et al. (2014). "Bioluminescent imaging of genetically selected induced pluripotent

stem cell-derived cardiomyocytes after transplantation into infarcted heart of syngeneic recipients." PLoS One **9**(9): e107363.

Cell loss after transplantation is a major limitation for cell replacement approaches in regenerative medicine. To assess the survival kinetics of induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) we generated transgenic murine iPSC lines which, in addition to CM-specific expression of puromycin N-acetyl-transferase and enhanced green fluorescent protein (EGFP), also constitutively express firefly luciferase (FLuc) for bioluminescence (BL) in vivo imaging. While undifferentiated iPSC lines generated by random integration of the transgene into the genome retained stable FLuc activity over many passages, the BL signal intensity was strongly decreased in purified iPS-CM compared to undifferentiated iPSC. Targeted integration of FLuc-expression cassette into the ROSA26 genomic locus using zinc finger nuclease (ZFN) technology strongly reduced transgene silencing in iPS-CM, leading to a several-fold higher BL compared to iPS-CM expressing FLuc from random genomic loci. To investigate the survival kinetics of iPS-CM in vivo, purified CM obtained from iPSC lines expressing FLuc from a random or the ROSA26 locus were transplanted into cryoinfarcted hearts of syngeneic mice. Engraftment of viable cells was monitored by BL imaging over 4 weeks. Transplanted iPS-CM were poorly retained in the myocardium independently of the cell line used. However, up to 8% of cells survived for 28 days at the site of injection, which was confirmed by immunohistological detection of EGFP-positive iPS-CM in the host tissue. Transplantation of iPS-CM did not affect the scar formation or capillary density in the periinfarct region of host myocardium. This report is the first to determine the survival kinetics of drug-selected iPS-CM in the infarcted heart using BL imaging and demonstrates that transgene silencing in the course of iPSC differentiation can be greatly reduced by employing genome editing technology. FLuc-expressing iPS-CM generated in this study will enable further studies to reduce their loss, increase long-term survival and functional integration upon transplantation.

Li, C., et al. (2018). "Reactivation of gamma-globin in adult beta-YAC mice after ex vivo and in vivo hematopoietic stem cell genome editing." Blood **131**(26): 2915-2928.

Disorders involving beta-globin gene mutations, primarily beta-thalassemia and sickle cell disease, represent a major target for hematopoietic stem/progenitor cell (HSPC) gene therapy. This includes CRISPR/Cas9-mediated genome editing approaches in adult CD34(+) cells aimed toward the reactivation of fetal gamma-globin expression in red blood cells. Because models involving erythroid differentiation of CD34(+) cells have limitations in assessing gamma-globin reactivation, we focused on human beta-globin locus-transgenic (beta-YAC) mice. We used a helper-dependent human CD46-targeting adenovirus vector expressing CRISPR/Cas9 (HDAd-HBG-CRISPR) to disrupt a repressor binding region within the gamma-globin promoter. We transduced HSPCs from beta-YAC/human CD46-transgenic mice ex vivo and subsequently transplanted them into irradiated recipients. Furthermore, we used an in vivo HSPC transduction approach that involves HSPC mobilization and the intravenous injection of HDAd-HBG-CRISPR into beta-YAC/CD46-transgenic mice. In both models, we demonstrated efficient target site disruption, resulting in a pronounced switch from human beta- to gamma-globin expression in red blood cells of adult mice that was maintained after secondary transplantation of HSPCs. In long-term follow-up studies, we did not detect hematological abnormalities, indicating that HBG promoter editing does not negatively affect hematopoiesis. This is the first study that shows successful in vivo HSPC genome editing by CRISPR/Cas9.

Li, S., et al. (2015). "Human Induced Pluripotent Stem Cell NEUROG2 Dual Knockin Reporter Lines Generated by the CRISPR/Cas9 System." Stem Cells Dev **24**(24): 2925-2942.

Human induced pluripotent stem cell (hiPSC) technologies are powerful tools for modeling development and disease, drug screening, and regenerative medicine. Faithful gene targeting in hiPSCs greatly facilitates these applications. We have developed a fast and precise clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) technology-based method and obtained fluorescent protein and antibiotic resistance dual knockin reporters in hiPSC lines for neurogenin2 (NEUROG2), an important proneural transcription factor. Gene targeting efficiency was greatly improved in CRISPR/Cas9-mediated homology directed recombination (approximately 33% correctly targeted clones) compared to conventional targeting protocol (approximately 3%) at the same locus. No off-target events were detected. In addition, taking the advantage of the versatile applications of the CRISPR/Cas9 system, we designed transactivation components to transiently induce NEUROG2 expression, which helps identify transcription factor binding sites and trans-regulation regions of human NEUROG2. The strategy of using CRISPR/Cas9 genome editing coupled with fluorescence-activated cell sorting of neural progenitor

cells in a knockin lineage hiPSC reporter platform might be broadly applicable in other stem cell derivatives and subpopulations.

Liang, N., et al. (2018). "Stem cell contributions to neurological disease modeling and personalized medicine." Prog Neuropsychopharmacol Biol Psychiatry **80**(Pt A): 54-62.

Human induced pluripotent stem cells (iPSCs) represent a revolutionary tool for disease modeling and drug discovery. The generation of tissue-relevant cell types exhibiting a patient's genetic and molecular background offers the ability to develop individual and effective therapies. In this review, we present some major achievements in the neuroscience field using iPSCs and discuss promising perspectives in personalized medicine. In addition to disease modeling, the understanding of the cellular and molecular basis of neurological disorders is explored, including the discovery of new targets and potential drugs. Ultimately, we highlight how iPSC technology, together with genome editing approaches, may bring a deep impact on pre-clinical trials by reducing costs and increasing the success of treatments in a personalized fashion.

Liang, P., et al. (2016). "Patient-Specific and Genome-Edited Induced Pluripotent Stem Cell-Derived Cardiomyocytes Elucidate Single-Cell Phenotype of Brugada Syndrome." J Am Coll Cardiol **68**(19): 2086-2096.

BACKGROUND: Brugada syndrome (BrS), a disorder associated with characteristic electrocardiogram precordial ST-segment elevation, predisposes afflicted patients to ventricular fibrillation and sudden cardiac death. Despite marked achievements in outlining the organ level pathophysiology of the disorder, the understanding of human cellular phenotype has lagged due to a lack of adequate human cellular models of the disorder. OBJECTIVES: The objective of this study was to examine single cell mechanism of Brugada syndrome using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). METHODS: This study recruited 2 patients with type 1 BrS carrying 2 different sodium voltage-gated channel alpha subunit 5 variants as well as 2 healthy control subjects. We generated iPSCs from their skin fibroblasts by using integration-free Sendai virus. We used directed differentiation to create purified populations of iPSC-CMs. RESULTS: BrS iPSC-CMs showed reductions in inward sodium current density and reduced maximal upstroke velocity of action potential compared with healthy control iPSC-CMs. Furthermore, BrS iPSC-CMs demonstrated increased burden of triggered activity, abnormal calcium (Ca (2+)) transients, and beating interval variation. Correction of the causative variant by genome editing was performed, and resultant iPSC-CMs showed resolution of triggered activity and abnormal Ca (2+) transients. Gene expression profiling of iPSC-CMs showed clustering of BrS compared with control subjects. Furthermore, BrS iPSC-CM gene expression correlated with gene expression from BrS human cardiac tissue gene expression. CONCLUSIONS: Patient-specific iPSC-CMs were able to recapitulate single-cell phenotype features of BrS, including blunted inward sodium current, increased triggered activity, and abnormal Ca (2+) handling. This novel human cellular model creates future opportunities to further elucidate the cellular disease mechanism and identify novel therapeutic targets.

Liu, F., et al. (2016). "Drug Discovery via Human-Derived Stem Cell Organoids." Front Pharmacol **7**: 334.

Patient-derived cell lines and animal models have proven invaluable for the understanding of human intestinal diseases and for drug development although both inherently comprise disadvantages and caveats. Many genetically determined intestinal diseases occur in specific tissue microenvironments that are not adequately modeled by monolayer cell culture. Likewise, animal models incompletely recapitulate the complex pathologies of intestinal diseases of humans and fall short in predicting the effects of candidate drugs. Patient-derived stem cell organoids are new and effective models for the development of novel targeted therapies. With the use of intestinal organoids from patients with inherited diseases, the potency and toxicity of drug candidates can be evaluated better. Moreover, owing to the novel clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 genome-editing technologies, researchers can use organoids to precisely modulate human genetic status and identify pathogenesis-related genes of intestinal diseases. Therefore, here we discuss how patient-derived organoids should be grown and how advanced genome-editing tools may be applied to research on modeling of cancer and infectious diseases. We also highlight practical applications of organoids ranging from basic studies to drug screening and precision medicine.

Maillet, A., et al. (2017). "Use of Human Pluripotent Stem Cell Derived-Cardiomyocytes to Study Drug-Induced Cardiotoxicity." Curr Protoc Toxicol **73**: 22 25 21-22 25 22.

Drug-induced cardiotoxicity is the one of the most common causes of drug withdrawal from market. A major barrier in managing the risk of drug-induced cardiotoxicity has been the lack of relevant models to

study cardiac safety. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have great potential in drug discovery and cardiotoxcity screens as they display many characteristics of the human myocardium and offer unlimited supply. This unit describes how to use pluripotent stem cells derived cardiomyocytes to study drug-induced cardiotoxicty using doxorubicin as an example. We present a workflow that explains procedure for editing hPSC using the CRISPR/Cas9 system and for differentiation of hPSC into cardiomyocytes. We also report protocols to study drug effect on ROS production, intracellular calcium concentration, formation of DNA double strand breaks, gene expression and electrophysiological properties of hPSC-CMs. (c) 2017 by John Wiley & Sons, Inc.

Martin Gonzalez, J., et al. (2018). "A new genetic tool to improve immune-compromised mouse models: Derivation and CRISPR/Cas9-mediated targeting of NRG embryonic stem cell lines." Genesis **56**(9): e23238.

Development of human hematopoietic stem cells and differentiation of embryonic stem (ES) cells/induced pluripotent stem (iPS) cells to hematopoietic stem cells are poorly understood. NOD (Non-obese diabetic)-derived mouse strains, such as NSG (NOD-Scid-il2Rg) or NRG (NOD-Rag1-il2Rg), are the best available models for studying the function of fetal and adult human hematopoietic cells as well as ES/iPS cell-derived hematopoietic stem cells. Unfortunately, engraftment of human hematopoietic stem cells is very variable in these models. Introduction of additional permissive mutations into these complex genetic backgrounds of the NRG/NSG mice by natural breeding is a very demanding task in terms of time and resources. Specifically, since the genetic elements defining the NSG/NRG phenotypes have not yet been fully characterized, intense backcrossing is required to ensure transmission of the full phenotype. Here we describe the derivation of embryonic stem cell (ESC) lines from NRG pre-implantation embryos generated by in vitro fertilization followed by the CRISPR/CAS9 targeting of the Gata-2 locus. After injection into morula stage embryos, cells from three tested lines gave rise to chimeric adult mice showing high contribution of the ESCs (70%-100%), assessed by coat color. Moreover, these lines have been successfully targeted using Cas9/CRISPR technology, and the mutant cells have been shown to remain germ line competent. Therefore, these new NRG ESC lines combined with genome editing nucleases bring a powerful genetic tool that facilitates the generation of new NOD-based mouse models with the aim to improve the existing xenograft models.

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

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.

Mosqueira, D., et al. (2018). "CRISPR/Cas9 editing in human pluripotent stem cell-cardiomyocytes highlights arrhythmias, hypocontractility, and energy depletion as potential therapeutic targets for hypertrophic cardiomyopathy." Eur Heart J **39**(43): 3879-3892.

Aims: Sarcomeric gene mutations frequently underlie hypertrophic cardiomyopathy (HCM), a prevalent and complex condition leading to left ventricle thickening and heart dysfunction. We evaluated isogenic genome-edited human pluripotent stem cell-cardiomyocytes (hPSC-CM) for their validity to model, and add clarity to, HCM. Methods and results: CRISPR/Cas9 editing produced 11

variants of the HCM-causing mutation c.C9123T-MYH7 [(p.R453C-beta-myosin heavy chain (MHC)] in 3 independent hPSC lines. Isogenic sets were differentiated to hPSC-CMs for high-throughput, non-subjective molecular and functional assessment using 12 approaches in 2D monolayers and/or 3D engineered heart tissues. Although immature, edited hPSC-CMs exhibited the main hallmarks of HCM (hypertrophy, multi-nucleation, hypertrophic marker expression, sarcomeric disarray). Functional evaluation supported the energy depletion model due to higher metabolic respiration activity, accompanied by abnormalities in calcium handling, arrhythmias, and contraction force. Partial phenotypic rescue was achieved with ranolazine but not omecamtiv mecarbil, while RNAseq highlighted potentially novel molecular targets. Conclusion: Our holistic and comprehensive approach showed that energy depletion affected core cardiomyocyte functionality. The engineered R453C-betaMHC-mutation triggered compensatory responses in hPSC-CMs, causing increased ATP production and alphaMHC to energy-efficient betaMHC switching. We showed that pharmacological rescue of arrhythmias was possible, while MHY7: MYH6 and mutant: wild-type MYH7 ratios may be diagnostic, and previously undescribed lncRNAs and gene modifiers are suggestive of new mechanisms.

Nordin, F., et al. (2017). "Transactivator protein: An alternative for delivery of recombinant proteins for safer reprogramming of induced Pluripotent Stem Cell." Virus Res **235**: 106-114.

Induced pluripotent stem cells (iPSC) are somatic cells reprogrammed to pluripotency by forced expression of pluripotency factors. These cells are shown to have the same pluripotent potential as embryonic stem cells (ESC) and considered as an alternative to the much controversial usage of ESC which involved human embryos. However, the traditional method in reprogramming cells into iPSC using genome-integrating retro- or lenti- viruses remains an obstacle for its application in clinical setting. Although numerous studies have been conducted for a safer DNA-based reprogramming, reprogramming of iPSC by genetic modifications may raise the possibility of malignant transformation and has been a major limitation for its usage in clinical applications. Therefore, there is a need for an alternative method to reprogram the cells without the use of gene editing and a much safer way to deliver transcription factors to induce pluripotency on target cells. Using protein transduction approach, a number of studies have demonstrated the generation of human iPSCs from human fibroblasts and mouse embryonic fibroblasts by direct delivery of reprogramming proteins. In this review, the definition and mechanism of HIV-TAT protein (a type of protein transduction domain) in delivering recombinant proteins, including the potential of protein-based delivery to induce iPSC were further discussed.

Osteil, P., et al. (2016). "Generation of genome-edited mouse epiblast stem cells via a detour through ES cell-chimeras." Differentiation **91**(4-5): 119-125.

Conventionally, mouse epiblast stem cells (EpiSCs) are derived directly from the epiblast or ectoderm germ layer of the post-implantation embryo. Self-renewing and multipotent EpiSC-like stem cells can also be derived by the conversion of embryonic stem cells (ESCs) via the provision of culture conditions that enable the maintenance of the EpiSCs. Here, we outline an experimental procedure for deriving EpiSCs from post-implantation chimeric embryos that are generated using genome-edited ESCs. This strategy enables the production of EpiSCs where (i) no genetically modified animals or ESCs are available, (ii) the impact of the genetic modification on post-implantation development, which may influence the property of the EpiSCs, is requisite knowledge for using the EpiSC for a specific investigation, and (iii) multiple editing of the genome is desirable to modify the biological attributes of the EpiSCs for studying, for example, the gene network activity on the trajectory of lineage differentiation and tissue morphogenesis.

Panch, S. R., et al. (2017). "Sources of Hematopoietic Stem and Progenitor Cells and Methods to Optimize Yields for Clinical Cell Therapy." Biol Blood Marrow Transplant **23**(8): 1241-1249.

Bone marrow (BM) aspirates, mobilized peripheral blood, and umbilical cord blood (UCB) have developed as graft sources for hematopoietic stem and progenitor cells (HSPCs) for stem cell transplantation and other cellular therapeutics. Individualized techniques are necessary to enhance graft HSPC yields and cell quality from each graft source. BM aspirates yield adequate CD34(+) cells but can result in relative delays in engraftment. Granulocyte colony-stimulating factor (G-CSF)-primed BM HSPCs may facilitate faster engraftment while minimizing graft-versus-host disease in certain patient subsets. The levels of circulating HSPCs are enhanced using mobilizing agents, such as G-CSF and/or plerixafor, which act via the stromal cell-derived factor 1/C-X-C chemokine receptor type 4 axis. Alternate niche pathway mediators, including very late antigen-4/vascular cell adhesion molecule-1, heparan sulfate proteoglycans, parathyroid hormone, and coagulation cascade intermediates, may offer promising alternatives for graft enhancement. UCB grafts have been expanded ex vivo with cytokines,

notch-ligand, or mesenchymal stromal cells, and most studies demonstrated greater quantities of CD34(+) cells ex vivo and improved short-term engraftment. No significant changes were observed in long-term repopulating potential or in patient survival. Early phase clinical trials using nicotinamide and StemReginin1 may offer improved short- and long-term repopulating ability. Breakthroughs in genome editing and stem cell reprogramming technologies may hasten the generation of pooled, third-party HSPC grafts. This review elucidates past, present, and potential future approaches to HSPC graft optimization.

Park, S. H., et al. (2019). "Highly efficient editing of the beta-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease." Nucleic Acids Res.

Sickle cell disease (SCD) is a monogenic disorder that affects millions worldwide. Allogeneic hematopoietic stem cell transplantation is the only available cure. Here, we demonstrate the use of CRISPR/Cas9 and a short single-stranded oligonucleotide template to correct the sickle mutation in the beta-globin gene in hematopoietic stem and progenitor cells (HSPCs) from peripheral blood or bone marrow of patients with SCD, with 24.5 +/- 7.6% efficiency without selection. Erythrocytes derived from gene-edited cells showed a marked reduction of sickle cells, with the level of normal hemoglobin (HbA) increased to 25.3 +/- 13.9%. Gene-corrected SCD HSPCs retained the ability to engraft when transplanted into non-obese diabetic (NOD)-SCID-gamma (NSG) mice with detectable levels of gene correction 16-19 weeks post-transplantation. We show that, by using a high-fidelity SpyCas9 that maintained the same level of on-target gene modification, the off-target effects including chromosomal rearrangements were significantly reduced. Taken together, our results demonstrate efficient gene correction of the sickle mutation in both peripheral blood and bone marrow-derived SCD HSPCs, a significant reduction in sickling of red blood cells, engraftment of gene-edited SCD HSPCs in vivo and the importance of reducing off-target effects; all are essential for moving genome editing based SCD treatment into clinical practice.

Patmanathan, S. N., et al. (2018). "CRISPR/Cas9 in Stem Cell Research: Current Application and Future Perspective." Curr Stem Cell Res Ther **13**(8): 632-644.

The clustered regularly interspaced short palindromic repeats-associated protein 9 or CRISPR/Cas9 system is one of the hottest topics discussed lately due to its robustness and effectiveness in genome editing. The technology has been widely used in life science research including microbial, plant, animal, and human cell studies. Combined with the pluripotency of stem cells, the technology represents a powerful tool to generate various cell types for disease modeling, drug screening, toxicology, and targeted therapies. Generally, the CRISPR/Cas9 system has been applied in genetic modification of pluripotent or multipotent stem cells, after which the cells are differentiated into specific cell types and used for functional analysis or even clinical transplantation. Recent advancement in CRISPR/Cas9 technology has widened the scope of stem cell research and its therapeutic application. This review provides an overview of the current application and the prospect of CRISPR/Cas9 technology, particularly in stem cell research and therapy.

Pei, W., et al. (2017). "Polylox barcoding reveals haematopoietic stem cell fates realized in vivo." Nature **548**(7668): 456-460.

Developmental deconvolution of complex organs and tissues at the level of individual cells remains challenging. Non-invasive genetic fate mapping has been widely used, but the low number of distinct fluorescent marker proteins limits its resolution. Much higher numbers of cell markers have been generated using viral integration sites, viral barcodes, and strategies based on transposons and CRISPR-Cas9 genome editing; however, temporal and tissue-specific induction of barcodes in situ has not been achieved. Here we report the development of an artificial DNA recombination locus (termed Polylox) that enables broadly applicable endogenous barcoding based on the Cre-loxP recombination system. Polylox recombination in situ reaches a practical diversity of several hundred thousand barcodes, allowing tagging of single cells. We have used this experimental system, combined with fate mapping, to assess haematopoietic stem cell (HSC) fates in vivo. Classical models of haematopoietic lineage specification assume a tree with few major branches. More recently, driven in part by the development of more efficient single-cell assays and improved transplantation efficiencies, different models have been proposed, in which unilineage priming may occur in mice and humans at the level of HSCs. We have introduced barcodes into HSC progenitors in embryonic mice, and found that the adult HSC compartment is a mosaic of embryo-derived HSC clones, some of which are unexpectedly large. Most HSC clones gave rise to multilineage or oligolineage fates, arguing against unilineage priming, and suggesting coherent usage of the potential of cells in a clone. The spreading of barcodes, both after induction in embryos and in adult mice, revealed a basic split between common myeloid-erythroid development and common lymphocyte development,

supporting the long-held but contested view of a tree-like haematopoietic structure.

Pernet, O., et al. (2016). "Stem cell-based therapies for HIV/AIDS." Adv Drug Deliv Rev **103**: 187-201.

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.

Piga, D., et al. (2019). "Human induced pluripotent stem cell models for the study and treatment of Duchenne and Becker muscular dystrophies." Ther Adv Neurol Disord **12**: 1756286419833478.

Duchenne and Becker muscular dystrophies are the most common muscle diseases and are both currently incurable. They are caused by mutations in the dystrophin gene, which lead to the absence or reduction/truncation of the encoded protein, with progressive muscle degeneration that clinically manifests in muscle weakness, cardiac and respiratory involvement and early death. The limits of animal models to exactly reproduce human muscle disease and to predict clinically relevant treatment effects has prompted the development of more accurate in vitro skeletal muscle models. However, the challenge of effectively obtaining mature skeletal muscle cells or satellite stem cells as primary cultures has hampered the development of in vitro models. Here, we discuss the recently developed technologies that enable the differentiation of skeletal muscle from human induced pluripotent stem cells (iPSCs) of Duchenne and Becker patients. These systems recapitulate key disease features including inflammation and scarce regenerative myogenic capacity that are partially rescued by genetic and pharmacological therapies and can provide a useful platform to study and realize future therapeutic treatments. Implementation of this model also takes advantage of the developing genome editing field, which is a promising approach not only for correcting dystrophin, but also for modulating the underlying mechanisms of skeletal muscle development, regeneration and disease. These data prove the possibility of creating an accurate Duchenne and Becker in vitro model starting from iPSCs, to be used for pathogenetic studies and for drug screening to identify strategies capable of stopping or reversing muscular dystrophinopathies and other muscle diseases.

Pollard, S. M. (2016). "Quantitative stem cell biology: the threat and the glory." Development **143**(22): 4097-4100.

Major technological innovations over the past decade have transformed our ability to extract quantitative data from biological systems at an unprecedented scale and resolution. These quantitative methods and associated large datasets should lead to an exciting new phase of discovery across many areas of biology. However, there is a clear threat: will we drown in these rivers of data? On 18th July 2016, stem cell biologists gathered in Cambridge for the 5th annual Cambridge Stem Cell Symposium to discuss 'Quantitative stem cell biology: from molecules to models'. This Meeting Review provides a summary of the data presented by each speaker, with a focus on quantitative techniques and the new biological insights that are emerging.

Qin, Y. and W. Q. Gao (2016). "Concise Review: Patient-Derived Stem Cell Research for Monogenic Disorders." Stem Cells **34**(1): 44-54.

Monogenic disorders (MGDs) are caused by a single gene mutation and have a serious impact on human health. At present, there are no effective therapeutic methods for MGDs. Stem cell techniques provide insights into potential treatments for MGDs. With the development of patient-derived stem cells, we can begin to progressively understand the molecular mechanism of MGDs and identify new drugs for MGD treatment. Using powerful genome editing tools, such as zinc finger nucleases, transcriptional activator-like effector nucleases, and the clustered regulatory interspaced short palindromic repeat/Cas9 system, MGD-associated gene mutations can be corrected in MGD stem cells in vitro and then transplanted into MGD animal models to assess their safety and therapeutic effects. Despite the continued challenges surrounding potential pluripotent stem cell tumorigenicity and concerns regarding the genetic modification of stem cells, the extensive clinical application of MGD patient-specific stem cells will be

pursued through further advances in basic research in the MGD field. In this review, we will summarize the latest progress in research into the use of patient-derived stem cells for the potential treatment of MGDs and provide predictions regarding the direction of future investigations.

Randolph, M. K. and W. Zhao (2015). "Genome editing and stem cell therapy pave the path for new treatment of sickle-cell disease." Stem Cell Investig **2**: 22.

Sickle-cell disease (SCD), also known as sickle-cell anemia, is a hereditary blood disorder characterized by the presence of abnormal hemoglobin, the oxygen-carrying protein found in red blood cells. This devastating hematologic disease affects millions of children worldwide. Currently the only available cure is an allogenic hematopoietic stem cell transplant (HSCT) which is limited by the scarcity of fully-matched donors. SCD is caused by a single nucleotide mutation in the beta-globin gene. Correction of this genetic defect would provide a cure for the disease. Two recent murine studies have provided proof of principle for such a strategy by correcting the mutation in hematopoietic stem cells (HSC) using genome editing techniques. With transformative advances being made in the genome editing field, effective and precise manipulation of cellular genomes is becoming highly feasible. Genome editing techniques in combination with stem cell therapy should provide a safe and curative treatment of various genetic diseases such as SCD.

Roberts, B., et al. (2017). "Systematic gene tagging using CRISPR/Cas9 in human stem cells to illuminate cell organization." Mol Biol Cell **28**(21): 2854-2874.

We present a CRISPR/Cas9 genome-editing strategy to systematically tag endogenous proteins with fluorescent tags in human induced pluripotent stem cells (hiPSC). To date, we have generated multiple hiPSC lines with monoallelic green fluorescent protein tags labeling 10 proteins representing major cellular structures. The tagged proteins include alpha tubulin, beta actin, desmoplakin, fibrillarin, nuclear lamin B1, nonmuscle myosin heavy chain IIB, paxillin, Sec61 beta, tight junction protein ZO1, and Tom20. Our genome-editing methodology using Cas9/crRNA ribonuclear protein and donor plasmid coelectroporation, followed by fluorescence-based enrichment of edited cells, typically resulted in <0.1-4% homology-directed repair (HDR). Twenty-five percent of clones generated from each edited population were precisely edited. Furthermore, 92% (36/39) of expanded clonal lines displayed robust morphology, genomic stability, expression and localization of the tagged protein to the appropriate subcellular structure, pluripotency-marker expression, and multilineage differentiation. It is our conclusion that, if cell lines are confirmed to harbor an appropriate gene edit, pluripotency, differentiation potential, and genomic stability are typically maintained during the clonal line-generation process. The data described here reveal general trends that emerged from this systematic gene-tagging approach. Final clonal lines corresponding to each of the 10 cellular structures are now available to the research community.

Roessler, R., et al. (2018). "JIP2 haploinsufficiency contributes to neurodevelopmental abnormalities in human pluripotent stem cell-derived neural progenitors and cortical neurons." Life Sci Alliance **1**(4): e201800094.

Phelan-McDermid syndrome (also known as 22q13.3 deletion syndrome) is a syndromic form of autism spectrum disorder and currently thought to be caused by heterozygous loss of SHANK3. However, patients most frequently present with large chromosomal deletions affecting several additional genes. We used human pluripotent stem cell technology and genome editing to further dissect molecular and cellular mechanisms. We found that loss of JIP2 (MAPK8IP2) may contribute to a distinct neurodevelopmental phenotype in neural progenitor cells (NPCs) affecting neuronal maturation. This is most likely due to a simultaneous down-regulation of c-Jun N-terminal kinase (JNK) proteins, leading to impaired generation of mature neurons. Furthermore, semaphorin signaling appears to be impaired in patient NPCs and neurons. Pharmacological activation of neuropilin receptor 1 (NRP1) rescued impaired semaphorin pathway activity and JNK expression in patient neurons. Our results suggest a novel disease-specific mechanism involving the JIP/JNK complex and identify NRP1 as a potential new therapeutic target.

Rubio, A., et al. (2016). "Rapid and efficient CRISPR/Cas9 gene inactivation in human neurons during human pluripotent stem cell differentiation and direct reprogramming." Sci Rep **6**: 37540.

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.

Saba-El-Leil, M. K., et al. (2017). "Isolation of Mouse Embryonic Stem Cell Lines in the Study of ERK1/2 MAP Kinase Signaling." Methods Mol Biol **1487**: 243-253.

Mouse embryonic stem (ES) cells have proven to be invaluable research tools for dissecting the role of signaling pathways in embryonic development, adult physiology, and various diseases. ES cells are amenable to genetic manipulation by classical gene targeting via homologous recombination or by genome editing technologies. These cells can be used to generate genetically modified mouse models or to study the signaling circuitry regulating self-renewal and early lineage commitment. In this chapter, we describe methods used for the isolation and establishment of mouse ES cell lines from blastocyst embryos and for the measurement of ERK1/2 activity in ES cells.

Sackett, S. D., et al. (2017). "The Nexus of Stem Cell-Derived Beta-Cells and Genome Engineering." Rev Diabet Stud **14**(1): 39-50.

Diabetes, type 1 and type 2 (T1D and T2D), are diseases of epidemic proportions, which are complicated and defined by genetics, epigenetics, environment, and lifestyle choices. Current therapies consist of whole pancreas or islet transplantation. However, these approaches require life-time immunosuppression, and are compounded by the paucity of available donors. Pluripotent stem cells have advanced research in the fields of stem cell biology, drug development, disease modeling, and regenerative medicine, and importantly allows for the interrogation of therapeutic interventions. Recent developments in beta-cell differentiation and genomic modifications are now propelling investigations into the mechanisms behind beta-cell failure and autoimmunity, and offer new strategies for reducing the propensity for immunogenicity. This review discusses the derivation of endocrine lineage cells from human pluripotent stem cells for the treatment of diabetes, and how the editing or manipulation of their genomes can transcend many of the remaining challenges of stem cell technologies, leading to superior transplantation and diabetes drug discovery platforms.

Sato, T. and T. Ogawa (2019). "Generating Genetically Engineered Mice Using a Spermatogonial Stem Cell-Mediated Method." Methods Mol Biol **1874**: 87-98.

Mouse spermatogonial stem cells (SSCs) can be grown in culture for long periods. Cultured SSCs, also called germline stem (GS) cells, maintain themselves by self-renewing proliferation while retaining the ability to differentiate into sperm. Thus, when transplanted into the seminiferous tubules of a host mouse testis, they settle in the basal compartment of the tubules and establish spermatogenenic colonies. The sperm produced in the host are competent to produce offspring. This can be exploited for the generation of genetically modified mice, through the transplantation of genetically modified GS cells. In this section, we describe a method of genome editing-mediated GS cell modification and transplantation.

Sato, T., et al. (2015). "Genome Editing in Mouse Spermatogonial Stem Cell Lines Using TALEN and Double-Nicking CRISPR/Cas9." Stem Cell Reports **5**(1): 75-82.

Mouse spermatogonial stem cells (SSCs) can be cultured for multiplication and maintained for long periods while preserving their spermatogenic ability. Although the cultured SSCs, named germline stem (GS) cells, are targets of genome modification, this process remains technically difficult. In the present study, we tested TALEN and double-nicking CRISPR/Cas9 on GS cells, targeting Rosa26 and Stra8 loci as representative genes dispensable and indispensable in spermatogenesis, respectively. Harvested GS cell colonies showed a high targeting efficiency with both TALEN and CRISPR/Cas9. The Rosa26-targeted GS cells differentiated into fertility-competent sperm following transplantation. On the other hand, Stra8-targeted GS cells showed defective spermatogenesis following transplantation, confirming its prime role in the initiation of meiosis. TALEN and CRISPR/Cas9, when applied in GS cells, will be valuable tools in the study of spermatogenesis and for revealing the genetic mechanism of spermatogenic failure.

Schiroli, G., et al. (2019). "Precise Gene Editing Preserves Hematopoietic Stem Cell Function following Transient p53-Mediated DNA Damage Response." Cell Stem Cell **24**(4): 551-565 e558.

Precise gene editing in hematopoietic stem and progenitor cells (HSPCs) holds promise for treating genetic diseases. However, responses triggered by programmable nucleases in HSPCs are poorly characterized and may negatively impact HSPC engraftment and long-term repopulation capacity. Here, we induced either one or several DNA double-stranded breaks (DSBs) with optimized zinc-finger and CRISPR/Cas9 nucleases and monitored DNA damage response (DDR) foci induction, cell-cycle progression, and transcriptional responses in HSPC subpopulations, with up to single-cell resolution. p53-mediated DDR pathway activation was the predominant response to even single-nuclease-induced DSBs across all HSPC subtypes analyzed. Excess DSB load and/or adeno-associated virus (AAV)-mediated delivery of DNA repair templates induced cumulative p53 pathway activation, constraining proliferation, yield, and engraftment of edited HSPCs. However, functional impairment was reversible when DDR burden was low and could be overcome by transient p53 inhibition. These findings provide molecular and functional evidence for feasible and seamless gene editing in HSPCs.

Schiroli, G., et al. (2017). "Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1." Sci Transl Med **9**(411).

Targeted genome editing in hematopoietic stem/progenitor cells (HSPCs) is an attractive strategy for treating immunohematological diseases. However, the limited efficiency of homology-directed editing in primitive HSPCs constrains the yield of corrected cells and might affect the feasibility and safety of clinical translation. These concerns need to be addressed in stringent preclinical models and overcome by developing more efficient editing methods. We generated a humanized X-linked severe combined immunodeficiency (SCID-X1) mouse model and evaluated the efficacy and safety of hematopoietic reconstitution from limited input of functional HSPCs, establishing thresholds for full correction upon different types of conditioning. Unexpectedly, conditioning before HSPC infusion was required to protect the mice from lymphoma developing when transplanting small numbers of progenitors. We then designed a one-size-fits-all IL2RG (interleukin-2 receptor common gamma-chain) gene correction strategy and, using the same reagents suitable for correction of human HSPC, validated the edited human gene in the disease model in vivo, providing evidence of targeted gene editing in mouse HSPCs and demonstrating the functionality of the IL2RG-edited lymphoid progeny. Finally, we optimized editing reagents and protocol for human HSPCs and attained the threshold of IL2RG editing in long-term repopulating cells predicted to safely rescue the disease, using clinically relevant HSPC sources and highly specific zinc finger nucleases or CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9). Overall, our work establishes the rationale and guiding principles for clinical translation of SCID-X1 gene editing and provides a framework for developing gene correction for other diseases.

Schwank, G., et al. (2013). "Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients." Cell Stem Cell **13**(6): 653-658.

Single murine and human intestinal stem cells can be expanded in culture over long time periods as genetically and phenotypically stable epithelial organoids. Increased cAMP levels induce rapid swelling of such organoids by opening the cystic fibrosis transmembrane conductor receptor (CFTR). This response is lost in organoids derived from cystic fibrosis (CF) patients. Here we use the CRISPR/Cas9 genome editing system to correct the CFTR locus by homologous recombination in cultured intestinal stem cells of CF patients. The corrected allele is expressed and fully functional as measured in clonally expanded organoids. This study provides proof of concept for gene correction by homologous recombination in primary adult stem cells derived from patients with a single-gene hereditary defect.

Seino, T., et al. (2018). "Human Pancreatic Tumor Organoids Reveal Loss of Stem Cell Niche Factor Dependence during Disease Progression." Cell Stem Cell **22**(3): 454-467 e456.

Despite recent efforts to dissect the inter-tumor heterogeneity of pancreatic ductal adenocarcinoma (PDAC) by determining prognosis-predictive gene expression signatures for specific subtypes, their functional differences remain elusive. Here, we established a pancreatic tumor organoid library encompassing 39 patient-derived PDACs and identified 3 functional subtypes based on their stem cell niche factor dependencies on Wnt and R-spondin. A Wnt-non-producing subtype required Wnt from cancer-associated fibroblasts, whereas a Wnt-producing subtype autonomously secreted Wnt ligands and an R-spondin-independent subtype grew in the absence of Wnt and R-spondin. Transcriptome analysis of PDAC organoids revealed gene-expression signatures that associated Wnt niche subtypes with GATA6-dependent gene expression subtypes, which were functionally supported by genetic perturbation of GATA6. Furthermore, CRISPR-Cas9-based genome editing of PDAC driver genes (KRAS, CDKN2A,

SMAD4, and TP53) demonstrated non-genetic acquisition of Wnt niche independence during pancreas tumorigenesis. Collectively, our results reveal functional heterogeneity of Wnt niche independency in PDAC that is non-genetically formed through tumor progression.

Shaheen, N., et al. (2017). "Pluripotent Stem Cell-Based Platforms in Cardiac Disease Modeling and Drug Testing." Clin Pharmacol Ther **102**(2): 203-208.

The ability to generate patient/disease-specific human pluripotent stem cell (hPSC)-derived cardiomyocytes (hPSC-CMs) brings a unique value to the fields of cardiac disease modeling, drug testing, drug discovery, and precision medicine. Further integration of emerging innovative technologies such as developmental-biology inspired differentiation into chamber-specific cardiomyocyte subtypes, genome-editing, tissue-engineering, and novel functional phenotyping methodologies should facilitate even more advanced investigations. Here, we review cornerstone concepts and recent highlights of hPSC-based cardiac disease modeling and drug testing.

Shen, Y., et al. (2018). "Generation of PTEN knockout bone marrow mesenchymal stem cell lines by CRISPR/Cas9-mediated genome editing." Cytotechnology **70**(2): 783-791.

The tumor suppressor PTEN is involved in the regulation of cell proliferation, lineage determination, motility, adhesion and apoptosis. Loss of PTEN in the bone mesenchymal stem cells (BMSCs) was shown to change their function in the repair tissue. So far, the CRISPR/Cas9 system has been proven extremely simple and flexible. Using this system to manipulate PTEN gene editing could produce the PTEN-Knocking-out (PTEN-KO) strain. We knocked out PTEN in MSCs and validated the expression by PCR and Western blot. To clarify the changes in proliferation, CCK-8 assay was applied. In support, living cell proportion was assessed by Trypan blue staining. For osteogenic and adipogenic induction, cells were cultured in different media for 2 weeks. Oil red staining and alizarin red staining were performed for assessment of osteogenic or adipogenic differentiation. The expression of Id4, Runx2, ALP and PPARgamma was examined by qPCR and immunocytochemistry staining. The PTEN-KO strain was identified by sequencing. The PTEN-KO cells had an increased cell viability and higher survival compared with the wild type. However, decreased expression of Runx2 and PPARgamma was found in the PTEN loss strain after induction, and consistently decreased osteogenic or adipogenic differentiation was observed by alizarin and oil red staining. Together, PTEN-KO strain showed an increased proliferation capability but decreased multi-directional differentiation potential. When BMSCs serve as seed cells for tissue engineering, the PTEN gene may be used as an indicator.

Sluch, V. M., et al. (2017). "Enhanced Stem Cell Differentiation and Immunopurification of Genome Engineered Human Retinal Ganglion Cells." Stem Cells Transl Med **6**(11): 1972-1986.

Human pluripotent stem cells have the potential to promote biological studies and accelerate drug discovery efforts by making possible direct experimentation on a variety of human cell types of interest. However, stem cell cultures are generally heterogeneous and efficient differentiation and purification protocols are often lacking. Here, we describe the generation of clustered regularly-interspaced short palindromic repeats (CRISPR)-Cas9 engineered reporter knock-in embryonic stem cell lines in which td Tomato and a unique cell-surface protein, THY1.2, are expressed under the control of the retinal ganglion cell (RGC)-enriched gene BRN3B. Using these reporter cell lines, we greatly improved adherent stem cell differentiation to the RGC lineage by optimizing a novel combination of small molecules and established an anti-THY1.2-based protocol that allows for large-scale RGC immunopurification. RNA-sequencing confirmed the similarity of the stem cell-derived RGCs to their endogenous human counterparts. Additionally, we developed an in vitro axonal injury model suitable for studying signaling pathways and mechanisms of human RGC cell death and for high-throughput screening for neuroprotective compounds. Using this system in combination with RNAi-based knockdown, we show that knockdown of dual leucine kinase (DLK) promotes survival of human RGCs, expanding to the human system prior reports that DLK inhibition is neuroprotective for murine RGCs. These improvements will facilitate the development and use of large-scale experimental paradigms that require numbers of pure RGCs that were not previously obtainable. Stem Cells Translational Medicine 2017;6:1972-1986.

Smith, L. J., et al. (2018). "Stem cell-derived clade F AAVs mediate high-efficiency homologous recombination-based genome editing." Proc Natl Acad Sci U S A **115**(31): E7379-E7388.

The precise correction of genetic mutations at the nucleotide level is an attractive permanent therapeutic strategy for human disease. However, despite significant progress, challenges to efficient and accurate genome editing persist. Here, we report a genome editing platform based upon a class of hematopoietic stem cell (HSC)-derived clade F adeno-

associated virus (AAV), which does not require prior nuclease-mediated DNA breaks and functions exclusively through BRCA2-dependent homologous recombination. Genome editing is guided by complementary homology arms and is highly accurate and seamless, with no evidence of on-target mutations, including insertion/deletions or inclusion of AAV inverted terminal repeats. Efficient genome editing was demonstrated at different loci within the human genome, including a safe harbor locus, AAVS1, and the therapeutically relevant IL2RG gene, and at the murine Rosa26 locus. HSC-derived AAV vector (AAVHSC)-mediated genome editing was robust in primary human cells, including CD34(+) cells, adult liver, hepatic endothelial cells, and myocytes. Importantly, high-efficiency gene editing was achieved in vivo upon a single i.v. injection of AAVHSC editing vectors in mice. Thus, clade F AAV-mediated genome editing represents a promising, highly efficient, precise, single-component approach that enables the development of therapeutic in vivo genome editing for the treatment of a multitude of human gene-based diseases.

Sterneckert, J. L., et al. (2014). "Investigating human disease using stem cell models." Nat Rev Genet **15**(9): 625-639.

Tractable and accurate disease models are essential for understanding disease pathogenesis and for developing new therapeutics. As stem cells are capable of self-renewal and differentiation, they are ideally suited both for generating these models and for obtaining the large quantities of cells required for drug development and transplantation therapies. Although proof of principle for the use of adult stem cells and embryonic stem cells in disease modelling has been established, induced pluripotent stem cells (iPSCs) have demonstrated the greatest utility for modelling human diseases. Furthermore, combining gene editing with iPSCs enables the generation of models of genetically complex disorders.

Sun, N. and H. Zhao (2014). "Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs." Biotechnol Bioeng **111**(5): 1048-1053.

Sickle cell disease (SCD) is the most common human genetic disease which is caused by a single mutation of human beta-globin (HBB) gene. The lack of long-term treatment makes the development of reliable cell and gene therapies highly desirable. Disease-specific patient-derived human induced pluripotent stem cells (hiPSCs) have great potential for developing novel cell and gene therapies. With the disease-causing mutations corrected in situ, patient-derived hiPSCs can restore normal cell functions and serve as a renewable autologous cell source for the treatment of genetic disorders. Here we successfully utilized transcription activator-like effector nucleases (TALENs), a recently emerged novel genome editing tool, to correct the SCD mutation in patient-derived hiPSCs. The TALENs we have engineered are highly specific and generate minimal off-target effects. In combination with piggyBac transposon, TALEN-mediated gene targeting leaves no residual ectopic sequences at the site of correction and the corrected hiPSCs retain full pluripotency and a normal karyotype. Our study demonstrates an important first step of using TALENs for the treatment of genetic diseases such as SCD, which represents a significant advance toward hiPSC-based cell and gene therapies.

Sun, Y. and Q. Ding (2017). "Genome engineering of stem cell organoids for disease modeling." Protein Cell **8**(5): 315-327.

Precision medicine emerges as a new approach that takes into account individual variability. Successful realization of precision medicine requires disease models that are able to incorporate personalized disease information and recapitulate disease development processes at the molecular, cellular and organ levels. With recent development in stem cell field, a variety of tissue organoids can be derived from patient specific pluripotent stem cells and adult stem cells. In combination with the state-of-the-art genome editing tools, organoids can be further engineered to mimic disease-relevant genetic and epigenetic status of a patient. This has therefore enabled a rapid expansion of sophisticated in vitro disease models, offering a unique system for fundamental and biomedical research as well as the development of personalized medicine. Here we summarize some of the latest advances and future perspectives in engineering stem cell organoids for human disease modeling.

Suzuki, K., et al. (2014). "Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones." Cell Stem Cell **15**(1): 31-36.

The utility of genome editing technologies for disease modeling and developing cellular therapies has been extensively documented, but the impact of these technologies on mutational load at the whole-genome level remains unclear. We performed whole-genome sequencing to evaluate the mutational load at single-base resolution in individual gene-corrected human induced pluripotent stem cell (hiPSC) clones in three different disease models. In single-cell clones, gene correction by helper-dependent adenoviral vector

(HDAdV) or Transcription Activator-Like Effector Nuclease (TALEN) exhibited few off-target effects and a low level of sequence variation, comparable to that accumulated in routine hiPSC culture. The sequence variants were randomly distributed and unique to individual clones. We also combined both technologies and developed a TALEN-HDAdV hybrid vector, which significantly increased gene-correction efficiency in hiPSCs. Therefore, with careful monitoring via whole-genome sequencing it is possible to apply genome editing to human pluripotent cells with minimal impact on genomic mutational load.

Tagliafierro, L., et al. (2019). "Lentiviral Vector Platform for the Efficient Delivery of Epigenome-editing Tools into Human Induced Pluripotent Stem Cell-derived Disease Models." J Vis Exp (145).

The use of hiPSC-derived cells represents a valuable approach to study human neurodegenerative diseases. Here, we describe an optimized protocol for the differentiation of hiPSCs derived from a patient with the triplication of the alpha-synuclein gene (SNCA) locus into Parkinson's disease (PD)-relevant dopaminergic neuronal populations. Accumulating evidence has shown that high levels of SNCA are causative for the development of PD. Recognizing the unmet need to establish novel therapeutic approaches for PD, especially those targeting the regulation of SNCA expression, we recently developed a CRISPR/dCas9-DNA-methylation-based system to epigenetically modulate SNCA transcription by enriching methylation levels at the SNCA intron 1 regulatory region. To deliver the system, consisting of a dead (deactivated) version of Cas9 (dCas9) fused with the catalytic domain of the DNA methyltransferase enzyme 3A (DNMT3A), a lentiviral vector is used. This system is applied to cells with the triplication of the SNCA locus and reduces the SNCA-mRNA and protein levels by about 30% through the targeted DNA methylation of SNCA intron 1. The fine-tuned downregulation of the SNCA levels rescues disease-related cellular phenotypes. In the current protocol, we aim to describe a step-by-step procedure for differentiating hiPSCs into neural progenitor cells (NPCs) and the establishment and validation of pyrosequencing assays for the evaluation of the methylation profile in the SNCA intron 1. To outline in more detail the lentivirus-CRISPR/dCas9 system used in these experiments, this protocol describes how to produce, purify, and concentrate lentiviral vectors and to highlight their suitability for epigenome- and genome-editing applications using hiPSCs and NPCs. The protocol is easily adaptable and can be used to produce high titer lentiviruses for in vitro and in vivo applications.

Tamburini, C. and M. Li (2017). "Understanding neurodevelopmental disorders using human pluripotent stem cell-derived neurons." Brain Pathol **27**(4): 508-517.

Research into psychiatric disorders has long been hindered by the lack of appropriate models. Induced pluripotent stem cells (iPSCs) offer an unlimited source of patient-specific cells, which in principle can be differentiated into all disease-relevant somatic cell types to create in vitro models of the disorder of interest. Here, neuronal differentiation protocols available for this purpose and the current progress on iPSCs-based models of schizophrenia, autism spectrum disorders and bipolar disorder were reviewed. We also discuss the impact of the recently developed CRISPR/Cas9 genome editing tool in the disease modeling field. Genetically engineered mutation of disease risk alleles in well characterized reference "control" hPSCs or correction of disease risk variants in patient iPSCs has been used as a powerful means to establish causality of the identified cellular pathology. Together, iPSC reprogramming and CRISPR/CAS9 genome editing technology have already significantly contributed to our understanding of the developmental origin of some major psychiatric disorders. The challenge ahead is the identification of shared mechanisms in their etiology, which will ultimately be relevant to the development of new treatments.

Tian, L., et al. (2016). "Efficient and Controlled Generation of 2D and 3D Bile Duct Tissue from Human Pluripotent Stem Cell-Derived Spheroids." Stem Cell Rev **12**(4): 500-508.

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.

Trevisan, M., et al. (2015). "Modeling Viral Infectious Diseases and Development of Antiviral Therapies Using Human Induced Pluripotent Stem Cell-Derived Systems." Viruses **7**(7): 3835-3856.

The recent biotechnology breakthrough of cell reprogramming and generation of induced pluripotent stem cells (iPSCs), which has revolutionized the approaches to study the mechanisms of human diseases and to test new drugs, can be exploited to generate patient-specific models for the investigation of host-pathogen interactions and to develop new antimicrobial and antiviral therapies. Applications of iPSC technology to the study of viral infections in humans have included in vitro modeling of viral infections of neural, liver, and cardiac cells; modeling of human genetic susceptibility to severe viral infectious diseases, such as encephalitis and severe influenza; genetic engineering and genome editing of patient-specific iPSC-derived cells to confer antiviral resistance.

Tu, J., et al. (2018). "Generation of human embryonic stem cell line with heterozygous RB1 deletion by CRIPSR/Cas9 nickase." Stem Cell Res **28**: 29-32.

The Retinoblastoma 1 (RB1) tumor suppressor, a member of the Retinoblastoma gene family, functions as a pocket protein for the functional binding of E2F transcription factors. About 1/3 of retinoblastoma patients harbor a germline RB1 mutation or deletion, leading to the development of retinoblastoma. Here, we demonstrate generation of a heterozygous deletion of the RB1 gene in the H1 human embryonic stem cell line using CRISPR/Cas9 nickase genome editing. The RB1 heterozygous knockout H1 cell line shows a normal karyotype, maintains a pluripotent state, and is capable of differentiation to the three germline layers.

Vasileva, E. A., et al. (2015). "Genome-editing tools for stem cell biology." Cell Death Dis **6**: e1831.

Human pluripotent stem cells provide a versatile platform for regenerative studies, drug testing and disease modeling. That the expression of only four transcription factors, Oct4, Klf4, Sox2 and c-Myc (OKSM), is sufficient for generation of induced pluripotent stem cells (iPSCs) from differentiated somatic cells has revolutionized the field and also highlighted the importance of OKSM as targets for genome editing. A number of novel genome-editing systems have been developed recently. In this review, we focus on successful applications of several such systems for generation of iPSCs. In particular, we discuss genome-editing systems based on zinc-finger fusion proteins (ZFs), transcription activator-like effectors (TALEs) and an RNA-guided DNA-specific nuclease, Cas9, derived from the bacterial defense system against viruses that utilizes clustered regularly interspaced short palindromic repeats (CRISPR).

Veres, A., et al. (2014). "Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing." Cell Stem Cell **15**(1): 27-30.

Genome editing has attracted wide interest for the generation of cellular models of disease using human pluripotent stem cells and other cell types. CRISPR-Cas systems and TALENs can target desired genomic sites with high efficiency in human cells, but recent publications have led to concern about the extent to which these tools may cause off-target mutagenic effects that could potentially confound disease-modeling studies. Using CRISPR-Cas9 and TALEN targeted human pluripotent stem cell clones, we performed whole-genome sequencing at high coverage in order to assess the degree of mutagenesis across the entire genome. In both types of clones, we found that off-target mutations attributable to the nucleases were very rare. From this analysis, we suggest that, although some cell types may be at risk for off-target mutations, the incidence of such effects in human pluripotent stem cells may be sufficiently low and thus not a significant concern for disease modeling and other applications.

Vo, L. T. and G. Q. Daley (2015). "De novo generation of HSCs from somatic and pluripotent stem cell sources." Blood **125**(17): 2641-2648.

Generating human hematopoietic stem cells (HSCs) from autologous tissues, when coupled with genome editing technologies, is a promising approach for cellular transplantation therapy and for in vitro disease modeling, drug discovery, and toxicology studies. Human pluripotent stem cells (hPSCs) represent a potentially inexhaustible supply of autologous tissue; however, to date, directed differentiation from hPSCs has yielded hematopoietic cells that lack robust and sustained multilineage potential. Cellular reprogramming technologies represent an alternative platform for the de novo

generation of HSCs via direct conversion from heterologous cell types. In this review, we discuss the latest advancements in HSC generation by directed differentiation from hPSCs or direct conversion from somatic cells, and highlight their applications in research and prospects for therapy.

Wang, G., et al. (2014). "Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies." Nat Med **20**(6): 616-623.

Study of monogenic mitochondrial cardiomyopathies may yield insights into mitochondrial roles in cardiac development and disease. Here, we combined patient-derived and genetically engineered induced pluripotent stem cells (iPSCs) with tissue engineering to elucidate the pathophysiology underlying the cardiomyopathy of Barth syndrome (BTHS), a mitochondrial disorder caused by mutation of the gene encoding tafazzin (TAZ). Using BTHS iPSC-derived cardiomyocytes (iPSC-CMs), we defined metabolic, structural and functional abnormalities associated with TAZ mutation. BTHS iPSC-CMs assembled sparse and irregular sarcomeres, and engineered BTHS 'heart-on-chip' tissues contracted weakly. Gene replacement and genome editing demonstrated that TAZ mutation is necessary and sufficient for these phenotypes. Sarcomere assembly and myocardial contraction abnormalities occurred in the context of normal whole-cell ATP levels. Excess levels of reactive oxygen species mechanistically linked TAZ mutation to impaired cardiomyocyte function. Our study provides new insights into the pathogenesis of Barth syndrome, suggests new treatment strategies and advances iPSC-based in vitro modeling of cardiomyopathy.

Wang, Y., et al. (2019). "Multiomics Analyses of HNF4alpha Protein Domain Function during Human Pluripotent Stem Cell Differentiation." iScience **16**: 206-217.

During mammalian development, liver differentiation is driven by signals that converge on multiple transcription factor networks. The hepatocyte nuclear factor signaling network is known to be essential for hepatocyte specification and maintenance. In this study, we have generated deletion and point mutants of hepatocyte nuclear factor-4alpha (HNF4alpha) to precisely evaluate the function of protein domains during hepatocyte specification from human pluripotent stem cells. We demonstrate that nuclear HNF4alpha is essential for hepatic progenitor specification, and the introduction of point mutations in HNF4alpha's Small Ubiquitin-like Modifier (SUMO) consensus motif leads to disrupted hepatocyte differentiation. Taking a multiomics approach, we identified key deficiencies in cell biology, which included dysfunctional metabolism, substrate adhesion, tricarboxylic acid cycle flux, microRNA transport, and mRNA processing. In summary, the combination of genome editing and multiomics analyses has provided valuable insight into the diverse functions of HNF4alpha during pluripotent stem cell entry into the hepatic lineage and during hepatocellular differentiation.

Wei, Y. D., et al. (2015). "Use of genome editing tools in human stem cell-based disease modeling and precision medicine." Yi Chuan **37**(10): 983-991.

Precision medicine emerges as a new approach that takes into account individual variability. The successful conduct of precision medicine requires the use of precise disease models. Human pluripotent stem cells (hPSCs), as well as adult stem cells, can be differentiated into a variety of human somatic cell types that can be used for research and drug screening. The development of genome editing technology over the past few years, especially the CRISPR/Cas system, has made it feasible to precisely and efficiently edit the genetic background. Therefore, disease modeling by using a combination of human stem cells and genome editing technology has offered a new platform to generate " personalized " disease models, which allow the study of the contribution of individual genetic variabilities to disease progression and the development of precise treatments. In this review, recent advances in the use of genome editing in human stem cells and the generation of stem cell models for rare diseases and cancers are discussed.

Weykopf, B., et al. (2019). "Induced pluripotent stem cell-based modeling of mutant LRRK2-associated Parkinson's disease." Eur J Neurosci **49**(4): 561-589.

Recent advances in cell reprogramming have enabled assessment of disease-related cellular traits in patient-derived somatic cells, thus providing a versatile platform for disease modeling and drug development. Given the limited access to vital human brain cells, this technology is especially relevant for neurodegenerative disorders such as Parkinson's disease (PD) as a tool to decipher underlying pathomechanisms. Importantly, recent progress in genome-editing technologies has provided an ability to analyze isogenic induced pluripotent stem cell (iPSC) pairs that differ only in a single genetic change, thus allowing a thorough assessment of the molecular and cellular phenotypes that result from monogenetic risk factors. In this review, we summarize the current state of iPSC-based modeling of PD with a focus on leucine-rich repeat kinase 2 (LRRK2), one of the most prominent monogenetic risk factors for PD linked to

both familial and idiopathic forms. The LRRK2 protein is a primarily cytosolic multi-domain protein contributing to regulation of several pathways including autophagy, mitochondrial function, vesicle transport, nuclear architecture and cell morphology. We summarize iPSC-based studies that contributed to improving our understanding of the function of LRRK2 and its variants in the context of PD etiopathology. These data, along with results obtained in our own studies, underscore the multifaceted role of LRRK2 in regulating cellular homeostasis on several levels, including proteostasis, mitochondrial dynamics and regulation of the cytoskeleton. Finally, we expound advantages and limitations of reprogramming technologies for disease modeling and drug development and provide an outlook on future challenges and expectations offered by this exciting technology.

Williams, C. A., et al. (2016). "Erk5 Is a Key Regulator of Naive-Primed Transition and Embryonic Stem Cell Identity." Cell Rep **16**(7): 1820-1828.

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.

Wong, O. G. and A. N. Cheung (2016). "Stem cell transcription factor NANOG in cancers--is eternal youth a curse?" Expert Opin Ther Targets **20**(4): 407-417.

INTRODUCTION: Targeting cancer stem cells can be a more effective approach to treat cancer. NANOG is one of the key factors for maintaining the self-renewal ability and pluripotency of stem cells, including cancer stem cells. Overexpression of NANOG has been observed in various human malignancies. Several reports have suggested that NANOG contributes to carcinogenesis by initiating and preserving cancer stem cells. It is obvious that NANOG is also involved in establishing other hallmarks of cancer such as uncontrolled cell growth, chemoresistance, metastasis, and immune evasion. AREAS COVERED: This review will discuss the molecular properties and oncogenic roles of NANOG. The idea of using agents that inhibit the transcription factor to treat cancer is presented. Interfering with NANOG-mediated transcriptions using small interfering RNA, transcription factor decoy, genome editing, and small-molecule inhibitors may provide novel strategies to target cancer stem cells. EXPERT OPINION: As a pivotal controller in cancer stem cell maintenance and a positive regulator of various oncogenic pathways, NANOG may be an important target for cancer therapy. However, as a transcription factor, it is inherently difficult to target by pharmacological means. Novel approaches need to be explored before the inhibition of NANOG can be applied in a clinical setting.

Wu, J. and J. C. Izpisua Belmonte (2015). "Dynamic Pluripotent Stem Cell States and Their Applications." Cell Stem Cell **17**(5): 509-525.

Embryonic pluripotency can be recapitulated in vitro by a spectrum of pluripotent stem cell states stabilized with different culture conditions. Their distinct spatiotemporal characteristics provide an unprecedented tool for the study of early human development. The newly unveiled ability of some stem cell types for crossing xeno-barriers will facilitate the generation of interspecies chimeric embryos from distant species, including humans. When combined with efficient zygote genome editing technologies, xenogeneic human pluripotent stem cells may also open new frontiers for regenerative medicine applications, including the possibility of generating human organs in animals via interspecies chimeric complementation.

Xia, G., et al. (2015). "Genome modification leads to phenotype reversal in human myotonic dystrophy type 1 induced pluripotent stem cell-derived neural stem cells." Stem Cells **33**(6): 1829-1838.

Myotonic dystrophy type 1 (DM1) is caused by expanded CTG repeats in the 3'-untranslated region (3' UTR) of the DMPK gene. Correcting the mutation in DM1 stem cells would be an important step toward autologous stem cell therapy. The objective of this study is to demonstrate in vitro genome editing to prevent production of toxic mutant transcripts and reverse phenotypes in DM1 stem cells. Genome editing was performed in DM1 neural stem cells (NSCs) derived from human DM1 induced pluripotent stem (iPS) cells. An editing cassette containing

SV40/bGH polyA signals was integrated upstream of the CTG repeats by TALEN-mediated homologous recombination (HR). The expression of mutant CUG repeats transcript was monitored by nuclear RNA foci, the molecular hallmarks of DM1, using RNA fluorescence in situ hybridization. Alternative splicing of microtubule-associated protein tau (MAPT) and muscleblind-like (MBNL) proteins were analyzed to further monitor the phenotype reversal after genome modification. The cassette was successfully inserted into DMPK intron 9 and this genomic modification led to complete disappearance of nuclear RNA foci. MAPT and MBNL 1, 2 aberrant splicing in DM1 NSCs were reversed to normal pattern in genome-modified NSCs. Genome modification by integration of exogenous polyA signals upstream of the DMPK CTG repeat expansion prevents the production of toxic RNA and leads to phenotype reversal in human DM1 iPS-cells derived stem cells. Our data provide proof-of-principle evidence that genome modification may be used to generate genetically modified progenitor cells as a first step toward autologous cell transfer therapy for DM1.

Yamashita, T., et al. (2019). "Pharmaceutical Research for Inherited Metabolic Disorders of the Liver Using Human Induced Pluripotent Stem Cell and Genome Editing Technologies." Biol Pharm Bull **42**(3): 312-318.

Orthotopic liver transplantation, rather than drug therapy, is the major curative approach for various inherited metabolic disorders of the liver. However, the scarcity of donated livers is a serious problem. To resolve this, there is an urgent need for novel drugs to treat inherited metabolic disorders of the liver. This requirement, in turn, necessitates the establishment of suitable disease models for many inherited metabolic disorders of the liver that currently lack such models for drug development. Recent studies have shown that human induced pluripotent stem (iPS) cells generated from patients with inherited metabolic disorders of the liver are an ideal cell source for models that faithfully recapitulate the pathophysiology of inherited metabolic disorders of the liver. By using patient iPS cell-derived hepatocyte-like cells, drug efficacy evaluation and drug screening can be performed. In addition, genome editing technology has enabled us to generate functionally recovered patient iPS cell-derived hepatocyte-like cells in vitro. It is also possible to identify the genetic mutations responsible for undiagnosed liver diseases using iPS cell and genome editing technologies. Finally, a combination of exhaustive analysis, iPS cells, and genome editing technologies would be a powerful approach to accelerate the identification of novel genetic mutations responsible for undiagnosed liver diseases. In this review, we will discuss the usefulness of iPS cell and genome editing technologies in the field of inherited metabolic disorders of the liver, such as alpha-1 antitrypsin deficiency and familial hypercholesterolemia.

Yang, L., et al. (2013). "Optimization of scarless human stem cell genome editing." Nucleic Acids Res **41**(19): 9049-9061.

Efficient strategies for precise genome editing in human-induced pluripotent cells (hiPSCs) will enable sophisticated genome engineering for research and clinical purposes. The development of programmable sequence-specific nucleases such as Transcription Activator-Like Effectors Nucleases (TALENs) and Cas9-gRNA allows genetic modifications to be made more efficiently at targeted sites of interest. However, many opportunities remain to optimize these tools and to enlarge their spheres of application. We present several improvements: First, we developed functional re-coded TALEs (reTALEs), which not only enable simple one-pot TALE synthesis but also allow TALE-based applications to be performed using lentiviral vectors. We then compared genome-editing efficiencies in hiPSCs mediated by 15 pairs of reTALENs and Cas9-gRNA targeting CCR5 and optimized ssODN design in conjunction with both methods for introducing specific mutations. We found Cas9-gRNA achieved 7-8x higher non-homologous end joining efficiencies (3%) than reTALENs (0.4%) and moderately superior homology-directed repair efficiencies (1.0 versus 0.6%) when combined with ssODN donors in hiPSCs. Using the optimal design, we demonstrated a streamlined process to generated seamlessly genome corrected hiPSCs within 3 weeks.

Yin, Y., et al. (2015). "Opposing Roles for the lncRNA Haunt and Its Genomic Locus in Regulating HOXA Gene Activation during Embryonic Stem Cell Differentiation." Cell Stem Cell **16**(5): 504-516.

Long noncoding RNAs (lncRNAs) have been implicated in controlling various aspects of embryonic stem cell (ESC) biology, although the functions of specific lncRNAs, and the molecular mechanisms through which they act, remain unclear. Here, we demonstrate discrete and opposing roles for the lncRNA transcript Haunt and its genomic locus in regulating the HOXA gene cluster during ESC differentiation. Reducing or enhancing Haunt expression, with minimal disruption of the Haunt locus, led to upregulation or downregulation of HOXA genes, respectively. In contrast, increasingly large genomic deletions within the Haunt locus attenuated HOXA activation. The Haunt DNA locus contains potential enhancers of HOXA activation, whereas Haunt RNA acts to prevent aberrant HOXA expression. This work

reveals a multifaceted model of lncRNA-mediated transcriptional regulation of the HOXA cluster, with distinct roles for a lncRNA transcript and its genomic locus, while illustrating the power of rapid CRISPR/Cas9-based genome editing for assigning lncRNA functions.

Yu, J., et al. (2016). "CHES-1-like, the orthologue of a non-obstructive azoospermia-associated gene, blocks germline stem cell differentiation by upregulating Dpp expression in Drosophila testis." Oncotarget **7**(27): 42303-42313.

Azoospermia is a high risk factor for testicular germ cell tumors, whose underlying molecular mechanisms remain unknown. In a genome-wide association study to identify novel loci associated with human non-obstructive azoospermia (NOA), we uncovered a single nucleotide polymorphism (rs1887102, P=2.60 x10-7) in a human gene FOXN3. FOXN3 is an evolutionarily conserved gene. We used Drosophila melanogaster as a model system to test whether CHES-1-like, the Drosophila FOXN3 ortholog, is required for male fertility. CHES-1-like knockout flies are viable and fertile, and show no defects in spermatogenesis. However, ectopic expression of CHES-1-like in germ cells significantly reduced male fertility. With CHES-1-like overexpression, spermatogonia fail to differentiate after four rounds of mitotic division, but continue to divide to form tumor like structures. In these testes, expression levels of differentiation factor, Bam, were reduced, but the expression region of Bam was expanded. Further reduced Bam expression in CHES-1-like expressing testes exhibited enhanced tumor-like structure formation. The expression of daughters against dpp (dad), a downstream gene of dpp signaling, was upregulated by CHES-1-like expression in testes. We found that CHES-1-like could directly bind to the dpp promoter. We propose a model that CHES-1-like overexpression in germ cells activates dpp expression, inhibits spermatocyte differentiation, and finally leads to germ cell tumors.

Zhou, R., et al. (2018). "A homozygous p53 R282W mutant human embryonic stem cell line generated using TALEN-mediated precise gene editing." Stem Cell Res **27**: 131-135.

The tumor suppressor gene TP53 is the most frequently mutated gene in human cancers. Many hot-spot mutations of TP53 confer novel functions not found in wild-type p53 and contribute to tumor development and progression. We report on the generation of a H1 human embryonic stem cell line carrying a homozygous TP53 R282W mutation using TALEN-mediated genome editing. The generated cell line demonstrates normal karyotype, maintains a pluripotent state, and is capable of generating a teratoma in vivo containing tissues from all three germ layers.

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