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Stem Cell

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Stem Cell and (CRISPR)/Cas9 Research Literatures (3)

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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. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

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

Angelos, M. G. and D. S. Kaufman (2015). "Pluripotent stem cell applications for regenerative medicine." Curr Opin Organ Transplant **20**(6): 663-670.

PURPOSE OF REVIEW: In this review, we summarize the current status of clinical trials using therapeutic cells produced from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). We also discuss combined cell and gene therapy via correction of defined mutations in human pluripotent stem cells and provide commentary on key obstacles facing widescale clinical adoption of pluripotent stem cell-based therapy. RECENT FINDINGS: Initial data suggest that hESC/hiPSC-derived cell products used for retinal repair and spinal cord injury are safe for human use. Early-stage studies for treatment of cardiac injury and diabetes are also in progress. However, there remain key concerns regarding the safety and efficacy of these cells that need to be addressed in additional well designed clinical trials. Advances using the clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas9 gene-editing system offer an improved tool for more rapid and on-target gene correction of genetic diseases. Combined gene and cell therapy using human pluripotent stem cells may provide an additional curative approach for disabling or lethal genetic and degenerative diseases wherein there are currently limited therapeutic opportunities. SUMMARY: Human pluripotent stem cells are emerging as a promising tool to produce cells and tissues suitable for regenerative therapy for a variety of genetic and degenerative diseases.

Bezzerides, V. J., et al. (2016). "Modeling Inherited Arrhythmia Disorders Using Induced Pluripotent Stem Cell-Derived Cardiomyocytes." Circ J **81**(1): 12-21.

Inherited arrhythmia disorders (IADs) are a group of potentially lethal diseases that remain diagnostic and management challenges. Although the genetic basis for many of these disorders is well known, the pathogenicity of individual mutations and the resulting clinical outcomes are difficult to predict. Treatment options remain imperfect, and optimizing therapy for individual patients can be difficult. Recent advances in the derivation of induced pluripotent stem cells (iPSCs) from patients and creation of genetically engineered human models using CRISPR/Cas9 has the potential to dramatically advance translational arrhythmia research. In this review, we discuss the current state of modeling IADs using human iPSC-

derived cardiomyocytes. We also discuss current limitations and areas for further study.

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.

Borestrom, C., et al. (2018). "A CRISP(e)R view on kidney organoids allows generation of an induced pluripotent stem cell-derived kidney model for drug discovery." Kidney Int.

Development of physiologically relevant cellular models with strong translatability to human pathophysiology is critical for identification and validation of novel therapeutic targets. Herein we describe a detailed protocol for generation of an advanced 3-dimensional kidney cellular model using induced pluripotent stem cells, where differentiation and maturation of kidney progenitors and podocytes can be monitored in live cells due to CRISPR/Cas9-mediated fluorescent tagging of kidney lineage markers (SIX2 and NPHS1). Utilizing these cell lines, we have refined the previously published procedures to generate a new, higher throughput protocol suitable for drug discovery. Using paraffin-embedded sectioning and whole-mount immunostaining, we demonstrated that organoids grown in suspension culture express key markers of kidney biology (WT1, ECAD, LTL, nephrin) and vasculature (CD31) within renal cortical structures with microvilli, tight junctions and podocyte foot processes visualized by electron microscopy. Additionally, the organoids resemble the adult kidney transcriptomics profile, thereby strengthening the translatability of our in vitro model. Thus, development of human nephron-like structures in vitro fills a major gap in our ability to assess the effect of potential treatment on key kidney structures, opening up a wide range of possibilities to improve clinical translation.

Bulstrode, H., et al. (2017). "Elevated FOXG1 and SOX2 in glioblastoma enforces neural stem cell identity through transcriptional control of cell cycle and epigenetic regulators." Genes Dev **31**(8): 757-773.

Glioblastoma multiforme (GBM) is an aggressive brain tumor driven by cells with hallmarks of neural stem (NS) cells. GBM stem cells frequently express high levels of the transcription factors FOXG1 and SOX2. Here we show that increased expression of these factors restricts astrocyte differentiation and can trigger dedifferentiation to a proliferative NS cell state. Transcriptional targets include cell cycle and epigenetic regulators (e.g., Foxo3, Plk1, Mycn, Dnmt1, Dnmt3b, and Tet3). Foxo3 is a critical repressed downstream effector that is controlled via a conserved FOXG1/SOX2-bound cis-regulatory element. Foxo3 loss, combined with exposure to the DNA methylation inhibitor 5-azacytidine, enforces astrocyte dedifferentiation. DNA methylation profiling in differentiating astrocytes identifies changes at multiple polycomb targets, including the promoter of Foxo3 In patient-derived GBM stem cells, CRISPR/Cas9 deletion of FOXG1 does not impact proliferation in vitro; however, upon transplantation in vivo, FOXG1-null cells display increased astrocyte differentiation and up-regulate FOXO3. In contrast, SOX2 ablation attenuates proliferation, and mutant cells cannot be expanded in vitro. Thus, FOXG1 and SOX2 operate in complementary but distinct roles to fuel unconstrained self-renewal in GBM stem cells via transcriptional control of core cell cycle and epigenetic regulators.

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.

Ceholski, D. K., et al. (2018). "Functional and transcriptomic insights into pathogenesis of R9C phospholamban mutation using human induced pluripotent stem cell-derived cardiomyocytes." J Mol Cell Cardiol **119**: 147-154.

Dilated cardiomyopathy (DCM) can be caused by mutations in the cardiac protein phospholamban (PLN). We used CRISPR/Cas9 to insert the R9C PLN mutation at its endogenous locus into a human induced pluripotent stem cell (hiPSC) line from an individual with no cardiovascular disease. R9C PLN hiPSC-CMs display a blunted beta-agonist response and defective calcium handling. In 3D human engineered cardiac tissues (hECTs), a blunted lusitropic response to beta-adrenergic stimulation was observed with R9C PLN. hiPSC-CMs harboring the R9C PLN mutation showed activation of a hypertrophic phenotype, as evidenced by expression of hypertrophic markers and increased cell size and capacitance of cardiomyocytes. RNA-seq suggests that R9C PLN results in an altered metabolic state and profibrotic signaling, which was confirmed by gene expression analysis and picrosirius staining of R9C PLN hECTs. The expression of several miRNAs involved in fibrosis, hypertrophy, and cardiac metabolism were also perturbed in R9C PLN hiPSC-CMs. This study contributes to better understanding of the pathogenic mechanisms of the hereditary R9C PLN mutation in the context of human cardiomyocytes.

Chen, K. Y., et al. (2017). "A Notch positive feedback in the intestinal stem cell niche is essential for stem cell self-renewal." Mol Syst Biol **13**(4): 927.

The intestinal epithelium is the fastest regenerative tissue in the body, fueled by fast-cycling stem cells. The number and identity of these dividing and migrating stem cells are maintained by a mosaic pattern at the base of the crypt. How the underlying regulatory scheme manages this dynamic stem cell niche is not entirely clear. We stimulated intestinal organoids with Notch ligands and inhibitors and discovered that intestinal stem cells employ a positive feedback mechanism via direct Notch binding to the second intron of the Notch1 gene. Inactivation of the positive feedback by CRISPR/Cas9 mutation of the binding sequence alters the mosaic stem cell niche pattern and hinders regeneration in organoids. Dynamical system analysis and agent-based multiscale stochastic modeling suggest that the positive feedback enhances the robustness of Notch-mediated niche patterning. This study highlights the importance of feedback mechanisms in spatiotemporal control of the stem cell niche.

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.

Choi, K. A., et al. (2018). "Stem cell transplantation for Huntington's diseases." Methods **133**: 104-112.

Therapeutic approaches based on stem cells have received considerable attention as potential treatments for Huntington's disease (HD), which is a fatal, inherited neurodegenerative disorder, caused by progressive loss of GABAergic medium spiny neurons (MSNs) in the striatum of the forebrain. Transplantation of stem cells or their derivatives in animal models of HD, efficiently improved functions by replacing the damaged or lost neurons. In particular, neural stem cells (NSCs) for HD treatments have been developed from various sources, such as the brain itself, the pluripotent stem cells (PSCs), and the somatic cells of the HD patients. However, the brain-derived NSCs are difficult to obtain, and the PSCs have to be differentiated into a population of the desired neuronal cells that may cause a risk of tumor formation after transplantation. In contrast, induced NSCs, derived from somatic cells as a new stem cell source for transplantation, are less likely to form tumors. Given that the stem cell transplantation strategy for treatment of HD, as a genetic disease, is to replace the dysfunctional or lost neurons, the correction of mutant genes containing the expanded CAG repeats is essential. In this review, we will describe the methods for obtaining the optimal NSCs for transplantation-based HD treatment and the differentiation conditions for the functional GABAergic MSNs as therapeutic cells. Also, we will discuss the valuable gene correction of the disease stem cells by the CRISPR/Cas9 system for HD treatment.

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.

Chu, L. F., et al. (2016). "Single-cell RNA-seq reveals novel regulators of human embryonic stem cell differentiation to definitive endoderm." Genome Biol **17**(1): 173.

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

Chung, K. M., et al. (2016). "Mediation of Autophagic Cell Death by Type 3 Ryanodine Receptor (RyR3) in Adult Hippocampal Neural Stem Cells." Front Cell Neurosci **10**: 116.

Cytoplasmic Ca(2+) actively engages in diverse intracellular processes from protein synthesis, folding and trafficking to cell survival and death. Dysregulation of intracellular Ca(2+) levels is observed in various neuropathological states including Alzheimer's and Parkinson's diseases. Ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP3Rs), the main Ca(2+) release channels located in endoplasmic reticulum (ER) membranes, are known to direct various cellular events such as

autophagy and apoptosis. Here we investigated the intracellular Ca(2+)-mediated regulation of survival and death of adult hippocampal neural stem (HCN) cells utilizing an insulin withdrawal model of autophagic cell death (ACD). Despite comparable expression levels of RyR and IP3R transcripts in HCN cells at normal state, the expression levels of RyRs-especially RyR3-were markedly upregulated upon insulin withdrawal. While treatment with the RyR agonist caffeine significantly promoted the autophagic death of insulin-deficient HCN cells, treatment with its inhibitor dantrolene prevented the induction of autophagy following insulin withdrawal. Furthermore, CRISPR/Cas9-mediated knockout of the RyR3 gene abolished ACD of HCN cells. This study delineates a distinct, RyR3-mediated ER Ca(2+) regulation of autophagy and programmed cell death in neural stem cells. Our findings provide novel insights into the critical, yet understudied mechanisms underlying the regulatory function of ER Ca(2+) in neural stem cell biology.

Cruz-Molina, S., et al. (2017). "PRC2 Facilitates the Regulatory Topology Required for Poised Enhancer Function during Pluripotent Stem Cell Differentiation." Cell Stem Cell **20**(5): 689-705 e689.

Poised enhancers marked by H3K27me3 in pluripotent stem cells have been implicated in the establishment of somatic expression programs during embryonic stem cell (ESC) differentiation. However, the functional relevance and mechanism of action of poised enhancers remain unknown. Using CRISPR/Cas9 technology to engineer precise genetic deletions, we demonstrate that poised enhancers are necessary for the induction of major anterior neural regulators. Interestingly, circularized chromosome conformation capture sequencing (4C-seq) shows that poised enhancers already establish physical interactions with their target genes in ESCs in a polycomb repressive complex 2 (PRC2)-dependent manner. Loss of PRC2 does not activate poised enhancers or induce their putative target genes in undifferentiated ESCs; however, loss of PRC2 in differentiating ESCs severely and specifically compromises the induction of major anterior neural genes representing poised enhancer targets. Overall, our work illuminates an unexpected function for polycomb proteins in facilitating neural induction by endowing major anterior neural loci with a permissive regulatory topology.

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.

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.

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.

Guo, D., et al. (2016). "Generation of an Abcc8 heterozygous mutation human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **17**(3): 670-672.

The gene of ATP-binding cassette subfamily C member 8 (Abcc8) is cytogenetically located at 11p15.1 and encodes the sulfonylurea receptor (SUR1). SUR1 is a subunit of ATP-sensitive potassium channel (KAPT) in the beta-cell regulating insulin secretion. Mutations of ABCC8 are responsible for congenital hyperinsulinism (CHI). Here we reported that an Abcc8 heterozygous mutant cell line was generated by CRISPR/Cas9 technique with 1bp insertion resulting in abnormal splicing on human embryonic stem cell line H1. The phenotypic characteristics of this cell line reveal defective KATP channel and diazoxide-responsive that provides ideal model for molecular pathology research and drug screening for CHI.

Guo, D., et al. (2016). "Generation of an Abcc8 homozygous mutation human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **17**(3): 640-642.

The gene of ATP-binding cassette subfamily C member 8 (Abcc8) is cytogenetically located at 11p15.1 and encodes the sulfonylurea receptor (SUR1). SUR1 is a subunit of ATP-sensitive potassium channel (KAPT) in the beta-cell regulating insulin secretion. Mutations of ABCC8 are responsible for congenital hyperinsulinism (CHI). Here we generated an Abcc8 homozygous mutant cell line by CRISPR/Cas9 technique with 22bp deletion resulting in abnormal splicing on human embryonic stem cell line H1. The phenotypic characteristics of this cell line reveal defective KATP channel and diazoxide-unresponsive that provides an ideal model for molecular pathology research and drug screening for CHI.

Guo, H., et al. (2017). "PBX3 is essential for leukemia stem cell maintenance in MLL-rearranged leukemia." Int J Cancer **141**(2): 324-335.

Interaction of HOXA9/MEIS1/PBX3 is responsible for hematopoietic system transformation in MLL-rearranged (MLL-r) leukemia. Of these genes, HOXA9 has been shown to be critical for leukemia cell survival, while MEIS1 has been identified as an essential regulator for leukemia stem cell (LSC) maintenance. Although significantly high expression of PBX3 was observed in clinical acute myeloid leukemia (AML) samples, the individual role of PBX3 in leukemia development is still largely unknown. In this study, we explored the specific role of PBX3 and its associated regulatory network in leukemia progression. By analyzing the clinical database, we found that the high expression of PBX3 is significantly correlated with a poor prognosis in AML patients. ChIP-Seq/qPCR analysis in MLL-r mouse models revealed aberrant epigenetic modifications with increased H3K79me2, and decreased H3K9me3 and H3K27me3 levels in LSCs, which may account for the high expression levels of Pbx3. To further examine the role of Pbx3 in AML maintenance and progression, we used the CRISPR/Cas9 system to delete Pbx3 in leukemic cells in the MLL-AF9 induced AML mouse model. We found that Pbx3 deletion significantly prolonged the survival of leukemic mice and decreased the leukemia burden by decreasing the capacity of LSCs and promoting LSC apoptosis. In conclusion, we found that PBX3 is epigenetically aberrant in the LSCs of MLL-r AML and is essential for leukemia development. Significantly, the differential expression of PBX3 in normal and malignant hematopoietic cells suggests PBX3 as a potential prognostic marker and therapeutic target for MLL-r leukemia.

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.

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.

Jayavaradhan, R., et al. (2018). "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.

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 shows its applicability in evaluating the different DDR outcomes utilized by human hematopoietic cell lines and primary human HSPC.

Jin, L. F. and J. S. Li (2016). "Generation of genetically modified mice using CRISPR/Cas9 and haploid embryonic stem cell systems." Dongwuxue Yanjiu **37**(4): 205-213.

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

Kim, H., et al. (2018). "Generation of a PXR reporter human induced pluripotent stem cell line (PXR-mCherry hiPSC) using the CRISPR/Cas9 system." Stem Cell Res **26**: 72-75.

Pregnane X receptor (PXR) is a key nuclear receptor that mediates drug metabolism and stimulates hepatocyte proliferation. However, the lack of PXR expression in human pluripotent stem cell-derived hepatocytes limits their application for drug screening and toxicity testing. Here, we generated a PXR-

mCherry reporter human induced pluripotent stem cell (hiPSC) line using the CRISPR/Cas9 system. PXR-mCherry hiPSCs were pluripotent and had differentiation potential and a normal karyotype. This cell line is an important tool for identifying factors that increase PXR-mediated drug metabolism and hepatocyte proliferation.

Kim, H. S., et al. (2017). "Endothelial-derived interleukin-6 induces cancer stem cell motility by generating a chemotactic gradient towards blood vessels." Oncotarget **8**(59): 100339-100352.

Recent evidence suggests that the metastatic spread of head and neck squamous cell carcinomas (HNSCC) requires the function of cancer stem cells endowed with multipotency, self-renewal, and high tumorigenic potential. We demonstrated that cancer stem cells reside in perivascular niches and are characterized by high aldehyde dehydrogenase (ALDH) activity and high CD44 expression (ALDH(high)CD44(high)) in HNSCC. Here, we hypothesize that endothelial cell-secreted interleukin-6 (IL-6) contributes to tumor progression by enhancing the migratory phenotype and survival of cancer stem cells. Analysis of tissue microarrays generated from the invasive fronts of 77 HNSCC patients followed-up for up to 11 years revealed that high expression of IL-6 receptor (IL-6R) ($p=0.0217$) or co-receptor gp130 ($p=0.0422$) correlates with low HNSCC patient survival. We observed that endothelial cell-secreted factors induce epithelial to mesenchymal transition (EMT) and enhance invasive capacity of HNSCC cancer stem cells. Conditioned medium from CRISPR/Cas9-mediated IL-6 knockout primary human endothelial cells is less chemotactic for cancer stem cells in a microfluidics-based system than medium from control endothelial cells ($p<0.05$). Blockade of the IL-6 pathway with a humanized anti-IL-6R antibody (tocilizumab) inhibited endothelial cell-induced motility in vitro and decreased the fraction of cancer stem cells in vivo. Notably, xenograft HNSCC tumors vascularized with IL-6-knockout endothelial cells exhibited slower tumor growth and smaller cancer stem cell fraction. These findings demonstrate that endothelial cell-secreted IL-6 enhances the motility and survival of highly tumorigenic cancer stem cells, suggesting that endothelial cells can create a chemotactic gradient that enables the movement of carcinoma cells towards blood vessels.

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.

Lai, F. P., et al. (2017). "Correction of Hirschsprung-Associated Mutations in Human Induced Pluripotent Stem Cells Via Clustered Regularly Interspaced Short Palindromic Repeats/Cas9, Restores Neural Crest Cell Function." Gastroenterology **153**(1): 139-153 e138.

BACKGROUND & AIMS: Hirschsprung disease is caused by failure of enteric neural crest cells (ENCCs) to fully colonize the bowel, leading to bowel obstruction and megacolon. Heterozygous mutations in the coding region of the RET gene cause a severe form of Hirschsprung disease (total colonic aganglionosis). However, 80% of HSCR patients have short-segment Hirschsprung disease (S-HSCR), which has not been associated with genetic factors. We sought to identify mutations associated with S-HSCR, and used the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing system to determine how mutations affect ENCC function. METHODS: We created induced pluripotent stem cell (iPSC) lines from 1 patient with total colonic aganglionosis (with the G731del mutation in RET) and from 2 patients with S-HSCR (without a RET mutation), as well as RET(+/-) and RET(-/-) iPSCs. IMR90-iPSC cells were used as the control cell line. Migration and differentiation capacities of iPSC-derived ENCCs were analyzed in differentiation and migration assays. We searched for mutation(s) associated with S-HSCR by combining genetic and transcriptome data from patient blood- and iPSC-

derived ENCCs, respectively. Mutations in the iPSCs were corrected using the CRISPR/Cas9 system. RESULTS: ENCCs derived from all iPSC lines, but not control iPSCs, had defects in migration and neuronal lineage differentiation. RET mutations were associated with differentiation and migration defects of ENCCs in vitro. Genetic and transcriptome analyses associated a mutation in the vinculin gene (VCL M209L) with S-HSCR. CRISPR/Cas9 correction of the RET G731del and VCL M209L mutations in iPSCs restored the differentiation and migration capacities of ENCCs. CONCLUSIONS: We identified mutations in VCL associated with S-HSCR. Correction of this mutation in iPSC using CRISPR/Cas9 editing, as well as the RET G731del mutation that causes Hirschsprung disease with total colonic aganglionosis, restored ENCC function. Our study demonstrates how human iPSCs can be used to identify disease-associated mutations and determine how they affect cell functions and contribute to pathogenesis.

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.

Li, Y., et al. (2017). "Transcriptome analysis reveals determinant stages controlling human embryonic stem cell commitment to neuronal cells." J Biol Chem **292**(48): 19590-19604.

Proper neural commitment is essential for ensuring the appropriate development of the human brain and for preventing neurodevelopmental diseases such as autism spectrum disorders, schizophrenia, and intellectual disorders. However, the molecular mechanisms underlying the neural commitment in humans remain elusive. Here, we report the establishment of a neural differentiation system based on human embryonic stem cells (hESCs) and on comprehensive RNA sequencing analysis of transcriptome dynamics during early hESC differentiation. Using weighted gene co-expression network analysis, we reveal that the hESC neurodevelopmental trajectory has five stages: pluripotency (day 0); differentiation initiation (days 2, 4, and 6); neural commitment (days 8-10); neural progenitor cell proliferation (days 12, 14, and 16); and neuronal differentiation (days 18, 20, and 22). These

stages were characterized by unique module genes, which may recapitulate the early human cortical development. Moreover, a comparison of our RNA-sequencing data with several other transcriptome profiling datasets from mice and humans indicated that Module 3 associated with the day 8-10 stage is a critical window of fate switch from the pluripotency to the neural lineage. Interestingly, at this stage, no key extrinsic signals were activated. In contrast, using CRISPR/Cas9-mediated gene knockouts, we also found that intrinsic hub transcription factors, including the schizophrenia-associated SIX3 gene and septo-optic dysplasia-related HESX1 gene, are required to program hESC neural determination. Our results improve the understanding of the mechanism of neural commitment in the human brain and may help elucidate the etiology of human mental disorders and advance therapies for managing these conditions.

Ling, K., et al. (2018). "Nanog interaction with the androgen receptor signaling axis induce ovarian cancer stem cell regulation: studies based on the CRISPR/Cas9 system." J Ovarian Res **11**(1): 36.

BACKGROUND: Ovarian cancer stem cells (OCSCs) contribute to the poor prognosis of ovarian cancer. Involvement of the androgen receptor (AR) in the malignant behaviors of other tumors has been reported. However, whether AR associates with Nanog (a stem cell marker) and participates in OCSC functions remain unclear. In this study, we investigated the interaction of Nanog with AR and examined whether this interaction induced stem-like properties in ovarian cancer cells. METHODS: AR and Nanog expression in ovarian tumors was evaluated. Using the CRISPR/Cas9 system, we constructed a Nanog green fluorescent protein (GFP) marker cell model to investigate the expression and co-localization of Nanog and AR. Then, we examined the effect of androgen on the Nanog promoter in ovarian cancer cell lines (A2780 and SKOV3). After androgen or anti-androgen treatment, cell proliferation, migration, sphere formation, colony formation and tumorigenesis were assessed in vitro and in vivo. RESULTS: Both AR and Nanog expression were obviously high in ovarian tumors. Our results showed that Nanog expression was correlated with AR expression. The androgen 5alpha-dihydrotestosterone (DHT) activated Nanog promoter transcription. Meanwhile, Nanog GFP-positive cells treated with DHT exhibited higher levels of proliferation, migration, sphere formation and colony formation. We also observed that the tumorigenesis of Nanog GFP-positive cells was significantly higher than that of the GFP-negative cells. Xenografts of Nanog GFP-positive cells showed significant differences when treated with androgen or anti-androgen drugs in vivo. CONCLUSIONS: The interaction of Nanog with the AR signaling axis might induce or contribute to OCSC regulation. In addition, androgen might promote stemness characteristics in ovarian cancer cells by activating the Nanog promoter. This finding merits further study because it may provide a new understanding of OCSC regulation from a hormone perspective and lead to the reevaluation of stem cell therapy for ovarian cancer.

Liu, Y., et al. (2017). "Lack of MTTP Activity in Pluripotent Stem Cell-Derived Hepatocytes and Cardiomyocytes Abolishes apoB Secretion and Increases Cell Stress." Cell Rep **19**(7): 1456-1466.

Abetalipoproteinemia (ABL) is an inherited disorder of lipoprotein metabolism resulting from mutations in microsomal triglyceride transfer protein (MTTP). In addition to expression in the liver and intestine, MTTP is expressed in cardiomyocytes, and cardiomyopathy has been reported in several ABL cases. Using induced pluripotent stem cells (iPSCs) generated from an ABL patient homozygous for a missense mutation (MTTP(R46G)), we show that human hepatocytes and cardiomyocytes exhibit defects associated with ABL disease, including loss of apolipoprotein B (apoB) secretion and intracellular accumulation of lipids. MTTP(R46G) iPSC-derived cardiomyocytes failed to secrete apoB, accumulated intracellular lipids, and displayed increased cell death, suggesting intrinsic defects in lipid metabolism due to loss of MTTP function. Importantly, these phenotypes were reversed after the correction of the MTTP(R46G) mutation by CRISPR/Cas9 gene editing. Together, these data reveal clear cellular defects in iPSC-derived hepatocytes and cardiomyocytes lacking MTTP activity, including a cardiomyocyte-specific regulated stress response to elevated lipids.

Liu, Y., et al. (2017). "Generation of two MEN1 knockout lines from a human embryonic stem cell line." Stem Cell Res **24**: 169-173.

The MEN1 gene is cytogenetically located at 11q13.1 and encodes the nuclear protein menin, which is involved in cell proliferation, apoptosis, differentiation, and metabolism. Here, we generated two MEN1 knockout human embryonic stem cell lines, WAe001-A-4 and WAe001-A-5, by targeting exon-2 and exon-9 of MEN1 using the CRISPR/Cas9 technique. These cell lines maintained their pluripotency, in vitro differentiation potential, normal morphology, and karyotype. These human MEN1-mutated cell lines not only enlarge the pool of lab resources but also provide ideal models to dissect the detailed physio-pathological roles of the menin protein.

Liu, Y., et al. (2017). "Generation of three miR-122 knockout lines from a human embryonic stem cell line." Stem Cell Res **24**: 164-168.

miR-122 is the most abundant miRNA in the human liver, accounting for 52% of the entire hepatic miRNome. Previous studies have demonstrated that miR-122 plays key roles in hepatocyte growth, metabolism, and homeostasis. Here, we created three miR-122 knockout human embryonic stem cell line lines, WAe001-A-7, WAe001-A-8, and WAe001-A-9, using the CRISPR/Cas9 technique. These mutated cell lines retained their pluripotency, in vitro differentiation potential, normal morphology, and karyotype.

Lu, R., et al. (2016). "Epigenetic Perturbations by Arg882-Mutated DNMT3A Potentiate Aberrant Stem Cell Gene-Expression Program and Acute Leukemia Development." Cancer Cell **30**(1): 92-107.

DNA methyltransferase 3A (DNMT3A) is frequently mutated in hematological cancers; however, the underlying oncogenic mechanism remains elusive. Here, we report that the DNMT3A mutational hotspot at Arg882 (DNMT3A(R882H)) cooperates with NRAS mutation to transform hematopoietic stem/progenitor cells and induce acute leukemia development. Mechanistically, DNMT3A(R882H) directly binds to and potentiates transactivation of stemness genes critical for leukemogenicity including Meis1, Mn1, and Hoxa gene cluster. DNMT3A(R882H) induces focal epigenetic alterations, including CpG hypomethylation and concurrent gain of active histone modifications, at cis-regulatory elements such as enhancers to facilitate gene transcription. CRISPR/Cas9-mediated ablation of a putative Meis1 enhancer carrying DNMT3A(R882H)-induced DNA hypomethylation impairs Meis1 expression. Importantly, DNMT3A(R882H)-induced gene-expression programs can be repressed through Dot1l inhibition, providing an attractive therapeutic strategy for DNMT3A-mutated leukemias.

Luanpitpong, S., et al. (2018). "Reactive oxygen species mediate cancer stem-like cells and determine bortezomib sensitivity via Mcl-1 and Zeb-1 in mantle cell lymphoma." Biochim Biophys Acta Mol Basis Dis **1864**(11): 3739-3753.

Mantle cell lymphoma (MCL) is an aggressive, incurable non-Hodgkin B-cell lymphoma with good initial response to therapy then subsequently relapse. Cancer stem cells (CSCs) are considered to be an underlying cause of these inevitable drug resistance and tumor regrowth, but how CSCs are regulated is largely unknown. We demonstrate here for the first time the existence of CSC-like subpopulations that are modulated by reactive oxygen species (ROS) in MCL cell lines and patient-derived primary cells in an inverse correlation with bortezomib (BTZ) sensitivity. Using various known donors and inhibitors of cellular superoxide (O2(-)), hydrogen peroxide (H2O2) and hydroxyl radical (OH), we unveil their distinct roles in the regulation of CSC-like subpopulations and thus MCL response to BTZ. O2(-) inhibits CSC-like cells and sensitizes BTZ-induced apoptosis, whereas H2O2 conversely enriches CSC-like cells and protects against apoptosis and OH has minimal effects. We further observed that an anti-apoptotic Mcl-1 and a transcription factor Zeb-1 are favorable targets of O2(-) and H2O2, respectively. Using small molecule inhibition, ectopic expression and CRISPR/Cas9-mediated gene manipulation, we verified the roles of Mcl-1 and Zeb-1 in CSC and apoptosis regulation by O2(-) and H2O2. Our findings provide a novel mechanistic insight into the significance of redox status of MCL cells in determining their drug response via CSC-like subpopulations, which are imperative to a better understanding of therapeutic resistance and relapse.

MacLean, G. A., et al. (2018). "Downregulation of Endothelin Receptor B Contributes to Defective B Cell Lymphopoiesis in Trisomy 21 Pluripotent Stem Cells." Sci Rep **8**(1): 8001.

Individuals with Trisomy 21 (T21) exhibit numerous hematological abnormalities, including reductions in numbers of circulating B and T lymphocytes. To elucidate molecular mechanisms underlying these phenotypes, we differentiated human isogenic disomic and trisomic pluripotent cells, and observed that trisomic cells showed defects in B cell, but not T cell differentiation. Global gene expression of differentiated, trisomic B cells revealed reduced expression of genes encoding endothelin signaling components, namely the Endothelin Receptor B (EDNRB), and its ligand Endothelin1 (EDN1). Depletion of EDNRB mRNA in cord blood-derived CD34(+) cells led to defective B cell differentiation, supporting a hypothesis that low EDNRB expression in T21 contributes to intrinsic lymphoid defects. Further evidence for the role of the EDNRB pathway in B cell differentiation was obtained through CRISPR/Cas9 gene targeting in disomic and trisomic iPS cells. Knockout of EDNRB in both cell backgrounds reduced the capacity for B cell differentiation. Collectively, this work identifies downregulation of EDNRB as a causative factor for impaired B lymphocyte generation in trisomic cells, which may contribute to defects in immune function associated with T21. Furthermore, a novel role for endothelin signaling in regulation of B cell development has been identified.

Mahony, C. B., et al. (2016). "tfec controls the hematopoietic stem cell vascular niche during zebrafish embryogenesis." Blood **128**(10): 1336-1345.

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

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.

Millette, K. and S. Georgia (2017). "Gene Editing and Human Pluripotent Stem Cells: Tools for Advancing Diabetes Disease Modeling and Beta-Cell Development." Curr Diab Rep **17**(11): 116.

PURPOSE OF REVIEW: This review will focus on the multiple approaches to gene editing and address the potential use of genetically modified human pluripotent stem cell-derived beta cells (SC-beta) as a tool to study human beta-cell development and model their function in diabetes. We will explore how new variations of CRISPR/Cas9 gene editing may accelerate our understanding of beta-cell developmental biology, elucidate novel mechanisms that establish and regulate beta-cell function, and assist in pioneering new therapeutic modalities for treating diabetes. RECENT FINDINGS: Improvements in CRISPR/Cas9 target specificity and homology-directed recombination continue to advance its use in engineering stem cells to model and potentially treat disease. We will review how CRISPR/Cas9 gene editing is informing our understanding of beta-cell development and expanding the therapeutic possibilities for treating diabetes and other diseases. Here we focus on the emerging use of gene editing technology, specifically CRISPR/Cas9, as a means of manipulating human gene expression to gain novel insights into the roles of key factors in beta-cell development and function. Taken together, the combined use of SC-beta cells and CRISPR/Cas9 gene editing will shed new light on human beta-cell development and function and accelerate our progress towards developing new therapies for patients with diabetes.

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.

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.

Murray, A., et al. (2016). "Plet1 is an epigenetically regulated cell surface protein that provides essential cues to direct trophoblast stem cell differentiation." Sci Rep **6**: 25112.

Gene loci that are hypermethylated and repressed in embryonic (ESCs) but hypomethylated and expressed in trophoblast (TSCs) stem cells are very rare and may have particularly important roles in early developmental cell fate decisions, as previously shown for Elf5. Here, we assessed another member of this

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

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.

Quintero, C. M., et al. (2018). "CARM1 (PRMT4) Acts as a Transcriptional Coactivator during Retinoic Acid-Induced Embryonic Stem Cell Differentiation." J Mol Biol **430**(21): 4168-4182.

Activation of the retinoic acid (RA) signaling pathway is important for controlling embryonic stem cell differentiation and development. Modulation of this pathway occurs through the recruitment of different epigenetic regulators at the retinoic acid receptors (RARs) located at RA-responsive elements and/or RA-responsive regions of RA-regulated genes. Coactivator-associated arginine methyltransferase 1 (CARM1, PRMT4) is a protein arginine methyltransferase that also functions as a transcriptional coactivator. Previous studies highlight CARM1's importance in the differentiation of different cell types. We address CARM1 function during RA-induced differentiation of murine embryonic stem cells (mESCs) using shRNA lentiviral transduction and CRISPR/Cas9 technology to deplete CARM1 in mESCs. We identify CARM1 as a novel transcriptional coactivator required for the RA-associated decrease in Rex1 (Zfp42) and for the RA induction of a subset of RA-regulated genes, including CRABP2 and NR2F1 (Coup-TF1). Furthermore, CARM1 is required for mESCs to differentiate into extraembryonic endoderm in response to RA. We next characterize the epigenetic mechanisms that contribute to RA-induced transcriptional activation of CRABP2 and NR2F1 in mESCs and show for the first time that CARM1 is required for this activation. Collectively, our data demonstrate that CARM1 is required for transcriptional activation of a subset of RA target genes, and we uncover changes in the recruitment of Suz12 and the epigenetic H3K27me3 and H3K27ac marks at gene regulatory regions for CRABP2 and NR2F1 during RA-induced differentiation.

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.

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.

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.

Schrenk-Siemens, K., et al. (2015). "PIEZO2 is required for mechanotransduction in human stem cell-derived touch receptors." Nat Neurosci **18**(1): 10-16.

Human sensory neurons are inaccessible for functional examination, and thus little is known about the mechanisms mediating touch sensation in humans. Here we demonstrate that the mechanosensitivity of human embryonic stem (hES) cell-derived touch receptors depends on PIEZO2. To recapitulate sensory neuron development in vitro, we established a multistep differentiation protocol and generated sensory neurons via the intermediate production of neural crest cells derived from hES cells or human induced pluripotent stem (hiPS) cells. The generated neurons express a distinct set of touch receptor-specific genes and convert mechanical stimuli into electrical signals, their most salient characteristic in vivo. Strikingly, mechanosensitivity is lost after CRISPR/Cas9-mediated PIEZO2 gene deletion. Our work establishes a model system that resembles human touch receptors, which may facilitate mechanistic analysis of other sensory subtypes and provide insight into developmental programs underlying sensory neuron diversity.

Schwach, V., et al. (2017). "A COUP-TFII Human Embryonic Stem Cell Reporter Line to Identify and Select Atrial Cardiomyocytes." Stem Cell Reports **9**(6): 1765-1779.

Reporter cell lines have already proven valuable in identifying, tracking, and purifying cardiac subtypes and progenitors during differentiation of human pluripotent stem cells (hPSCs). We previously showed that chick ovalbumin upstream promoter transcription factor II (COUP-TFII) is highly enriched in human atrial cardiomyocytes (CMs), but not ventricular. Here, we targeted mCherry to the COUP-TFII genomic locus in hPSCs expressing GFP from the NKX2.5 locus. This dual atrial NKX2.5(EGFP/+)-COUP-TFII(mCherry/+) reporter line allowed identification and selection of GFP(+) (G(+))/mCherry(+) (M(+)) CMs following cardiac differentiation. These cells exhibited transcriptional and functional properties of atrial CMs, whereas G(+)/M(-) CMs displayed ventricular characteristics. Via CRISPR/Cas9-mediated knockout, we demonstrated that COUP-TFII is not required for atrial specification in hPSCs. This new tool allowed selection of human atrial and ventricular CMs from mixed populations, of relevance for studying cardiac

specification, developing human atrial disease models, and examining distinct effects of drugs on the atrium versus ventricle.

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.

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.

Shetty, D. K. and M. S. Inamdar (2016). "Generation of a heterozygous knockout human embryonic stem cell line for the OCIAD1 locus using CRISPR/CAS9 mediated targeting: BJNhem20-OCIAD1-CRISPR-20." Stem Cell Res **16**(2): 207-209.

Ovarian carcinoma immuno-reactive antigen domain containing 1(OCIAD1) single copy was knocked out generating an OCIAD1 heterozygous knockout human embryonic stem line named BJNhem20-OCIAD1-CRISPR-20. The line was generated using CRISPR-Cas9D10A double nickase knockout strategy (Mali et al., 2013).

Shetty, D. K. and M. S. Inamdar (2016). "Generation of a heterozygous knockout human embryonic stem cell line for the OCIAD1 locus using CRISPR/CAS9 mediated targeting: BJNhem20-OCIAD1-CRISPR-39." Stem Cell Res **16**(2): 308-310.

Ovarian carcinoma immuno-reactive antigen domain containing 1 (OCIAD1) single copy was knocked out generating an OCIAD1 heterozygous knockout human embryonic stem line named BJNhem20-OCIAD1-CRISPR-39. The line was generated using CRISPR-Cas9D10A double nickase knockout strategy (Mali et al., 2013).

Sweeney, C. L., et al. (2017). "CRISPR-Mediated Knockout of Cybb in NSG Mice Establishes a Model of Chronic Granulomatous Disease for Human Stem-Cell Gene Therapy Transplants." Hum Gene Ther **28**(7): 565-575.

Chronic granulomatous disease (CGD) is characterized by defects in the production of microbicidal reactive oxygen species (ROS) by phagocytes. Testing of gene and cell therapies for the treatment of CGD in human hematopoietic cells requires preclinical transplant models. The use of the lymphocyte-deficient NOD.Cg-Prkdc(scid) Il2rg(tm1Wjl/)SzJ (NSG) mouse strain for human hematopoietic cell xenografts to test CGD therapies is complicated by the presence of functional mouse granulocytes capable of producing ROS for subsequent bacterial and fungal killing. To establish a phagocyte-defective mouse model of X-linked CGD (X-CGD) in NSG mice, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 was utilized for targeted knockout of mouse Cybb on the X-chromosome by microinjection of NSG mouse zygotes with Cas9 mRNA and CRISPR single-guide RNA targeting Cybb exon 1 or exon 3. This resulted in a high incidence of indel formation at the CRISPR target site, with all mice exhibiting deletions in at least one

Cybb allele based on sequence analysis of tail snip DNA. A female mouse heterozygous for a 235-bp deletion in Cybb exon 1 was bred to an NSG male to establish the X-CGD NSG mouse strain, NSG.Cybb[KO]. Resulting male offspring with the 235 bp deletion were found to be defective for production of ROS by neutrophils and other phagocytes, and demonstrated increased susceptibility to spontaneous bacterial and fungal infections with granulomatous inflammation. The establishment of the phagocyte-defective NSG.Cybb[KO] mouse model enables the in vivo assessment of gene and cell therapy strategies for treating CGD in human hematopoietic cell transplants without obfuscation by functional mouse phagocytes, and may also be useful for modeling other phagocyte disorders in humanized NSG mouse xenografts.

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.

Tang, C. C., et al. (2017). "Generation of a Bag1 homozygous knockout mouse embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **21**: 29-31.

Bag1 transcribes a multifunctional protein that participates in many important biological processes such as cell apoptosis, proliferation, differentiation and embryo development. Despite numerous published studies, the role of Bag1 in the context of embryonic stem (ES) cells, has not been explored. To investigate the function of Bag1 in ES cells, we generated mutant Bag1(-/-) ES cells using the CRISPR/Cas9 system. We established that the Bag1 double knockout ES cell line maintained their pluripotency, possessed a normal karyotype and the ability to differentiate into all three germ layers.

Tang, Z. H., et al. (2016). "Genetic Correction of Induced Pluripotent Stem Cells From a Deaf Patient With MYO7A Mutation Results in Morphologic and Functional Recovery of the Derived Hair Cell-Like Cells." Stem Cells Transl Med **5**(5): 561-571.

UNLABELLED: The genetic correction of induced pluripotent stem cells (iPSCs) induced from somatic cells of patients with sensorineural hearing loss (caused by hereditary factors) is a promising method for its treatment. The correction of gene mutations in iPSCs could restore the normal function of cells and provide a rich source of cells for transplantation. In the present study, iPSCs were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T; P-iPSCs), the asymptomatic father of the patient (MYO7A c.1184G>A mutation; CF-iPSCs), and a normal donor (MYO7A(WT/WT); C-iPSCs). One of MYO7A mutation sites (c.4118C>T) in the P-iPSCs was corrected using CRISPR/Cas9. The corrected iPSCs (CP-iPSCs) retained cell pluripotency and normal karyotypes. Hair cell-like cells induced from CP-iPSCs showed restored organization of stereocilia-like protrusions; moreover, the electrophysiological function of these cells was similar to that of cells induced from C-iPSCs and CF-iPSCs. These results might facilitate the development of iPSC-based gene therapy for genetic disorders. SIGNIFICANCE: Induced pluripotent stem cells (iPSCs) were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T). One of the MYO7A mutation sites (c.4118C>T) in the iPSCs was corrected using CRISPR/Cas9. The genetic correction of MYO7A mutation resulted in morphologic and functional recovery of hair cell-like cells derived from iPSCs. These findings confirm the hypothesis that MYO7A plays an important role in the assembly of stereocilia into stereociliary bundles. Thus, the present study might provide further insight into the pathogenesis of sensorineural hearing loss and facilitate the development of therapeutic strategies against monogenic disease through the genetic repair of patient-specific iPSCs.

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.

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.

Wei, H., et al. (2018). "CRISPR/Cas9 Gene editing of RyR2 in human stem cell-derived cardiomyocytes provides a novel approach in investigating dysfunctional Ca(2+) signaling." Cell Calcium **73**: 104-111.

Type-2 ryanodine receptors (RyR2s) play a pivotal role in cardiac excitation-contraction coupling by releasing Ca(2+) from sarcoplasmic reticulum (SR) via a Ca(2+) -induced Ca(2+) release (CICR) mechanism. Two strategies have been used to study the structure-function characteristics of RyR2 and its disease associated mutations: (1) heterologous cell expression of the recombinant mutant RyR2s, and (2) knock-in mouse models harboring RyR2 point mutations. Here, we establish an alternative approach where Ca(2+) signaling aberrancy caused by the RyR2 mutation is studied in human cardiomyocytes with robust CICR mechanism. Specifically, we introduce point mutations in wild-type RYR2 of human induced pluripotent stem cells (hiPSCs) by CRISPR/Cas9 gene editing, and then differentiate them into cardiomyocytes. To verify the reliability of this approach, we introduced the same disease-associated RyR2 mutation, F2483I, which was studied by us in hiPSC-derived cardiomyocytes (hiPSC-CMs) from a patient biopsy. The gene-edited F2483I hiPSC-CMs exhibited longer and wandering Ca(2+) sparks, elevated diastolic Ca(2+) leaks, and smaller SR Ca(2+) stores, like those of patient-derived cells. Our CRISPR/Cas9 gene editing approach validated the feasibility of creating myocytes expressing the various RyR2 mutants, making comparative mechanistic analysis and pharmacotherapeutic approaches for RyR2 pathologies possible.

Wei, R., et al. (2018). "Construction of a GLI3 compound heterozygous knockout human embryonic stem cell line WAe001-A-20 by CRISPR/Cas9 editing." Stem Cell Res **32**: 139-144.

The human GLI3 protein has a dual function as a transcriptional activator or repressor of hedgehog signaling, depending on the proteolytic processing forms of GLI3. In this study, we established a compound heterozygous GLI3 mutant human embryonic stem cell line (WAe001-A-20) through CRISPR/Cas9 editing. The WAe001-A-20 cells carried two deletions on two different alleles of exon 2 of GLI3, respectively, which resulted in a frame shift and early termination in the translation of GLI3. Moreover, WAe001-A-20 maintains a normal karyotype, parental cell morphology, pluripotent phenotype and the ability to differentiate into three germ layers. Resource table.

Wettstein, R., et al. (2016). "Generation of a Knockout Mouse Embryonic Stem Cell Line Using a Paired CRISPR/Cas9 Genome Engineering Tool." Methods Mol Biol **1341**: 321-343.

CRISPR/Cas9, originally discovered as a bacterial immune system, has recently been engineered into the latest tool to successfully introduce site-specific mutations in a variety of different organisms.

Composed only of the Cas9 protein as well as one engineered guide RNA for its functionality, this system is much less complex in its setup and easier to handle than other guided nucleases such as Zinc-finger nucleases or TALENs.Here, we describe the simultaneous transfection of two paired CRISPR sgRNAs-Cas9 plasmids, in mouse embryonic stem cells (mESCs), resulting in the knockout of the selected target gene. Together with a four primer-evaluation system, it poses an efficient way to generate new independent knockout mouse embryonic stem cell lines.

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.

Wu, F., et al. (2018). "Generation of a SMO homozygous knockout human embryonic stem cell line WAe001-A-16 by CRISPR/Cas9 editing." Stem Cell Res **27**: 5-9.

The human SMO protein encoded by the smoothened (SMO) gene acts as a positive mediator for Hedgehog signaling. This pathway regulates many cellular activities, developmental morphogenesis, and tumorigenesis. Using CRISPR/Cas9 to edit human embryonic stem cell line WA01 (H1), we established a SMO mutant cell line (WAe001-A-16). This cell line has a 40bp homozygous deletion in exon 2 of SMO leading to a shift in the open reading frame and early termination at amino acid position 287. WAe001-A-16 maintains a normal karyotype, parental cell morphology, pluripotency markers, and the capacity to differentiate into all three germline layers.

Xing, Y., et al. (2018). "A novel piperidine identified by stem cell-based screening attenuates pulmonary arterial hypertension by regulating BMP2 and PTGS2 levels." Eur Respir J **51**(4).

Genetic defects in bone morphogenetic protein type II receptor (BMPRII) signalling and inflammation contribute to the pathogenesis of pulmonary arterial hypertension (PAH). The receptor is activated by bone morphogenetic protein (BMP) ligands, which also enhance BMPR2 transcription. A small-molecule BMP upregulator with selectivity on vascular endothelium would be a desirable therapeutic intervention for PAH.We assayed compounds identified in the screening of BMP2 upregulators for their ability to increase the expression of inhibitor of DNA binding 1 (Id1), using a dual reporter driven specifically in human embryonic stem cell-derived endothelial cells. These assays identified a novel piperidine, BMP upregulator 1 (BUR1), that increased endothelial Id1 expression with a half-maximal effective concentration of 0.098 mumol.L(-1) Microarray analyses and immunoblotting showed that BUR1 induced BMP2 and prostaglandin-endoperoxide synthase 2 (PTGS2) expression.

Xu, G., et al. (2018). "Generation of a GDE heterozygous mutation human embryonic stem cell line WAe001-A-14 by CRISPR/Cas9 editing." Stem Cell Res **27**: 38-41.

Glycogen debranching enzyme (GDE) plays a critical role in glycogenolysis. Mutations in the GDE gene are associated with a metabolic disease known as glycogen storage disease type III (GSDIII). We generated a mutant GDE human embryonic stem cell line, WAe001-A-14, using the CRISPR/Cas9 editing system. This cell line contains a 24-nucleotide deletion within exon-13 of GDE, resulting in 8 amino acids (TRLGISSL) missing of the GDE protein from amino acid position 567 to 575. The WAe001-A-14 cell line maintains typical stem cell morphology, pluripotency and in vitro differentiation potential, and a normal karyotype.

Xu, Y., et al. (2017). "Generation of an ASGR1 homozygous mutant human embryonic stem cell line WAe001-A-6 using CRISPR/Cas9." Stem Cell Res **22**: 29-32.

The gene asialoglycoprotein receptor 1 (ASGR1) encodes a subunit of the asialoglycoprotein receptor. Here we report the generation of a human embryonic stem cell line WAe001-A-6 harbouring homozygous ASGR1 mutations using CRISPR/Cas9. The mutation involves a 37bp deletion, resulting in a frame shift. The homozygous knockout WA01 cell line maintains a normal karyotype, typical stem cell

morphology, pluripotency and differentiation potential in vitro.

Yang, D., et al. (2016). "Enrichment of G2/M cell cycle phase in human pluripotent stem cells enhances HDR-mediated gene repair with customizable endonucleases." Sci Rep **6**: 21264.

Efficient gene editing is essential to fully utilize human pluripotent stem cells (hPSCs) in regenerative medicine. Custom endonuclease-based gene targeting involves two mechanisms of DNA repair: homology directed repair (HDR) and non-homologous end joining (NHEJ). HDR is the preferred mechanism for common applications such knock-in, knock-out or precise mutagenesis, but remains inefficient in hPSCs. Here, we demonstrate that synchronizing synchronizing hPSCs in G2/M with ABT phase increases on-target gene editing, defined as correct targeting cassette integration, 3 to 6 fold. We observed improved efficiency using ZFNs, TALENs, two CRISPR/Cas9, and CRISPR/Cas9 nickase to target five genes in three hPSC lines: three human embryonic stem cell lines, neural progenitors and diabetic iPSCs. neural progenitors and diabetic iPSCs. Reversible synchronization has no effect on pluripotency or differentiation. The increase in on-target gene editing is locus-independent and specific to the cell cycle phase as G2/M phase enriched cells show a 6-fold increase in targeting efficiency compared to cells in G1 phase.

Yang, Z., et al. (2017). "Single-cell Sequencing Reveals Variants in ARID1A, GPRC5A and MLL2 Driving Self-renewal of Human Bladder Cancer Stem Cells." Eur Urol **71**(1): 8-12.

Cancer stem cells are considered responsible for many important aspects of tumors such as their self-renewal, tumor-initiating, drug-resistance and metastasis. However, the genetic basis and origination of human bladder cancer stem cells (BCSCs) remains unknown. Here, we conducted single-cell sequencing on 59 cells including BCSCs, bladder cancer non-stem cells (BCNSCs), bladder epithelial stem cells (BESCs) and bladder epithelial non-stem cells (BENSCs) from three bladder cancer (BC) specimens. Specifically, BCSCs demonstrate clonal homogeneity and suggest their origin from BESCs or BCNSCs through phylogenetic analysis. Moreover, 21 key altered genes were identified in BCSCs including six genes not previously described in BC (ETS1, GPRC5A, MKL1, PAWR, PITX2 and RGS9BP). Co-mutations of ARID1A, GPRC5A and MLL2 introduced by CRISPR/Cas9 significantly enhance the capabilities of self-renewal and tumor-initiating of BCNSCs. To our knowledge, our study first provides an overview of the genetic basis of human BCSCs with single-cell sequencing and demonstrates the biclonal origin of human BCSCs via evolution analysis. PATIENT SUMMARY: Human bladder cancer stem cells show the high level of consistency and may derived from bladder epithelial stem cells or bladder cancer non-stem cells. Mutations of ARID1A, GPRC5A and MLL2 grant bladder cancer non-stem cells the capability of self-renewal.

Yeung, A. T. Y., et al. (2017). "Exploiting induced pluripotent stem cell-derived macrophages to unravel host factors influencing Chlamydia trachomatis pathogenesis." Nat Commun **8**: 15013.

Chlamydia trachomatis remains a leading cause of bacterial sexually transmitted infections and preventable blindness worldwide. There are, however, limited in vitro models to study the role of host genetics in the response of macrophages to this obligate human pathogen. Here, we describe an approach using macrophages derived from human induced pluripotent stem cells (iPSdMs) to study macrophage-Chlamydia interactions in vitro. We show that iPSdMs support the full infectious life cycle of C. trachomatis in a manner that mimics the infection of human blood-derived macrophages. Transcriptomic and proteomic profiling of the macrophage response to chlamydial infection highlighted the role of the type I interferon and interleukin 10-mediated responses. Using CRISPR/Cas9 technology, we generated biallelic knockout mutations in host genes encoding IRF5 and IL-10RA in iPSCs, and confirmed their roles in limiting chlamydial infection in macrophages. This model can potentially be extended to other pathogens and tissue systems to advance our understanding of host-pathogen interactions and the role of human genetics in influencing the outcome of infections.

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.

Yuan, F., et al. (2017). "Generation of a KCNJ11 homozygous knockout human embryonic stem cell line WAe001-A-12 using CRISPR/Cas9." Stem Cell Res **24**: 89-93.

The ATP-sensitive potassium channel is an octameric complex, and one of its subunits, namely Kir6.2, is encoded by the KCNJ11 gene. Mutations in KCNJ11 result in hyperinsulinism or diabetes mellitus, associated with abnormal insulin secretion. Here, using CRISPR/Cas9 editing, we established a homozygous mutant KCNJ11 cell line, WAe001-A-12, which was generated by a 62-bp deletion in the coding sequence of the human embryonic stem cell line H1. It was confirmed that this deletion in the KCNJ11 gene did not affect the protein expression levels of key pluripotent factors. Additionally, normal karyotype and differentiation potency were observed for the cell line.

Yuan, F., et al. (2018). "Generation of an ASS1 heterozygous knockout human embryonic stem cell line, WAe001-A-13, using CRISPR/Cas9." Stem Cell Res **26**: 67-71.

The ASS1 gene encodes argininosuccinate synthetase-1, a cytosolic enzyme with a critical role in the urea cycle. Mutations are found in all ASS1 exons and cause the autosomal recessive disorder citrullinemia. Using CRISPR/Cas9-editing, we established the WAe001-A-13 cell line, which was heterozygous for an ASS1 mutation, from the human embryonic stem cell line H1. The WAe001-A-13 cell line maintained the pluripotent phenotype, the ability to differentiate into all three germ layers and a normal karyotype.

Yuan, X., et al. (2017). "Extracellular vesicles from human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs) protect against renal ischemia/reperfusion injury via delivering specificity protein (SP1) and transcriptional activating of sphingosine kinase 1 and inhibiting necroptosis." Cell Death Dis **8**(12): 3200.

Renal ischemia-reperfusion is a main cause of acute kidney injury (AKI), which is associated with high mortality. Here we show that extracellular vesicles (EVs) secreted from hiPSC-MSCs play a critical role in protection against renal I/R injury. hiPSC-MSCs-EVs can fuse with renal cells and deliver SP1 into target cells, subsequently active SK1 expression and increase S1P formation. Chromatin immunoprecipitation (ChIP) analyses and luciferase assay were used to confirm SP1 binds directly to the SK1 promoter region and promote promoter activity. Moreover, SP1 inhibition (MIT) or SK1 inhibition (SKI-II) completely abolished the renal protective effect of hiPSC-MSCs-EVs in rat I/R injury mode. However, pre-treatment of necroptosis inhibitor Nec-1 showed no difference with the administration of hiPSC-MSCs-EVs only. We then generated an SP1 knockout hiPSC-MSC cell line by CRISPR/Cas9 system and found that SP1 knockout failed to show the protective effect of hiPSC-MSCs-EVs unless restoring the level of SP1 by Ad-SP1 in vitro and in vivo. In conclusion, this study describes an anti-necroptosis effect of hiPSC-MSCs-EVs against renal I/R injury via delivering SP1 into target renal cells and intracellular activating the expression of SK1 and the generation of S1P. These findings suggest a novel mechanism for renal protection against I/R injury, and indicate a potential therapeutic approach for a variety of renal diseases and renal transplantation.

Zhang, X., et al. (2018). "Generation of an induced pluripotent stem cell line from a patient with non-syndromic CLN3-associated retinal degeneration and a coisogenic control line." Stem Cell Res **29**: 245-249.

We report the generation of the human iPSC line LEIi004-A from a patient with late-onset non-syndromic retinitis pigmentosa caused by compound heterozygous mutations in the CLN3 gene. Reprogramming of primary dermal fibroblasts was performed using episomal plasmids containing OCT4, SOX2, KLF4, L-MYC, LIN28, shRNA for p53 and mir302/367 microRNA. To create a coisogenic control line, one CLN3 variant was corrected in the patient-iPSC using CRISPR/Cas9 gene editing to generate the iPSC line LEIi004-A-1.

Zhong, C., et al. (2018). "Barhl1 is required for the differentiation of inner ear hair cell-like cells from mouse embryonic stem cells." Int J Biochem Cell Biol **96**: 79-89.

Inner ear hair cells are mechanoreceptors responsible for hearing. Pathogenic defects of hair cell-specific genes are one of the major causes of deafness. The BarH class homeobox gene Barhl1 is a deafness gene expressed in developing hair cells, yet the role of Barhl1 during hair cell development remains poorly understood. In the present study, we first established an in vitro differentiation system to efficiently obtain mouse embryonic stem cell (mESC)-derived hair cell-like cells. Subsequently, a mESC line carrying a targeted disruption of Barhl1 was generated using CRISPR/Cas9 technology and subjected to the established in vitro hair cell differentiation protocol. Targeted disruption of Barhl1 does not affect the induction of mESCs toward early primitive ectoderm-

like (EPL) cells and otic progenitors but strongly inhibits the differentiation of hair cell-like cells. Using RNA-sequencing and bioinformatics, we further unravel the molecular mechanism underlying Barhl1-mediated hair cell development. Our data demonstrate the essential role of Barhl1 during hair cell development and provide a basis for the treatment of Barhl1 mutation-based deafness.

Zhong, C. and J. Li (2017). "Efficient Generation of Gene-Modified Mice by Haploid Embryonic Stem Cell-Mediated Semi-cloned Technology." Methods Mol Biol **1498**: 121-133.

Haploid embryonic stem cells can be derived from androgenetic embryos produced by injection of sperm into enucleated oocytes or by removal of the female pronucleus from zygotes. These cells, termed AG-haESCs, can be used in place of sperm to produce the so-called semi-cloned (SC) mice. Importantly, AG-haESCs carrying H19-DMR and IG-DMR knockouts (DKO-AG-haESCs) can efficiently and stably support the generation of SC mice via intracytoplasmic AG-haESCs injection (ICAHCI), which provides a new route to obtain genetically modified mice. In this chapter, we describe the procedures for AG-haESCs culturing, enrichment of haploid cells by FACS, genomic manipulation in DKO-AG-haESCs by CRISPR/Cas9 and generation of live SC mice with gene-modified DKO-AG-haESCs.

Zhou, H. Y., et al. (2014). "A Sox2 distal enhancer cluster regulates embryonic stem cell differentiation potential." Genes Dev **28**(24): 2699-2711.

The Sox2 transcription factor must be robustly transcribed in embryonic stem (ES) cells to maintain pluripotency. Two gene-proximal enhancers, Sox2 regulatory region 1 (SRR1) and SRR2, display activity in reporter assays, but deleting SRR1 has no effect on pluripotency. We identified and functionally validated the sequences required for Sox2 transcription based on a computational model that predicted transcriptional enhancer elements within 130 kb of Sox2. Our reporter assays revealed three novel enhancers--SRR18, SRR107, and SRR111--that, through the formation of chromatin loops, form a chromatin complex with the Sox2 promoter in ES cells. Using the CRISPR/Cas9 system and F1 ES cells (Mus musculus(129) x Mus castaneus), we generated heterozygous deletions of each enhancer region, revealing that only the distal cluster containing SRR107 and SRR111, located >100 kb downstream from Sox2, is required for cis-regulation of Sox2 in ES cells. Furthermore, homozygous deletion of this distal Sox2 control region (SCR) caused significant reduction in Sox2 mRNA and protein levels, loss of ES cell colony morphology, genome-wide changes in gene expression, and impaired neuroectodermal formation upon spontaneous differentiation to embryoid bodies. Together, these data identify a distal control region essential for Sox2 transcription in ES cells.

Zhou, J., et al. (2016). "Generation of Human Embryonic Stem Cell Line Expressing zsGreen in Cholinergic Neurons Using CRISPR/Cas9 System." Neurochem Res **41**(8): 2065-2074.

Lineage specific human embryonic stem cell (hESC) reporter cell line is a versatile tool for biological studies on real time monitoring of differentiation, physiological and biochemical features of special cell types and pathological mechanism of disease. Here we report the generation of ChAT-zsGreen reporter hESC line that express zsGreen under the control of the choline acetyltransferase (ChAT) promoter using CRISPR (Clustered Regularly Interspersed Short Palindromic Repeats)/Cas9 system. We show that the ChAT-zsGreen hESC reporter cell lines retain the features of undifferentiated hESC. After cholinergic neuronal differentiation, cholinergic neurons were clearly labeled with green fluorescence protein (zsGreen). The ChAT-zsGreen reporter hESC lines are invaluable not only for the monitoring cholinergic neuronal differentiation but also for study physiological and biochemical hallmarks of cholinergic neurons.

Zuo, Q., et al. (2017). "CRISPR/Cas9-Mediated Deletion of C1EIS Inhibits Chicken Embryonic Stem Cell Differentiation Into Male Germ Cells (Gallus gallus)." J Cell Biochem **118**(8): 2380-2386.

We previously found that C1EIS is preferentially expressed in Chicken spermatogonial stem cells (SSCs) by RNA sequencing (RNA-seq), so our current study focused on C1EIS's role in Chicken embryonic stem cells (ESCs) differentiation into male germ cells. We constructed a CRISPR/Cas9 vector targeting C1EIS. T7 endonuclease I (T7EI) digestion method and sequencing of TA cloning were used to detect the knock-out efficiency of the Single guide RNA (sgRNA) after the cas9/gRNA vector transfected into D fibroblasts 1(DF-1), ESCs, and Chicken embryos. The results showed that CRISPR/Cas9 gene knockout efficiency is about 40%. Differentiation of the targeted ESCs into SSCs was inhibited at the embryoid body stage due to C1EIS deficiency. Immunofluorescent staining revealed that the mutagenized ESCs (RA (Retinoic Acid) with C1EIS Knock out) expressed lower levels of integrin alpha6 and integrin beta1 compared to wild type cells. Quantitative real-time PCR (QRT-PCR) revealed Oct4 and Sox2 expression significantly increased, contrarily integrin beta1 and Stra8 expression significantly decreased than RA induced group and RA with C1EIS

Overexpression. During retinoic acid-induced differentiation, knockout of C1EIS in ESCs inhibited formation of SSC-like cells, suggesting C1EIS plays a vital role in promoting differentiation of avian ESCs to SSCs by regulating expression of multiple pluripotency-related genes. J. Cell. Biochem. 118: 2380-2386, 2017. (c) 2017 Wiley Periodicals, Inc.

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