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Stem Cell

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# Stem Cell Technology Research Literatures

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**Abstract**: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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**Key words**: stem cell; technology; 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.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

Aalders, J., et al. (2023). "Generation of human induced pluripotent stem cell line UGENTi001-A from a patient with Marfan syndrome carrying a heterozygous c.7754 T > C variant in FBN1 and the isogenic control UGENT001-A-1 using CRISPR/Cas9 editing." Stem Cell Res **67**: 103036.

Marfan syndrome is an autosomal dominant genetic disorder resulting from pathogenic variants in FBN1 gene. FBN1 encodes for fibrillin-1, an important extracellular matrix protein. Impaired fibrillin-1 affects multiple organ systems, including the cardiovascular system. We generated an iPSC line carrying a heterozygous variant c.7754 T > C (p.Ile2585Thr, missense) in FBN1 from a patient with Marfan syndrome. Also, an isogenic control is generated, where the pathogenic variant is repaired using CRISPR-Cas9. This isogenic pair provides a valuable resource for in vitro disease modelling.

Ababneh, N. A., et al. (2020). "Correction of amyotrophic lateral sclerosis related phenotypes in induced pluripotent stem cell-derived motor neurons carrying a hexanucleotide expansion mutation in C9orf72 by CRISPR/Cas9 genome editing using homology-directed repair." Hum Mol Genet **29**(13): 2200-2217.

The G4C2 hexanucleotide repeat expansion (HRE) in C9orf72 is the commonest cause of familial amyotrophic lateral sclerosis (ALS). A number of different methods have been used to generate isogenic control lines using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 and non-homologous end-joining by deleting the repeat region, with the risk of creating indels and genomic instability. In this study, we demonstrate complete correction of an induced pluripotent stem cell (iPSC) line derived from a C9orf72-HRE positive ALS/frontotemporal dementia patient using CRISPR/Cas9 genome editing and homology-directed repair (HDR), resulting in replacement of the excised region with a donor template carrying the wild-type repeat size to maintain the genetic architecture of the locus. The isogenic correction of the C9orf72 HRE restored normal gene expression and methylation at the C9orf72 locus,

reduced intron retention in the edited lines and abolished pathological phenotypes associated with the C9orf72 HRE expansion in iPSC-derived motor neurons (iPSMNs). RNA sequencing of the mutant line identified 2220 differentially expressed genes compared with its isogenic control. Enrichment analysis demonstrated an over-representation of ALS relevant pathways, including calcium ion dependent exocytosis, synaptic transport and the Kyoto Encyclopedia of Genes and Genomes ALS pathway, as well as new targets of potential relevance to ALS pathophysiology. Complete correction of the C9orf72 HRE in iPSMNs by CRISPR/Cas9-mediated HDR provides an ideal model to study the earliest effects of the hexanucleotide expansion on cellular homeostasis and the key pathways implicated in ALS pathophysiology.

Acun, A. and P. Zorlutuna (2019). "CRISPR/Cas9 Edited Induced Pluripotent Stem Cell-Based Vascular Tissues to Model Aging and Disease-Dependent Impairment." Tissue Eng Part A **25**(9-10): 759-772.

Modeling human disease as precisely as possible is of upmost importance in understanding the underlying pathology and discovering effective therapies. Therefore, disease models that are highly controlled and composed of human-origin cells that present the disease phenotype are crucial. The human induced pluripotent stem cell (hiPSC)-based tissue model we present in this study is an important example of human-origin tissue model with controlled gene expression. Through CRISPR/Cas9 editing of hypoxia inducible factor 1alpha in hiPSCs, we developed tissue models that show the age and disease-dependent endothelial deterioration. This model holds promise for various biomedical applications as more realistic disease phenotypes can be created using fully human-origin platforms.

Ader, F., et al. (2022). "Generation of CRISPR-Cas9 edited human induced pluripotent stem cell line carrying FLNC exon skipping variant." Stem Cell Res **58**: 102616.

Loss-of-function (LoF) mutations in FLNC are strongly associated with dilated cardiomyopathy (DCM). Using CRISPR/Cas9 mediated edition in an healthy donor derived iPSC (ICAN-403.3) we subcloned 1 iPSC line harboring LoF mutation in FLNC. All lines are fully pluripotent and isogenic except at edited site where it presents a homozygous (ICAN-FLNC42.1) deletion of splice site leading to skipping of exon 42 traduced into a short filamin form with reduced expression in derived cardiomyocytes. This line would serve for FLNC mutation DCM modeling after differentiation into cardiocytes or beating organoids.

Alda-Catalinas, C., et al. (2021). "Pooled CRISPR-activation screening coupled with single-cell RNA-seq in mouse embryonic stem cells." STAR Protoc **2**(2): 100426.

CRISPR/Cas9 screens are a powerful approach to identify key regulators of biological processes. By combining pooled CRISPR/Cas9 screening with single-cell RNA-sequencing readout, individual perturbations can be assessed in parallel both comprehensively and at scale. Importantly, this allows gene function and regulation to be interrogated at a cellular level in an unbiased manner. Here, we present a protocol to perform pooled CRISPR-activation screens in mouse embryonic stem cells using 10x Genomics scRNA-seq as a readout. For complete information on the generation and use of this protocol, please refer to Alda-Catalinas et al. (2020).

Anderson, R. H., et al. (2018). "Generation of a CLTA reporter human induced pluripotent stem cell line, CRMi001-A-1, using the CRISPR/Cas9 system to monitor endogenous clathrin trafficking." Stem Cell Res **33**: 95-99.

The most highly studied endocytic pathway, clathrin-dependent endocytosis, mediates a wide range of fundamental processes including nutrient internalization, receptor recycling, and signal transduction. In order to model tissue specific and developmental aspects of this process, CRISPR/Cas9 genomic editing was utilized to fluorescently label the C-terminus of clathrin light chain A (CLTA) within the phenotypically normal, parental CRMi001-A human induced pluripotent stem cell line. Successfully edited cells were isolated by fluorescently activated cell sorting, remained karyotypically normal, and maintained their differentiation potential. This cell line facilitates imaging of endogenous clathrin trafficking within varied cell types.

Angsutararux, P., et al. (2019). "Generation of human induced pluripotent stem cell line carrying SCN5AC2204>T Brugada mutation (MUSli009-A-1) introduced by CRISPR/Cas9-mediated genome editing." Stem Cell Res **41**: 101618.

Human induced pluripotent stem cells (hiPSCs) derived from dermal fibroblasts having wild type (WT) SCN5A were engineered by CRISPR/Cas9-mediated genome editing to harbor a specific point mutation (C2204>T) in SCN5A, which results in a substitution of the WT alanine by valine at codon 735 (A735V). The established MUSli009-A-1 hiPSC line has a homozygous C2204>T mutation on exon 14 of SCN5A that was confirmed by DNA sequencing analysis. The cells exhibited normal karyotype, expressed pluripotent markers and retained its

capability to differentiate into three germ layers. The cardiomyocytes derived from this line would be a useful model for investigating cardiac channelopathy.

Antao, A. M., et al. (2020). "Disease modeling and stem cell immunoengineering in regenerative medicine using CRISPR/Cas9 systems." Comput Struct Biotechnol J **18**: 3649-3665.

CRISPR/Cas systems are popular genome editing tools that belong to a class of programmable nucleases and have enabled tremendous progress in the field of regenerative medicine. We here outline the structural and molecular frameworks of the well-characterized type II CRISPR system and several computational tools intended to facilitate experimental designs. The use of CRISPR tools to generate disease models has advanced research into the molecular aspects of disease conditions, including unraveling the molecular basis of immune rejection. Advances in regenerative medicine have been hindered by major histocompatibility complex-human leukocyte antigen (HLA) genes, which pose a major barrier to cell- or tissue-based transplantation. Based on progress in CRISPR, including in recent clinical trials, we hypothesize that the generation of universal donor immune-engineered stem cells is now a realistic approach to tackling a multitude of disease conditions.

Balboa, D., et al. (2017). "Generation of a SOX2 reporter human induced pluripotent stem cell line using CRISPR/SaCas9." Stem Cell Res **22**: 16-19.

SOX2 is an important transcription factor involved in pluripotency maintenance, pluripotent reprogramming and differentiation towards neural lineages. Here we engineered the previously described HEL24.3 hiPSC to generate a SOX2 reporter by knocking-in a T2A fused nuclear tdTomato reporter cassette before the STOP codon of the SOX2 gene coding sequence. CRISPR/SaCas9-mediated stimulation of homologous recombination was utilized to facilitate faithful targeted insertion. This line accurately reports the expression of endogenous SOX2 and therefore constitutes a useful tool to study the SOX2 expression dynamics upon hiPSC culture, differentiation and somatic cell reprogramming.

Balboa, D., et al. (2017). "Generation of an OCT4 reporter human induced pluripotent stem cell line using CRISPR/SpCas9." Stem Cell Res **23**: 105-108.

OCT4 is a crucial transcription factor in the pluripotent stem cell gene regulatory network and an essential factor for pluripotent reprogramming. We engineered the previously reported HEL24.3 hiPSC to generate an OCT4 reporter cell line by knocking-in a T2A nuclear EmGFP reporter cassette before the OCT4 gene STOP codon sequence. To enhance targeted insertion, homologous recombination was stimulated using targeted cutting at the OCT4 STOP codon with CRISPR/SpCas9. This HEL24.3-OCT4-nEmGFP cell line faithfully reports endogenous OCT4 expression, serving as a useful tool to examine temporal changes in OCT4 expression in live cells during hiPSC culture, differentiation and somatic cell reprogramming.

Bara, A. M., et al. (2016). "Generation of a TLE3 heterozygous knockout human embryonic stem cell line using CRISPR-Cas9." Stem Cell Res **17**(2): 441-443.

Here, we generated a monoallelic mutation in the TLE3 (Transducin Like Enhancer of Split 3) gene using CRISPR-Cas9 editing in the human embryonic stem cell (hESC) line WA01. The heterozygous knockout cell line, TLE3-447-D08-A01, displays partial loss of TLE3 protein expression while maintaining pluripotency, differentiation potential and genomic integrity.

Ben Jehuda, R., et al. (2018). "CRISPR correction of the PRKAG2 gene mutation in the patient's induced pluripotent stem cell-derived cardiomyocytes eliminates electrophysiological and structural abnormalities." Heart Rhythm **15**(2): 267-276.

BACKGROUND: Mutations in the PRKAG2 gene encoding the gamma-subunit of adenosine monophosphate kinase (AMPK) cause hypertrophic cardiomyopathy (HCM) and familial Wolff-Parkinson-White (WPW) syndrome. Patients carrying the R302Q mutation in PRKAG2 present with sinus bradycardia, escape rhythms, ventricular preexcitation, supraventricular tachycardia, and atrioventricular block. This mutation affects AMPK activity and increases glycogen storage in cardiomyocytes. The link between glycogen storage, WPW syndrome, HCM, and arrhythmias remains unknown. OBJECTIVE: The purpose of this study was to investigate the pathological changes caused by the PRKAG2 mutation. We tested the hypothesis that patient's induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) display clinical aspects of the disease. METHODS: Using clustered regularly interspaced short palindromic repeats (CRISPR) technology, we corrected the mutation and then generated isogenic iPSC-CMs. Action potentials were recorded from spontaneously firing and paced cardiomyocytes using the patch clamp technique. Using a microelectrode array setup, we recorded electrograms from iPSC-CMs clusters. Transmission electron microscopy was used to detect ultrastructural abnormalities in the mutated iPSC-CMs. RESULTS: PRKAG2-mutated iPSC-CMs exhibited abnormal firing patterns, delayed afterdepolarizations, triggered arrhythmias, and augmented beat rate variability. Importantly, CRISPR

correction eliminated the electrophysiological abnormalities, the augmented glycogen, storage, and cardiomyocyte hypertrophy. CONCLUSION: PRKAG2-mutated iPSC-CMs displayed functional and structural abnormalities, which were abolished by correcting the mutation in the patient's iPSCs using CRISPR technology.

Beneto, N., et al. (2020). "Generation of two NAGLU-mutated homozygous cell lines from healthy induced pluripotent stem cells using CRISPR/Cas9 to model Sanfilippo B syndrome." Stem Cell Res **42**: 101668.

Bharucha, N., et al. (2021). "Generation of AAVS1 integrated doxycycline-inducible CRISPR-Prime Editor human induced pluripotent stem cell line." Stem Cell Res **57**: 102610.

Prime editing uses the Cas9 nickase fused to a reverse transcriptase to copy a DNA sequence into a specific locus from a 'prime editing' guide RNA (pegRNA), eliminating the need for double-stranded DNA breaks and donor DNA templates. To facilitate prime editing in human induced pluripotent stem cells (iPSCs), we integrated a doxycycline-inducible Prime Editor protein (PE2) into the AAVS1 genomic safe harbor locus. Prime editing of iPSCs resulted in precise insertion of three nucleotides in HEK3 locus with high efficiency, demonstrating the utility of this approach. This engineered cell line can be used to edit a single or multiple genomic loci by introducing a target-specific pegRNA for precise and effective genome editing to facilitate disease modeling and functional genetics studies.

Bivalkar-Mehla, S., et al. (2021). "Understanding the role of Beclin1 in mouse embryonic stem cell differentiation through CRISPR-Cas9-mediated gene editing." J Biosci **46**.

Autophagy is a vacuolar pathway for the regulated degradation and recycling of cellular components. Beclin1, a Bcl2-interacting protein, is a well-studied autophagy regulator. Homozygous loss of Beclin1 in mice leads to early embryonic lethality. However, the role of Beclin1 in regulating the pluripotency of embryonic stem cells and their differentiation remains poorly explored. To study this, we generated Beclin1-Knockout (KO) mouse embryonic stem cells (mESCs) using the CRISPR-Cas9 genome-editing tool. Interestingly, Beclin1-KO mESCs did not show any change in the expression of pluripotency marker genes. Beclin1-KO mESCs also displayed active autophagy, suggesting the presence of Beclin1-independent autophagy in mESCs. However, loss of Beclin1 resulted in compromised differentiation of mESCs in vitro and in vivo due to misregulated expression of transcription factors. Our results suggest that Beclin1 may play an autophagy-independent role in regulating the differentiation of mESCs.

Bu, Q., et al. (2020). "Generation of an NANS homozygous knockout human induced pluripotent stem cell line by the insertion of GFP-P2A-Puro via CRISPR/Cas9 editing." Stem Cell Res **49**: 102052.

N-acetylneuraminic acid synthase (NANS), the gene encoding the synthase for N-acetylneuraminic acid (NeuNAc; sialic acid), is closely associated with infantile-onset severe developmental delay and skeletal dysplasia. However, the role and the involved mechanisms of NANS functioning have not been fully understood to date. Here, we generated a homozygous NANS-knockout human induced pluripotent stem cell (iPSC) line, NCCSEDi001-A-1, via the CRISPR/Cas9-based gene editing method. The NCCSEDi001-A-1 cell line does not express NANS protein, but maintains a normal karyotype, pluripotency, and trilineage differentiation potential.

C, X. Q. C., et al. (2022). "Generation of patient-derived pluripotent stem cell-lines and CRISPR modified isogenic controls with mutations in the Parkinson's associated GBA gene." Stem Cell Res **64**: 102919.

The GBA gene encodes the lysosomal enzyme glucocerebrosidase (GCase), responsible for the hydrolysis of glucocerebroside to glucose and ceramide. Heterozygous GBA mutations have been associated with the development of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). We generated two induced pluripotent stem cell (iPSC) lines from PD patients carrying heterozygous GBA W378G or N370S mutations and subsequently produced isogenic control lines using CRISPR/Cas9 genome editing. The patient-derived iPSCs and isogenic control lines maintained full pluripotency, normal karyotypes, and differentiation capacity. All iPSC lines could be differentiated into dopaminergic neurons, thus providing valuable tools for studying PD pathogenesis.

Canac, R., et al. (2022). "Generation of three human induced pluripotent stem cell lines with IRX5 knockout and knockin genetic editions using CRISPR-Cas9 system." Stem Cell Res **58**: 102627.

Studies on animal models have shown that Irx5 is an important regulator of cardiac development and that it regulates ventricular electrical repolarization gradient in the adult heart. Mutations in IRX5 have also been linked in humans to cardiac conduction defects. In order to fully characterize the role of IRX5 during cardiac development and in cardiomyocyte function, we generated three genetically-modified human induced pluripotent stem cell lines: two knockout lines

(heterozygous and homozygous) and a knockin HA-tagged line (homozygous).

Chang, Y. J., et al. (2023). "CRISPR Manipulations in Stem Cell Lines." Methods Mol Biol **2560**: 249-256.

Insights into genome engineering in cells have allowed researchers to cultivate and modify cells as organoids that display structural and phenotypic features of human diseases or normal health status. The generation of targeted mutants is a crucial step toward studying the biomedical effect of genes of interest. Modified organoids derived from patients' tissue cells are used as models to study diseases and test novel drugs. CRISPR-Cas9 technology has contributed to an explosion of advances that have the ability to edit genomes for the study of monogenic diseases and cancers. The generation of such mutants in human induced pluripotent stem cells (iPSCs) is of utmost importance as these cells carry the potential to be differentiated into any cell lineage. We describe recent developments that are broadening our understanding and extend DNA specificity, product selectivity, and fundamental capabilities. Furthermore, fundamental capabilities and remarkable advancements in basic research, biotechnology, and therapeutics development in cell engineering are detailed within this chapter. Using the CRISPR/Cas9 nuclease system for induction of targeted double-strand breaks, gene editing of target loci in iPSCs can be achieved with high efficiency. This chapter includes detailed protocols for the preparation of reagents to target loci of interest and transfection to genotype single cell-derived iPSC clones. Furthermore, we provide a protocol for the convenient generation of ribonucleoprotein (RNP) delivered directly to cells.

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

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

Chen, C. X., et al. (2022). "Generation of homozygous PRKN, PINK1 and double PINK1/PRKN knockout cell lines from healthy induced pluripotent stem cells using CRISPR/Cas9 editing." Stem Cell Res **62**: 102806.

Autosomal recessive mutations in either PRKN or PINK1 are associated with early-onset Parkinson's disease. The corresponding proteins, PRKN, an E3 ubiquitin ligase, and the mitochondrial serine/threonine-protein kinase PINK1 play a role in mitochondrial quality control. Using CRISPR/CAS9 technology we generated three human iPSC lines from the well characterized AIW002-02 control line. These isogenic iPSCs contain homozygous knockouts of PRKN (PRKN-KO, CBIGi001-A-1), PINK1 (PINK1-KO, CBIGi001-A-2) or both PINK1 and PRKN (PINK1-KO/PRKN-KO, CBIGi001-A-3). The knockout lines display normal karyotypes, express pluripotency markers and upon differentiation into relevant brain cells or midbrain organoids may be valuable tools to model Parkinson's disease.

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.

Coombe, L., et al. (2018). "Current approaches in regenerative medicine for the treatment of diabetes: introducing CRISPR/CAS9 technology and the case for non-embryonic stem cell therapy." Am J Stem Cells **7**(5): 104-113.

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which the body destroys its pancreatic beta cells. Since these cells are responsible for insulin production, dysfunction or destruction of these cells necessitates blood glucose control through exogenous insulin shots. Curative treatment involves pancreas transplantation, but due to the incidence of transplant rejection and complications associated with immunosuppression, alternatives are being explored. Despite facing clinical challenges and issues with public perception, the field of regenerative stem cell therapy shows great promise for the treatment of diabetes. The idea of harnessing pluripotency to derive cells and tissues with characteristics of choice is astounding but feasible, and this review seeks to determine which method of stem cell derivation is preferable for diabetes treatment. In this report, we outline the methods for deriving human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), and adult stem cells or progenitor cells to generate functional islet cells and related tissues. We discuss the specific uses and advantages of each method, and we comment on the ethics and public perceptions surrounding these methods and how they may affect the future of stem cell research. For the reasons outlined in this paper, we believe that non-embryonic stem cell lines, including iPSCs, somatic cell nuclear transfer lines, and adult tissue derived stem cells, offer the highest therapeutic potential for treating diabetes.

Daniel-Moreno, A., et al. (2019). "CRISPR/Cas9-modified hematopoietic stem cells-present and future perspectives for stem cell transplantation." Bone Marrow Transplant **54**(12): 1940-1950.

Allogeneic hematopoietic stem cell transplantation (HSCT) is a standard therapeutic intervention for hematological malignancies and several monogenic diseases. However, this approach has limitations related to lack of a suitable donor, graft-versus-host disease and infectious complications due to immune suppression. On the contrary, autologous HSCT diminishes the negative effects of allogeneic HSCT. Despite the good efficacy, earlier gene therapy trials with autologous HSCs and viral vectors have raised serious safety concerns. However, the CRISPR/Cas9-edited autologous HSCs have been proposed to be an alternative option with a high safety profile. In this review, we summarized the possibility of CRISPR/Cas9-mediated autologous HSCT as a potential treatment option for various diseases supported by preclinical gene-editing studies. Furthermore, we discussed future clinical perspectives and possible clinical grade improvements of CRISPR/cas9-mediated autologous HSCT.

Dettmer, R. and O. Naujok (2020). "Design and Derivation of Multi-Reporter Pluripotent Stem Cell Lines via CRISPR/Cas9n-Mediated Homology-Directed Repair." Curr Protoc Stem Cell Biol **54**(1): e116.

During the past decade, RNA-guided Cas9 nuclease from microbial clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) has become a powerful tool for gene editing of human pluripotent stem cells (PSCs). Using paired CRISPR/Cas9 nickases (CRISPR/Cas9n) it is furthermore possible to reduce off-target effects that may typically occur with traditional CRISPR/Cas9 systems while maintaining high on-target efficiencies. With this technology and a well-designed homology-directed repair vector (HDR), we are now able to integrate transgenes into specific gene loci of PSCs in an allele conserving way. In this protocol we describe CRISPR/Cas9n design and homology directed repair vector design, transfection of human pluripotent stem cells and selection and expansion of generated cell clones. (c) 2020 The Authors. Basic Protocol 1: Repair template design and CRISPR/Cas9n construction Basic Protocol 2: Transfection of human pluripotent stem cells by electroporation Basic Protocol 3: Genotyping of generated cell clones.

Ding, T., et al. (2023). "Establishment of a PPP2CA homozygous knockout human embryonic stem cell line via CRISPR/Cas9 system." Stem Cell Res **67**: 103029.

Protein phosphatase 2A (PPP2CA) is one of the four main Ser/Thr phosphatase enzymes, which involved in the negative control of cell growth and division. PPP2CA is the main protein phosphatase in the heart, which regulates Ca (2+) through a series of ion channels and transporters. In this study, we generated a PPP2CA homozygous knockout human embryonic stem cell line WAe009-A-25 based on the transient expression CRISPR/Cas9 system to investigate functional effect of PP1 deficiency. This cell line has multidirectional differentiation potential, normal karyotypic and trilineage differentiation potential in vivo.

Dong, T., et al. (2021). "The establishment of a homozygous SNTA1 knockout human embryonic stem cell line (WAe009-A-50) using the CRISPR/Cas9 system." Stem Cell Res **51**: 102196.

SNTA1 encodes alpha1-syntrophin, a scaffold protein, which is a component of the dystrophin-associated protein complex. Additionally, alpha1-

syntrophin interacts with SCN5A and nNOS-PMCA4b complex in cardiomyocytes. SNTA1 is a susceptibility locus for arrhythmia and cardiomyopathy. We generated a homozygous SNTA1 knockout human embryonic stem cell (H9SNTA1KO) using the CRISPR/Cas9 system. H9SNTA1KO maintained pluripotency and a normal karyotype and differentiated into three germ layers in vivo.

Drost, J., et al. (2017). "Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer." Science **358**(6360): 234-238.

Mutational processes underlie cancer initiation and progression. Signatures of these processes in cancer genomes may explain cancer etiology and could hold diagnostic and prognostic value. We developed a strategy that can be used to explore the origin of cancer-associated mutational signatures. We used CRISPR-Cas9 technology to delete key DNA repair genes in human colon organoids, followed by delayed subcloning and whole-genome sequencing. We found that mutation accumulation in organoids deficient in the mismatch repair gene MLH1 is driven by replication errors and accurately models the mutation profiles observed in mismatch repair-deficient colorectal cancers. Application of this strategy to the cancer predisposition gene NTHL1, which encodes a base excision repair protein, revealed a mutational footprint (signature 30) previously observed in a breast cancer cohort. We show that signature 30 can arise from germline NTHL1 mutations.

Fei, J. F., et al. (2014). "CRISPR-mediated genomic deletion of Sox2 in the axolotl shows a requirement in spinal cord neural stem cell amplification during tail regeneration." Stem Cell Reports **3**(3): 444-459.

The salamander is the only tetrapod that functionally regenerates all cell types of the limb and spinal cord (SC) and thus represents an important regeneration model, but the lack of gene-knockout technology has limited molecular analysis. We compared transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) in the knockout of three loci in the axolotl and find that CRISPRs show highly penetrant knockout with less toxic effects compared to TALENs. Deletion of Sox2 in up to 100% of cells yielded viable F0 larvae with normal SC organization and ependymoglial cell marker expression such as GFAP and ZO-1. However, upon tail amputation, neural stem cell proliferation was inhibited, resulting in spinal-cord-specific regeneration failure. In contrast, the mesodermal blastema formed normally. Sox3 expression during development, but not regeneration, most likely allowed embryonic survival and the regeneration-specific phenotype. This analysis represents the first tissue-specific regeneration phenotype from the genomic deletion of a gene in the axolotl.

Feng, Y., et al. (2020). "Generation of a RGS18 gene knockout cell line from a human embryonic stem cell line by CRISPR/Cas9." Stem Cell Res **49**: 102072.

RGS18 is a member of the RGS (Regulators of G-protein signaling) protein family, involved in megakaryopoiesis, megakaryocyte differentiation and chemotaxis. Here, we created a RGS18 knockout cell line from a human embryonic stem cell line by CRISPR/Cas9 mediated gene targeting, to further understand roles of RGS18 in these processes. The cell line maintains stem cell morphology and normal karyotype, and retains expression of pluripotent marker genes and differentiation potential in vivo. The RGS18(-/-) cell line will facilitate investigation of the role of RGS18 during multiple cellular processes in human pluripotent stem cell modeled hematopoiesis.

Galdos, F. X., et al. (2021). "Purification of Pluripotent Stem Cell-Derived Cardiomyocytes Using CRISPR/Cas9-Mediated Integration of Fluorescent Reporters." Methods Mol Biol **2158**: 223-240.

Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes have become critically important for the detailed study of cardiac development, disease modeling, and drug screening. However, directed differentiation of hiPSCs into cardiomyocytes often results in mixed populations of cardiomyocytes and other cell types, which may confound experiments that require pure populations of cardiomyocytes. Here, we detail the use of a CRISPR/Cas9 genome editing strategy to develop cardiomyocyte-specific reporters that allow for the isolation of hiPSC-derived cardiomyocytes and chamber-specific myocytes. Moreover, we describe a cardiac differentiation protocol to derive cardiomyocytes from hiPSCs, as well as a strategy to use fluorescence-activated cell sorting to isolate pure populations of fluorescently labeled cardiomyocytes for downstream applications.

Gao, Y., et al. (2021). "CRISPR/Cas9-edited triple-fusion reporter gene imaging of dynamics and function of transplanted human urinary-induced pluripotent stem cell-derived cardiomyocytes." Eur J Nucl Med Mol Imaging **48**(3): 708-720.

PURPOSE: To investigate the post-transplantation behaviour and therapeutic efficacy of human urinary-induced pluripotent stem cell-derived cardiomyocytes (hUiCMs) in infarcted heart. METHODS: We used clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9 (CRISPR/Cas9) technology to integrate a triple-

fusion (TF) reporter gene into the AAVS1 locus in human urine-derived hiPSCs (hUiPSCs) to generate TF-hUiPSCs that stably expressed monomeric red fluorescent protein for fluorescence imaging, firefly luciferase for bioluminescence imaging (BLI) and herpes simplex virus thymidine kinase for positron emission tomography (PET) imaging. RESULTS: Transplanted cardiomyocytes derived from TF-hUiPSCs (TF-hUiCMs) engrafted and proliferated in the infarcted heart as monitored by both BLI and PET imaging and significantly improved cardiac function. Under ischaemic conditions, TF-hUiCMs enhanced cardiomyocyte (CM) glucose metabolism and promoted angiogenic activity. CONCLUSION: This study established a CRISPR/Cas9-mediated multimodality reporter gene imaging system that can determine the dynamics and function of TF-hUiCMs in myocardial infarction, which is helpful for investigating the application of stem cell therapy.

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

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

Getachew, A., et al. (2021). "Generation of a TLR2 homozygous knockout human embryonic stem cell line WAe001-A-64 using CRISPR/Cas9 editing." Stem Cell Res **54**: 102401.

Toll-like receptor 2 (TLR2) is a pattern recognition receptor which plays an important role in innate immune system. In humans it's encoded by the TLR2 gene and also been designated as CD282. Using CRISPR/Cas9 gene editing technology, we have established a TLR2 mutant WAe001-A-64 cell line from the original embryonic stem cell line H1. It has adopted two biallelic deletions in exon 3 of TLR2 which resulted in a frame shift and early termination in the translation of TLR2. Moreover, WAe001-A-64 has maintained the normal karyotype, pluripotent phenotype, parental cell morphology and the ability to differentiate into three germ layers.

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

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

Gizon, M., et al. (2022). "Generation of a heterozygous SCN5A knockout human induced pluripotent stem cell line by CRISPR/Cas9 edition." Stem Cell Res **60**: 102680.

Mutations leading to haploinsufficiency in SCN5A, the gene encoding the cardiac sodium channel Na(v)1.5 alpha-subunit, are involved in life-threatening cardiac disorders. Using CRISPR/Cas9-mediated genome edition, we generated here a human induced-pluripotent stem cell (hiPSC) line carrying a heterozygous mutation in exon 2 of SCN5A, which leads to apparition of a premature stop codon. SCN5A-clone 5 line maintained normal karyotype, morphology and pluripotency and differentiated into three germ layers. Cardiomyocytes derived from these hiPSCs would be a useful model for investigating channelopathies related to SCN5A heterozygous deficiency.

Golchin, A., et al. (2020). "Advancing Mesenchymal Stem Cell Therapy with CRISPR/Cas9 for Clinical Trial Studies." Adv Exp Med Biol **1247**: 89-100.

Currently, regenerative medicine and cellular-based therapy have been in the center of attention worldwide in advanced medical technology. Mesenchymal stem cell (MSC) as a suitable stem cell source for cell-based therapy has been shown to be safe and effective in multiple clinical trial studies (CTSs) of

several diseases. Despite the advantages, MSC needs more investigation to enhance its therapeutic application. The CRISPR/Cas system is a novel technique for editing of genes that is being explored as a means to improve MSCs therapeutic usage. In this study, we review the recent studies that explore CRISPR potency in gene engineering of MSCs, which have great relevance in MSC-based therapies. However, CRISPR/Cas technology make possible specific targeting of loci in target genes, but next-generation MSC-based therapies to achieve extensive clinical application need dedicated efforts.

Gopal, S., et al. (2020). "Exploiting CRISPR Cas9 in Three-Dimensional Stem Cell Cultures to Model Disease." Front Bioeng Biotechnol **8**: 692.

Three-dimensional (3D) cell culture methods have been widely used on a range of cell types, including stem cells to modulate precisely the cellular biophysical and biochemical microenvironment and control various cell signaling cues. As a result, more in vivo-like microenvironments are recapitulated, particularly through the formation of multicellular spheroids and organoids, which may yield more valid mechanisms of disease. Recently, genome-engineering tools such as CRISPR Cas9 have expanded the repertoire of techniques to control gene expression, which complements external signaling cues with intracellular control elements. As a result, the combination of CRISPR Cas9 and 3D cell culture methods enhance our understanding of the molecular mechanisms underpinning several disease phenotypes and may lead to developing new therapeutics that may advance more quickly and effectively into clinical candidates. In addition, using CRISPR Cas9 tools to rescue genes brings us one step closer to its use as a gene therapy tool for various degenerative diseases. Herein, we provide an overview of bridging of CRISPR Cas9 genome editing with 3D spheroid and organoid cell culture to better understand disease progression in both patient and non-patient derived cells, and we address potential remaining gaps that must be overcome to gain widespread use.

Gosstola, N. C., et al. (2020). "Characterization of UMi028-A-1 stem cell line that contains a CRISPR/Cas9 induced hearing loss-associated variant (V60L (c.178G > T)) in the P2RX2 gene." Stem Cell Res **49**: 102017.

UMi028-A-1 hiPSC line contains a CRISPR/Cas9-induced heterozygous, hearing loss-associated variant (V60L (GTA > TTA)) in the Purinergic Receptor P2X2 (P2RX2) gene. This line, derived from an unaffected male iPSC line, has been successfully characterized for its cellular and genetic properties. The c.178G > T variant in P2RX2 is associated with non-syndromic, dominant, progressive hearing loss. Once differentiated into inner ear cell types, UMi028-A-1 will serve as a resource for understanding the molecular mechanisms underlying hearing loss and serve as a potential platform for testing therapeutic approaches to restore inner ear function.

Gross, T., et al. (2021). "Characterization of CRISPR/Cas9 RANKL knockout mesenchymal stem cell clones based on single-cell printing technology and Emulsion Coupling assay as a low-cellularity workflow for single-cell cloning." PLoS One **16**(3): e0238330.

The homogeneity of the genetically modified single-cells is a necessity for many applications such as cell line development, gene therapy, and tissue engineering and in particular for regenerative medical applications. The lack of tools to effectively isolate and characterize CRISPR/Cas9 engineered cells is considered as a significant bottleneck in these applications. Especially the incompatibility of protein detection technologies to confirm protein expression changes without a preconditional large-scale clonal expansion creates a gridlock in many applications. To ameliorate the characterization of engineered cells, we propose an improved workflow, including single-cell printing/isolation technology based on fluorescent properties with high yield, a genomic edit screen (Surveyor assay), mRNA RT-PCR assessing altered gene expression, and a versatile protein detection tool called emulsion-coupling to deliver a high-content, unified single-cell workflow. The workflow was exemplified by engineering and functionally validating RANKL knockout immortalized mesenchymal stem cells showing bone formation capacity of these cells. The resulting workflow is economical, without the requirement of large-scale clonal expansions of the cells with overall cloning efficiency above 30% of CRISPR/Cas9 edited cells. Nevertheless, as the single-cell clones are comprehensively characterized at an early, highly parallel phase of the development of cells including DNA, RNA, and protein levels, the workflow delivers a higher number of successfully edited cells for further characterization, lowering the chance of late failures in the development process.

Gulimiheranmu, M., et al. (2022). "Generation of a MIR5004 knockout cell line from human induced pluripotent stem cells by CRISPR/Cas9 gene editing." Stem Cell Res **62**: 102805.

MIR5004 is located in the intronic region of SYNGAP1, a genetic risk factor for Autism Spectrum Disorders (ASD), and co-expressed with SYNGAP1 in brain tissue, which indicates that MIR5004 may play an important role in ASD pathogenesis through the regulation of SYNGAP1. Here, we generated a

MIR5004 knockout human induced pluripotent stem cell (iPSC) line SHCDCLi001-B. SHCDCLi001-B shows expression of pluripotent markers, three lineage differentiation capacity, normal morphology and karyotypes, the same DNA origin with wild type iPSC (iPSC-WT) and no off-target effects, making it as a valuable tool for studying the interplay between MIR5004 and SYNGAP1 in ASD pathogenesis.

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, R., et al. (2023). "Generation of NANOS3-mCherry reporter human embryonic stem cell line SYSUe-009-A using CRISPR/Cas9." Stem Cell Res **67**: 103022.

NANOS3 is a zinc-finger containing RNA-binding protein that has been demonstrated to be highly expressed in human primordial germ cell(hPGC), thus NANOS3 can serve as a marker for hPGC development. Due to the ethical and technical restrictions, it is difficult to get primary human germline cells. Human primordial germ cell-like cells (hPGCLCs) generated from pluripotent stem cells is an excellent alternatives in human germ cell-related studies. This hESC line with an mCherry knock-in at the site before NANOS3's stop codon serves as a useful tool to learn human PGC specification.

Guo, T., et al. (2021). "Generation of a homozygous MYH7 gene knockout human embryonic stem cell line (WAe009-A-69) using an episomal vector-based CRISPR/Cas9 system." Stem Cell Res **57**: 102566.

Myosin heavy chain 7 (MYH7) encodes the human heart myosin heavy chain subunit, which plays an important role in myocardial contraction. MYH7 is the main pathogenic gene that causes Hypertrophic cardiomyopathy (HCM) and Dilated cardiomyopathy (DCM). In this experiment, a MYH7 homozygous knockout human embryonic stem cell (hESC) line, WAe009-A-69, was generated using an episomal vector-based CRISPR/Cas9 system. It can be an ideal tool to further study the function of MYH7. The cell line was confirmed with pluripotency, normal karyotype and trilineage differentiation potential.

Guo, X., et al. (2021). "CBLB ablation with CRISPR/Cas9 enhances cytotoxicity of human placental stem cell-derived NK cells for cancer immunotherapy." J Immunother Cancer **9**(3).

BACKGROUND: Tumors often develop resistance to surveillance by endogenous immune cells, which include natural killer (NK) cells. Ex vivo activated and/or expanded NK cells demonstrate cytotoxicity against various tumor cells and are promising therapeutics for adoptive cancer immunotherapy. Genetic modification can further enhance NK effector cell activity or activation sensitization. Here, we evaluated the effect of the genetic deletion of ubiquitin ligase Casitas B-lineage lymphoma pro-oncogene-b (CBLB), a negative regulator of lymphocyte activity, on placental CD34(+) cell-derived NK (PNK) cell cytotoxicity against tumor cells. METHODS: Using CRISPR/Cas9 technology, CBLB was knocked out in placenta-derived CD34(+) hematopoietic stem cells, followed by differentiation into PNK cells. Cell expansion, phenotype and cytotoxicity against tumor cells were characterized in vitro. The antitumor efficacy of CBLB knockout (KO) PNK cells was tested in an acute myeloid leukemia (HL-60) tumor model in NOD-scid IL2R gamma(null) (NSG) mice. PNK cell persistence, biodistribution, proliferation, phenotype and antitumor activity were evaluated. RESULTS: 94% of CBLB KO efficacy was achieved using CRISPR/Cas9 gene editing technology. CBLB KO placental CD34(+) cells differentiated into PNK cells with high cell yield and >90% purity determined by CD56(+) CD3(-) cell identity. Ablation of CBLB did not impact cell proliferation, NK cell

differentiation or phenotypical characteristics of PNK cells. When compared with the unmodified PNK control, CBLB KO PNK cells exhibited higher cytotoxicity against a range of liquid and solid tumor cell lines in vitro. On infusion into busulfan-conditioned NSG mice, CBLB KO PNK cells showed in vivo proliferation and maturation as evidenced by increased expression of CD16, killer Ig-like receptors and NKG2A over 3 weeks. Additionally, CBLB KO PNK cells showed greater antitumor activity in a disseminated HL60-luciferase mouse model compared with unmodified PNK cells. CONCLUSION: CBLB ablation increased PNK cell effector function and proliferative capacity compared with non-modified PNK cells. These data suggest that targeting CBLB may offer therapeutic advantages via enhancing antitumor activities of NK cell therapies.

Han, H. J., et al. (2019). "Generation of a KSCBi005-A-5(TLR8KO-A10) homozygous knockout human induced pluripotent stem cell line using CRISPR/Cas9." Stem Cell Res **40**: 101561.

The Toll like Receptor (TLR) family plays an essential role in pathogen recognition and innate immunity activation. TLR8, an endosomal receptor, can recognize single-stranded RNA viruses, such as influenza virus, Sendai virus, Coxsackie B virus, HIV, and HCV. TLR8 binding to the viral RNA recruits MyD88 and leads to activation of the transcription factor NF-kB and antiviral response. We generated biallelic mutants of the TLR8 gene using a CRISPR-Cas9 genome editing method in human induced pluripotent stem cells (hiPSCs). The TLR8 homozygous-knockout hiPSCs retained normal morphology, gene expression, and in vivo differentiation potential.

Han, H. J. and J. H. Kim (2021). "Establishment of a TLR3 homozygous knockout human induced pluripotent stem cell line using CRISPR/Cas9." Stem Cell Res **52**: 102187.

The Toll-like receptor (TLR) family plays an important role in the recognition of pathogens, including bacteria, viruses, fungi, and parasites, followed by the activation of innate immunity. TLR3 recognizes double-stranded RNA, a form of genetic material produced by positive-strand RNA viruses and DNA viruses, and is activated by viral infection. Upon recognition, TLR3 promotes the activation of interferon regulatory factor 3 to enhance the expression and secretion of type I interferons that signal other cells to enhance their antiviral defenses. We generated biallelic mutants of the TLR3 gene using a CRISPR-Cas9 genome editing method in human induced pluripotent stem cells (hiPSCs). TLR3 homozygous-knockout hiPSCs retained normal morphology, gene expression, and in vivo differentiation potential.

Han, H. J. and J. H. Kim (2021). "Generation of a TLR2 knockout human induced pluripotent stem cell line using CRISPR/Cas9." Stem Cell Res **57**: 102578.

The TLR family plays a fundamental function in the recognition of pathogens and activation of innate immunity. TLR2 is a membrane protein that is expressed on the surface of certain cells and recognizes foreign substances; it initiates a cascade of innate immune responses. TLR2 recognizes many bacterial, fungal, and viral components, as well as certain endogenous substances. We generated biallelic mutants of the TLR2 gene in human induced pluripotent stem cells (hiPSCs) using a CRISPR-Cas9 genome editing method. The TLR2 heterozygous-knockout hiPSCs retained normal morphology, gene expression, and in vivo differentiation potential.

Han, H. J., et al. (2019). "Generation of a TLR7 homozygous knockout human induced pluripotent stem cell line using CRISPR/Cas9." Stem Cell Res **40**: 101520.

Toll Like Receptor (TLR) family plays an important role in the activation of innate immunity against pathogens. TLR7 mediates the recognition of single-stranded RNA viruses, such as human immunodeficiency virus, hepatitis C virus, and influenza virus in endosomes. Here, we generated a TLR7 homozygous knockout human induced pluripotent cell (hiPSC) line, hiPSC-TLR7KO-A59, using the CRISPR/Cas9 genome editing method. The hiPSC-TLR7KO-A59 line maintains normal morphology, pluripotency, and differentiation capacity into three germ layers.

Hazrati, A., et al. (2022). "CRISPR/Cas9-engineered mesenchymal stromal/stem cells and their extracellular vesicles: A new approach to overcoming cell therapy limitations." Biomed Pharmacother **156**: 113943.

Cell therapy is one of the newest therapeutic approaches for treating tissue destruction diseases and replacing damaged parts in defective tissues. Among different cells, mesenchymal stem cells (MSCs) have received a lot of attention due to their advantages and desirable properties. Also, MSCs-derived secretome, which includes various growth factors, cytokines, and extracellular vesicles (EVs), is used in the treatment of different types of diseases. However, the application of MSCs in an intact form brings their functionality with limitations. For this reason, different methods are recommended to increase their efficiency and the extracellular vesicles derived from them. One of these methods is gene editing of these cells. Among the different techniques for MSCs gene editing,

CRISPR/Cas9 can increase the therapeutic potential of MSCs in a targeted manner due to its advantages. In order to achieve the desired result, various genes have been manipulated in MSCs, including genes involved in stemness, aging, migration, proliferation, survival, and inflammatory responses. Engineering MSCs with this method affects the cells' characteristics, changes their cytokine and different growth factors secretions, and increases their therapeutic efficiency.

He, L., et al. (2021). "CRISPR/Cas9/AAV9-mediated in vivo editing identifies MYC regulation of 3D genome in skeletal muscle stem cell." Stem Cell Reports **16**(10): 2442-2458.

Skeletal muscle satellite cells (SCs) are stem cells responsible for muscle development and regeneration. Although CRISPR/Cas9 has been widely used, its application in endogenous SCs remains elusive. Here, we generate mice expressing Cas9 in SCs and achieve robust editing in juvenile SCs at the postnatal stage through AAV9-mediated short guide RNA (sgRNA) delivery. Additionally, we reveal that quiescent SCs are resistant to CRISPR/Cas9-mediated editing. As a proof of concept, we demonstrate efficient editing of master transcription factor (TF) Myod1 locus using the CRISPR/Cas9/AAV9-sgRNA system in juvenile SCs. Application on two key TFs, MYC and BCL6, unveils distinct functions in SC activation and muscle regeneration. Particularly, we reveal that MYC orchestrates SC activation through regulating 3D genome architecture. Its depletion results in strengthening of the topologically associating domain boundaries thus may affect gene expression. Altogether, our study establishes a platform for editing endogenous SCs that can be harnessed to elucidate the functionality of key regulators governing SC activities.

Heider, J., et al. (2022). "Generation of two induced pluripotent stem cell lines (TMOi001-A-5, TMOi001-A-6) carrying variants in DISC1 exon 2 using CRISPR/Cas9 gene editing." Stem Cell Res **64**: 102925.

DISC1 is a scaffold protein involved in key developmental processes such as neuronal migration, differentiation and neurogenesis. Genetic variants of the DISC1 gene have been linked to neuropsychiatric disorders like schizophrenia, bipolar disorder and major depression. Here, we generated two isogenic iPSC lines carrying mutations in DISC1 exon 2 using CRISPR/Cas9 gene editing. Both lines express pluripotency markers, can be differentiated into the three germ layers and present a normal karyotype. The generated iPSC lines can be used to study the implications of DISC1 mutations in the context of neuropsychiatric diseases in vitro.

Herring, A., et al. (2016). "Generation of a TLE1 homozygous knockout human embryonic stem cell line using CRISPR-Cas9." Stem Cell Res **17**(2): 430-432.

Here, we generated a biallelic mutation in the TLE1 (Transducin Like Enhancer of Split 1) gene using CRISPR-Cas9 editing in the human embryonic stem cell (hESC) line WA01. The homozygous knockout cell line, TLE1-464-G04, displays loss of TLE1 protein expression while maintaining pluripotency, differentiation potential and genomic integrity.

Hommersom, M. P., et al. (2022). "Generation of induced pluripotent stem cell lines carrying monoallelic (UCSFi001-A-60) or biallelic (UCSFi001-A-61; UCSFi001-A-62) frameshift variants in CACNA1A using CRISPR/Cas9." Stem Cell Res **61**: 102730.

CACNA1A encodes a P/Q-type voltage-gated calcium channel. Heterozygous loss-of-function variants in this gene have been associated with episodic ataxia type 2. In this study, we used CRISPR/Cas9 to generate isogenic human induced pluripotent stem cell lines with a gene-dosage dependent deficiency of CACNA1A. We obtained one clone with monoallelic (UCSFi001-A-60) and two clones with biallelic (UCSFi001-A-61; UCSFi001-A-62) frameshift variants in CACNA1A. All three lines showed expression of pluripotency markers and a normal karyotype.

Hsu, M. N., et al. (2019). "CRISPR technologies for stem cell engineering and regenerative medicine." Biotechnol Adv **37**(8): 107447.

CRISPR/Cas9 system exploits the concerted action of Cas9 nuclease and programmable single guide RNA (sgRNA), and has been widely used for genome editing. The Cas9 nuclease activity can be abolished by mutation to yield the catalytically deactivated Cas9 (dCas9). Coupling with the customizable sgRNA for targeting, dCas9 can be fused with transcription repressors to inhibit specific gene expression (CRISPR interference, CRISPRi) or fused with transcription activators to activate the expression of gene of interest (CRISPR activation, CRISPRa). Here we introduce the principles and recent advances of these CRISPR technologies, their delivery vectors and review their applications in stem cell engineering and regenerative medicine. In particular, we focus on in vitro stem cell fate manipulation and in vivo applications such as prevention of retinal and muscular degeneration, neural regeneration, bone regeneration, cartilage tissue engineering, as well as treatment of diseases in blood, skin and liver. Finally, the challenges to translate CRISPR to regenerative medicine and future perspectives are discussed and proposed.

Hsu, M. N., et al. (2019). "CRISPR-based Activation of Endogenous Neurotrophic Genes in Adipose Stem Cell Sheets to Stimulate Peripheral Nerve Regeneration." Theranostics **9**(21): 6099-6111.

Background: Peripheral nerve regeneration requires coordinated functions of neurotrophic factors and neuronal cells. CRISPR activation (CRISPRa) is a powerful tool that exploits inactive Cas9 (dCas9), single guide RNA (sgRNA) and transcription activator for gene activation, but has yet to be harnessed for tissue regeneration. Methods: We developed a hybrid baculovirus (BV) vector to harbor and deliver the CRISPRa system for multiplexed activation of 3 neurotrophic factor genes (BDNF, GDNF and NGF). The hybrid BV was used to transduce rat adipose-derived stem cells (ASC) and functionalize the ASC sheets. We further implanted the ASC sheets into sciatic nerve injury sites in rats. Results: Transduction of rat ASC with the hybrid BV vector enabled robust, simultaneous and prolonged activation of the 3 neurotrophic factors for at least 21 days. The CRISPRa-engineered ASC sheets were able to promote Schwann cell (SC) migration, neuron proliferation and neurite outgrowth in vitro. The CRISPRa-engineered ASC sheets further enhanced in vivo functional recovery, nerve reinnervation, axon regeneration and remyelination. Conclusion: These data collectively implicated the potentials of the hybrid BV-delivered CRISPRa system for multiplexed activation of endogenous neurotrophic factor genes in ASC sheets to promote peripheral nerve regeneration.

Huang, Y., et al. (2021). "Generation of an EFNB2-2A-mCherry reporter human embryonic stem cell line using CRISPR/Cas9-mediated site-specific homologous recombination." Stem Cell Res **52**: 102241.

Ephrin B2 (EFNB2) is the first identified and most widely used marker for arterial endothelial cells (AECs). We generated a heterozygous EFNB2-2A-mCherry reporter H1 cell line, H1-EFNB2-2A-mCherry(+/-) (WAe001-A-57), by CRISPR/Cas9-mediated insertion of 2A-mCherry cassette into the EFNB2 gene locus, immediately before the translation stop codon. The H1-EFNB2-2A-mCherry reporter cells were pluripotent and could differentiate into all three germ layer lineages. Simultaneous expression of mCherry was observed when expression of EFNB2 was increased during endothelial cell differentiation. Thus, the generated reporter cells enable live identification of EFNB2-positive AECs, and screening of small molecule compound and target genes that promote AEC differentiation.

Hunt, C. P. J., et al. (2017). "Characterising the developmental profile of human embryonic stem cell-derived medium spiny neuron progenitors and assessing mature neuron function using a CRISPR-generated human DARPP-32(WT/eGFP-AMP) reporter line." Neurochem Int **106**: 3-13.

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

Itoh, M., et al. (2020). "Footprint-free gene mutation correction in induced pluripotent stem cell (iPSC) derived from recessive dystrophic epidermolysis bullosa (RDEB) using the CRISPR/Cas9 and piggyBac transposon system." J Dermatol Sci **98**(3): 163-172.

BACKGROUND: Recessive dystrophic epidermolysis bullosa (RDEB) is a monogenic skin blistering disorder caused by mutations in the type VII collagen gene. A combination of biological technologies, including induced pluripotent stem cells (iPSCs) and several gene-editing tools, allows us to develop gene and cell therapies for such inherited diseases. However, the methodologies for gene and cell therapies must be continuously innovated for safe clinical use. OBJECTIVE: In this study, we used the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology to correct the pathogenic mutation in RDEB-specific iPSCs, and the piggyBac transposon system so that no residual gene fragments remained in the genome of iPSCs after

correcting the mutation. METHODS: For homologous recombination (HR)-based gene editing using CRISPR/Cas9, we designed guide RNA and template DNA including homologous sequences with drug-mediated selection cassette flanked by inverted repeat sequences of the transposon. HR reaction using CRISPR/Cas9 was induced in RDEB-specific iPSCs, and mutation-corrected iPSCs (MC-iPSCs) was obtained. Consequently, the selection cassette in the genome of MC-iPSCs was removed by transposase expression. RESULTS: After CRISPR/Cas9-induced gene editing, we confirmed that the pathogenic mutation in RDEB-specific iPSCs was properly corrected. In addition, MC-iPSCs had no genetic footprint after removing the selection cassette by transposon system, and maintained their "stemness". When differentiating MC-iPSCs into keratinocytes, the expression of type VII collagen was restored. CONCLUSIONS: Our study demonstrated one of the safer approaches to establish gene and cell therapies for skin hereditary disorders for future clinical use.

Iwagawa, T., et al. (2023). "Evaluation of CRISPR/Cas9 exon-skipping vector for choroideremia using human induced pluripotent stem cell-derived RPE." J Gene Med **25**(2): e3464.

BACKGROUND: Exon-skipping is a powerful genetic tool, especially when delivering genes using an AAV-mediated full-length gene supplementation strategy is difficult owing to large length of genes. Here, we used engineered human induced pluripotent stem cells and artificial intelligence to evaluate clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9-based exon-skipping vectors targeting genes of the retinal pigment epithelium (RPE). The model system was choroideremia; this is an X-linked inherited retinal disease caused by mutation of the CHM gene. METHODS: We explored whether artificial intelligence detected differentiation of human OTX2, PAX6 and MITF (hOPM) cells, in which OTX2, PAX6 and MITF expression was induced by doxycycline treatment, into RPE. Plasmid encoding CHM exon-skipping modules targeting the splice donor sites of exons 6 were constructed. A clonal hOPM cell line with a frameshift mutation in exon 6 was generated and differentiated into RPE. CHM exon 6-skipping was induced, and the effects of skipping on phagocytic activity, cell death and prenylation of Rab small GTPase (RAB) were evaluated using flow cytometry, an in vitro prenylation assay and western blotting. RESULTS: Artificial intelligence-based evaluation of RPE differentiation was successful. Retinal pigment epithelium cells with a frameshift mutation in exon 6 showed increased cell death, reduced phagocytic activity and increased cytosolic unprenylated RABs only when oxidative stress was in play. The latter two phenotypes were partially rescued by exon 6-skipping of CHM. CONCLUSIONS: CHM exon 6-skipping contributed to RPE phagocytosis probably by increasing RAB38 prenylation under oxidative stress.

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

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

Jelinkova, S., et al. (2021). "Derivation of human pluripotent stem cell line via CRISPR/Cas9 mediated deletion of exon 3 LAMA2 gene (DMBi001-A-1)." Stem Cell Res **56**: 102529.

LAMA2-related muscular dystrophy (LAMA2-MD) results from mutations in LAMA2 gene, encoding laminin alpha-2. It is a congenital disease characterized by muscle wasting, with the most severe version being diagnosed within first few months after birth. To generate LAMA2-DM in vitro model, we excised exon 3 from the LAMA2 gene in our previously derived healthy human induced pluripotent stem cells (hiPSCs). Obtained hiPSCs show expression of pluripotency markers, differentiation capacity into all three germ layers, normal karyotype and lack of LAMA2 expression on mRNA and protein level after differentiation into skeletal myocytes. Accordingly, it may provide novel insight into the molecular basis of LAMA2-MD.

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.

Jo, S., et al. (2021). "Generation of an ACTA2-EGFP reporter human induced pluripotent stem cell line, KITi001-C-41, using CRISPR/Cas9-mediated homologous recombination." Stem Cell Res **56**: 102524.

Alpha-smooth muscle actin (alpha-SMA) is encoded by ACTA2 and is a key protein in the cellular contractile system of various mesodermal cell types, including hepatic stellate cells (HSCs), smooth muscle cells, and cardiomyocytes. alpha-SMA, which is a key protein in the development of hepatic fibrosis, is widely used as a reliable marker of HSC activation. Here, we generated an ACTA2-EGFP reporter human induced pluripotent stem cell line, KITi001-C-41, using a CRISPR/Cas9-based knock-in system. These reporter hiPSC lines can be used for lineage tracing of mesodermal cells and for screening of HSC activation factors.

Jung, J., et al. (2021). "Generation of Brachyury-mCherry knock-in reporter human pluripotent stem cell line (SNUe003-A-2) using CRISPR/CAS9 nuclease." Stem Cell Res **53**: 102321.

Brachyury is an embryonic nuclear transcription factor required for mesoderm formation and differentiation. Here, we introduced an mCherry reporter into the C-terminus of Brachyury in the human pluripotent stem cell line SNUhES3 using the CRISPR/Cas9 nuclease approach. Successful gene editing was verified by DNA sequencing. SNUhES3-Brachyury-mCherry cells expressed pluripotent stem cell markers, exhibited a normal karyotype, and could generate all three germ layers. This cell line expressed the red fluorescence protein mCherry upon the induction of mesoderm differentiation. This reporter cell line could be used to monitor mesodermal population enrichment during mesodermal differentiation.

Kamat, K. and M. S. Inamdar (2023). "Generation of OCIAD2 homozygous knockout (BJNhem20-OCIAD2-CRISPR-33) and heterozygous knockout (BJNhem20-OCIAD2-CRISPR-40) human embryonic stem cell lines using CRISPR-Cas9 mediated targeting." Stem Cell Res **67**: 103026.

Ovarian Carcinoma Immunoreactive Antigen domain containing 2 (OCIAD2) was knocked out by targeting its exon 4 through CRISPR-Cas9 paired nickase strategy to generate two OCIAD2 knockout human embryonic stem cell lines- one homozygous (BJNhem20-OCIAD2-CRISPR-33) and one heterozygous (BJNhem20-OCIAD2-CRISPR-40) for mutant ociad2. Both lines maintain pluripotency, normal karyotype, and trilineage differentiation potential.

Kang, B., et al. (2022). "Generation of a GFI1-flag knock-in human embryonic stem cell line using CRISPR-Cas9 technology." Stem Cell Res **60**: 102724.

GFI1 is a DNA binding transcriptional repressor, it is shown to be an important gene associated with blood cells development and many blood diseases (Moroy et al., 2015). But the role of GFI1 in human hematopoieticdevelopment has not been known (Thambyrajah et al., 2016). To illustrate the function of GFI1 in human hematopoieticdevelopment, we constructed a GFI1-2 x flag-tag knock-in human embryonic stem cell line by CRISPR/Cas9 mediated gene targeting, and it would be the effective tool to study GFI1. The cell line could express GFI1-2 x flag-tag and can be identified with western blot and immunofluorescence. This cell line maintains stem cell morphology, and displays normal karyotype, pluripotent stem cell marker expression and differentiation potential.

Kang, J. Y., et al. (2022). "Generation of a heterozygous TPM1-E192K knock-in human induced pluripotent stem cell line using CRISPR/Cas9 system." Stem Cell Res **63**: 102878.

E192K missense mutation of TPM1 has been found in different types of cardiomyopathies (e.g., hypertrophic cardiomyopathy, dilated cardiomyopathy, and left ventricular non-compaction), leading to systolic dysfunction, diastolic dysfunction, and/or tachyarrhythmias. Here, we generated a heterozygous TPM1-E192K knock-in human induced pluripotent stem cell (iPSC) line using CRISPR/Cas9-based genome editing system. The cells exhibit normal karyotype, typical stem cell morphology, expression of pluripotency markers and differentiation ability into three germ layers. Accordingly, this cell line could provide a useful cell resource for exploring the pathogenic role of TPM1-E192K mutation in different types of cardiomyopathies.

Kang, J. Y., et al. (2022). "Generation of three TTN knock-out human induced pluripotent stem cell lines using CRISPR/Cas9 system." Stem Cell Res **64**: 102901.

TTN mutations are the common genetic cause for various types of cardiomyopathies (e.g., dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy) and skeletal myopathies. Here, we generated three TTN knock-out human induced pluripotent stem cell (iPSC) lines using CRISPR/Cas9 system. These cell lines, which exhibit normal karyotype, typical morphology and pluripotency, could provide useful platform for investigating the role of TTN in associated disorders.

Khampang, S., et al. (2023). "CRISPR/Cas9 mediated approach to generate YAP-depleted human embryonic stem cell line (MUSIe002-A-1)." Stem Cell Res **66**: 102990.

Yes-associated protein (YAP), an important effector protein of the Hippo signaling pathway, acts as a molecular switch in controlling cell proliferation and apoptosis. In this study, a YAP-targeted isogenic sub-clone of the MUSIe002-A was generated, designated as MUSIe002-A-1. The MUSIe002-1 cell line had normal pluripotent stem cell characteristics and karyotype. Its ability to differentiate into three germ layers was confirmed. As reduction of YAP does not disturb the pluripotency of hESCs, this cell line serves as a valuable model to extrapolate the functional role of YAP in stem cell biology and its applications.

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

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

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

Nuclear factor erythroid 2-related factor 2 (NFE2L2 or Nrf2) is a well-known transcription factor that regulates the expression of a large number of anti-oxidant genes in mammalian cells (J.H. Kim et al., 2014). Here, we generated a homozygous Nrf2 knockout human embryonic stem cell (hESC) line, H9Nrf2KO-A13, using the CRISPR/Cas9 genome editing method. The Nrf2 homozygous knockout H9 cell line maintains pluripotency, differentiation potential into three germ layers, and a normal karyotype.

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

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

Kim, Y. K., et al. (2020). "Generation of a GLA knock-out human-induced pluripotent stem cell line, KSBCi002-A-1, using CRISPR/Cas9." Stem Cell Res **42**: 101676.

Fabry disease is an X-linked inherited disease caused by a mutation in the galactosidase alpha (GLA) gene. Here, we generated a GLA knock-out cell line (GLA-KO hiPSCs) from normal human-induced pluripotent stem cells (hFSiPS1) using the CRISPR-Cas9 genome-editing tool. The GLA-KO hiPSCs maintained normal morphology, karyotypes, expression of stemness markers, and trilineage differentiation potential. Furthermore, the GLA-KO hiPSCs exhibited dissipation of GLA activity and abnormal Globotriaosylceramide (Gb3) accumulation. Our GLA-KO hiPSC line represents a valuable tool for studying the mechanisms involved in Fabry disease and the development of novel therapeutic alternatives to treat this rare condition.

Lahm, H., et al. (2022). "Generation of a CRISPR/Cas edited human induced pluripotent stem cell line DHMi005-A-1 carrying a patient-specific disease-causing point mutation in the TBX5 gene." Stem Cell Res **60**: 102691.

A number of mutations in the human TBX5 gene have been described which cause Holt-Oram syndrome, a severe congenital disease associated with abnormalities in heart and upper limb development. We have used a prime-editing approach to introduce a patient-specific disease-causing TBX5 mutation (c.920_C > A) into an induced pluripotent stem cell (iPSC) line from a healthy donor. The resulting iPSC line provides a powerful tool to identify and analyze the biological and molecular impact of this specific TBX5 mutation in comparison to the isogenic control iPSC line during cardiac development.

Lahm, H., et al. (2023). "Generation of two CRISPR/Cas edited human induced pluripotent stem cell lines (DHMi005-A-3 and DHMi005-A-4) carrying a FLAG-tag after exon 9 of the TBX5 gene." Stem Cell Res **66**: 103011.

Although TBX5 plays a major role during human cardiogenesis and initiates and controls limb development, many of its interactions with genomic DNA and the resulting biological consequences are not well known. Existing anti-TBX5-antibodies work very inefficiently in certain applications such as ChIP-Seq analysis. To circumvent this drawback, we introduced a FLAG-tag sequence into the TBX5 locus at the end of exon 9 prior to the stop codon by CRISPR/Cas9. The expressed TBX5-FLAG fusion protein can effectively be precipitated by anti-FLAG antibodies. Therefore, these gene-edited iPSC lines represent powerful cellular in vitro tools to unravel TBX5:DNA interactions in detail.

Laverde-Paz, M. J., et al. (2021). "Derivation of stem cell line UMi028-A-2 containing a CRISPR/Cas9 induced Alzheimer's disease risk variant p.S1038C in the TTC3 gene." Stem Cell Res **52**: 102258.

The UMi028-A-2 human induced pluripotent stem cell line carries a homozygous mutation (rs377155188, C>G, p.S1038C) in the tetratricopeptide repeat domain 3 (TTC3) gene that was introduced via CRISPR/Cas9 genome editing. The line was originally derived from a neurologically normal male and has been thoroughly characterized following editing. The p.S1038C variant has been shown to potentially contribute to the risk of late onset Alzheimer's disease and is a resource to further investigate the consequences of TTC3 and this alteration in disease pathology.

Lee, H. M., et al. (2022). "Generation of alphaMHC-EGFP knock-in in human pluripotent stem cell line, SNUe003-A-3 using CRISPR/Cas9-based gene targeting." Stem Cell Res **61**: 102779.

The cardiac muscle-specific protein, alpha-myosin heavy chain (alphaMHC), is a major component of cardiac muscle filaments involved in cardiac muscle contraction. Here, we established an alphaMHC-enhanced fluorescent protein (EGFP) knock-in human pluripotent stem cell (hPSC) line by linking the EGFP gene to the C-terminal region of alphaMHC via a 2A non-joining peptide using CRISPR/Cas9 nuclease. The EGFP reporter precisely reflected the endogenous level of alphaMHC upon the induction of cardiac differentiation. This reporter cell line will be a valuable platform for cardiotoxicity tests, drug screening, and investigating the pathological mechanisms of cardiomyocytes.

Lee, M., et al. (2020). "Generation of OCT4-EGFP, NANOG-tdTomato dual reporter human induced pluripotent stem cell line, KKUi001-A, using the CRISPR/Cas9 system." Stem Cell Res **48**: 101943.

OCT4 and NANOG are core transcription factor genes in self-renewal, differentiation, and reprogramming. Here, we generated an OCT4-EGFP, NANOG-tdTomato dual reporter hiPSC line, KKUi001-A, on the basis of human induced pluripotent stem cells using CRISPR/Cas9 technology. EGFP and tdTomato reporter were inserted into before the stop codon of OCT4 and NANOG, respectively. Simultaneous expression of EGFP and tdTomato was observed when expression of OCT4 and NANOG was changed during differentiation and reprogramming. KKUi001-A hiPSC line will be a useful tool to find initial time point of OCT4 and NANOG expression

during reprogramming process and to screen small molecules that promote reprogramming.

Lee, O. H., et al. (2021). "Generation of a B2M homozygous knockout human somatic cell nuclear transfer-derived embryonic stem cell line using the CRISPR/Cas9 system." Stem Cell Res **59**: 102643.

Beta2-microglobulin (B2M) is a subunit of human leukocyte antigen class-I (HLA-I) heterodimer that mediates immune rejection through activation of cytotoxic T cells. B2M binding to HLA-I proteins is essential for functional HLA-I on the cell surface. Here, we generated a B2M homozygous knockout somatic cell nuclear transfer-induced embryonic stem cell (SCNT-ESC) line using CRISPR/Cas9-mediated gene targeting. B2M KO cell line, which does not express HLA-I molecules on cell surface, has pluripotency and differentiation ability to three germ layers. This cell line provides a useful cell source for investigating immunogenicity of allogeneic ESCs and their derivatives for tissue regeneration.

Lee, Y., et al. (2019). "Generation of a NESTIN-EGFP reporter human induced pluripotent stem cell line, KSCBi005-A-1, using CRISPR/Cas9 nuclease." Stem Cell Res **40**: 101554.

NESTIN, an intermediate filament, is a neuroectodermal marker involved in induced pluripotent stem cell (iPSC) differentiation toward neural lineages. Here, we introduced an EGFP reporter into the C-terminus of NESTIN in KSCBi005-A hiPSCs through homologous recombination using CRISPR/Cas9 nuclease. The successfully edited line was confirmed by sequencing and had a normal karyotype. It expressed EGFP upon induction of neural differentiation and exhibited potential for differentiation into three germ layers. KSCBi005-A-1 cells could be used to monitor the expression of NESTIN in differentiated cell types. This cell line is available at the National Stem Cell Bank, Korea National Institute of Health.

Lee, Y., et al. (2019). "Generation of a PDX1-EGFP reporter human induced pluripotent stem cell line, KSCBi005-A-3, using the CRISPR/Cas9 system." Stem Cell Res **41**: 101632.

PDX1 plays a crucial role in the development and maintenance of beta-cells and directly regulates pancreatic beta-cell-specific transcription factors by binding to the insulin gene. Here, we introduced an EGFP reporter into the C-terminus of PDX1 in KSCBi005-A human induced pluripotent stem cells through homologous recombination using CRISPR/Cas9 nuclease. The cells had a normal karyotype, expressed several pluripotency markers, and maintained their differentiation potential. KSCBi005-A-3 cells can be used to monitor PDX1 expression in live cells during beta-cell differentiation; the cell line has been registered at the National Stem Cell Bank, Korea National Institute of Health.

Leidy-Davis, T., et al. (2018). "Viable Mice with Extensive Gene Humanization (25-kbp) Created Using Embryonic Stem Cell/Blastocyst and CRISPR/Zygote Injection Approaches." Sci Rep **8**(1): 15028.

Here, we describe an expansion of the typical DNA size limitations associated with CRISPR knock-in technology, more specifically, the physical extent to which mouse genomic DNA can be replaced with donor (in this case, human) DNA at an orthologous locus by zygotic injection. Driving our efforts was the desire to create a whole animal model that would replace 17 kilobase pairs (kbp) of the mouse Bcl2l11 gene with the corresponding 25-kbp segment of human BCL2L11, including a conditionally removable segment (2.9-kbp) of intron 2, a cryptic human exon immediately 3' of this, and a native human exon some 20 kbp downstream. Using two methods, we first carried out the replacement by employing a combination of bacterial artificial chromosome recombineering, classic embryonic stem cell (ESC) targeting, dual selection, and recombinase-driven cassette removal (ESC/Blastocyst Approach). Using a unique second method, we employed the same vector (devoid of its selectable marker cassettes), microinjecting it along with redundant single guide RNAs (sgRNAs) and Cas9 mRNA into mouse zygotes (CRISPR/Zygote Approach). In both instances, we were able to achieve humanization of Bcl2l11 to the extent designed, remove all selection cassettes, and demonstrate the functionality of the conditionally removable, loxP-flanked, 2.9-kbp intronic segment.

Li, C., et al. (2019). "Generation of FOS gene knockout lines from a human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **39**: 101479.

FOS is component of the AP-1 complex and has been reported to be involved in many cellular functions, including cell proliferation, differentiation, survival, angiogenesis, hematopoiesis and cancer progress. To further understand the exact role of FOS in these processes, here we created two FOS knockout human embryonic stem cell lines by CRISPR/Cas9 mediated gene targeting. These cell lines retained normal morphology and karyotype, normal expression of pluripotent markers, and differentiation potential both in vivo and in vitro. These cell lines can be used to verify whether the FOS mutated produces any affect on endothelial cells and hematopoietic progenitor cells during the hematopoietic differentiation.

Li, J., et al. (2020). "Generation of GADD45A gene knockout human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **49**: 102090.

GADD45A is a DNA damage and stressful growth arrest inducible protein, also it is shown to a be tumor suppressor gene and a chromatin relaxer associated with opening chromatin during the somatic reprogramming. However, its role in human embryonic stem cells and human embryonic stem cell modeled development has been merely documented. To illustrate the function of GADD45A in the human embryonic stem cell biology, we reported a GADD45A knockout human embryonic stem cell line by CRISPR/Cas9 mediated gene targeting. This cell line displayed normal karyotype, pluripotent stem cell marker expression and differentiation potential both in vivo and vitro.

Li, J., et al. (2021). "Generation of SHMT2 knockout human embryonic stem cell line (WAe009-A-67) using CRISPR/Cas9 technique." Stem Cell Res **57**: 102581.

Serine hydroxymethyltransferase 2 (SHMT2), a catalytic enzyme playing an important role in aerobic cellular respiration and mitochondrial metabolism, might be pivotal in self-renewal and differentiation of human pluripotent stem cells. Herein, we used the CRISPR/Cas9 editing system to construct a homozygous SHMT2 knockout (SHMT2-KO) human embryonic stem cell (hESC) line, exhibiting a normal karyotype, colony morphology, and high expression levels of pluripotent proteins. Furthermore, SHMT2 knockout did not impact the self-renewal ability or differentiation potential into three germ layers of hESCs. Accordingly, this cell line provides a valuable model for further assessing SHMT2 functions in human embryonic development.

Li, Q., et al. (2022). "Generation of a human embryonic stem cell line targeted homozygous deletion of BMP10 (WAe007-A-2) by CRISPR/Cas9-dgRNA." Stem Cell Res **65**: 102942.

BMP10 signaling has been implicated in regulation of cardiovascular cell fate determination and diseases, while the underlying molecular mechanism still remains uncertain. Here, the human embryonic stem cell line (H7-BMP10(del)) with homozygous deletion of BMP10 was generated by CRISPR/Cas9 method. Thus, the crosstalk related to BMP10 signaling could be investigated in cell fate determination and the molecular pathogenesis of cardiovascular disease.

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, X. F., et al. (2019). "CRISPR/Cas9 facilitates genomic editing for large-scale functional studies in pluripotent stem cell cultures." Hum Genet **138**(11-12): 1217-1225.

Pluripotent stem cell (PSC) cultures form an integral part of biomedical and medical research due to their capacity to rapidly proliferate and differentiate into hundreds of highly specialized cell types. This makes them a highly useful tool in exploring human physiology and disease. Genomic editing of PSC cultures is an essential method of attaining answers to basic physiological functions, developing in vitro models of human disease, and exploring potential therapeutic strategies and the identification of drug targets. Achieving reliable and efficient genomic editing is an important aspect of using large-scale PSC cultures. The CRISPR/Cas9 genomic editing tool has facilitated highly efficient gene knockout, gene correction, or gene modifications through the design and use of single-guide RNAs which are delivered to the target DNA via Cas9. CRISPR/Cas9 modification of PSCs has furthered the understanding of basic physiology and has been utilized to develop in vitro disease models, to test therapeutic strategies, and to facilitate regenerative or tissue repair approaches. In this review, we discuss the benefits of the CRISPR/Cas9 system in large-scale PSC cultures.

Li, Y., et al. (2019). "Generation of NERCe003-A-3, a p53 compound heterozygous mutation human embryonic stem cell line, by CRISPR/Cas9 editing." Stem Cell Res **34**: 101371.

p53 is a tumor suppressor gene involved mainly in the regulation of the G1/S cell cycle phase, DNA repair, and senescence. Although p53 is frequently altered in human cancer, the consequences of its depletion in human embryonic stem cells (hESCs) are unknown. We generated NERCe003-A-3, a p53 knockout hESC line, from the normal NERCe003-A hESC line by using CRISPR/Cas9 editing. This cell line maintained a normal 46, XY karyotype. Further analysis suggested that the cells expressed pluripotency-related markers and had the capacity to differentiate in vitro into derivatives of all three germ layers.

Li, Y., et al. (2021). "Generation of PARP1 gene knockout human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **53**: 102288.

PARP1 encodes a chromatin-associated enzyme which responsible for post-translational poly(ADP-ribosyl)ation modification (Hsieh et al., 2017). It plays an important role in nucleotide excision repair, non-homologous end joining, DNA mismatch repair and many other DNA repair process. Also, PARP1 participates in inflammation and aging. However, its role in human embryonic stem cell biology has not been fully resolved. To clarify the function of PARP1 in human embryonic stem cells, we reported a PARP1 knockout human embryonic stem cell line, generated by CRISPR/Cas9 mediated gene targeting. This cell line shows normal karyotype, pluripotent stem cell marker expression and differentiation potential in vitro.

Liao, H. K., et al. (2015). "Stem cell, CRISPR and HIV." Cell Cycle **14**(13): 1991-1992.

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.

Luo, Y., et al. (2016). "Targeted Inhibition of the miR-199a/214 Cluster by CRISPR Interference Augments the Tumor Tropism of Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells under Hypoxic Condition." Stem Cells Int **2016**: 3598542.

The human induced pluripotent stem cell (hiPSC) provides a breakthrough approach that helps overcoming ethical and allergenic challenges posed in application of neural stem cells (NSCs) in targeted cancer gene therapy. However, the tumor-tropic capacity of hiPSC-derived NSCs (hiPS-NSCs) still has much room to improve. Here we attempted to promote the tumor tropism of hiPS-NSCs by manipulating the activity of endogenous miR-199a/214 cluster that is involved in regulation of hypoxia-stimulated cell migration. We first developed a baculovirus-delivered CRISPR interference (CRISPRi) system that sterically blocked the E-box element in the promoter of the miR-199a/214 cluster with an RNA-guided catalytically dead Cas9 (dCas9). We then applied this CRISPRi system to hiPS-NSCs and successfully suppressed the expression of miR-199a-5p, miR-199a-3p, and miR-214 in the microRNA gene cluster. Meanwhile, the expression levels of their targets related to regulation of hypoxia-stimulated cell migration, such as HIF1A,

MET, and MAPK1, were upregulated. Further migration assays demonstrated that the targeted inhibition of the miR-199a/214 cluster significantly enhanced the tumor tropism of hiPS-NSCs both in vitro and in vivo. These findings suggest a novel application of CRISPRi in NSC-based tumor-targeted gene therapy.

Ma, S., et al. (2022). "Generation of a COL1A2 homozygous knockout stem cell line via CRISPR/Cas9 system." Stem Cell Res **59**: 102652.

The loss of function of the COL1A2 gene can result in osteogenesis imperfecta (OI) types I, II, III, and IV and Ehlers-Danlos syndrome (cardiac valvular and arthrochalasia type).To further investigate the significance of COL1A2 in osteogenesis imperfecta and cardiac valve disease, we created a homozygous COL1A2(-/-) human embryonic stem cell line (WAe009-A-72) using CRISPR/Cas9. In vivo, the WAe009-A-72 cell line retained typical colony form, a normal karyotype, and robustly expressed pluripotency markers while differentiating into all three germ layers. This cell line is a potential tool for investigating the role of the COL1A2 gene in associated disorders.

Ma, S., et al. (2021). "Generation of a TPM1 homozygous knockout embryonic stem cell line by CRISPR/Cas9 editing." Stem Cell Res **55**: 102470.

Alpha-Tropomyosin (TPM1) plays a crucial role in actin regulation and stability and contributes fundamental functions to heart development: without TPM1 expressing, mice embryos will die early in embryogenesis. To further identify the role of TPM1 in human cardiac development, here we generated a homozygous TPM1 knockout (TPM1(-/-)) human embryonic stem cell (hESC) line using CRISPR/Cas9-based genome editing system. The generated TPM1(-/-) hESC line maintained normal karyotype, highly expressed pluripotency markers and was able to differentiate into all three germ layers in vivo. This cell line provides a powerful tool to investigate the role of TPM1 in heart development in future.

Ma, Y., et al. (2022). "CRISPR-mediated MECOM depletion retards tumor growth by reducing cancer stem cell properties in lung squamous cell carcinoma." Mol Ther **30**(11): 3341-3357.

Targeted therapy for lung squamous cell carcinoma (LUSC) remains a challenge due to the lack of robust targets. Here, we identified MECOM as a candidate of therapeutic target for LUSC by screening 38 genes that were commonly amplified in three pairs of primary tumors and patient-derived xenografts (PDXs) using a clustered regularly interspaced short palindromic repeats (CRISPR)-mediated approach. High MECOM expression levels were associated with poor prognosis. Forced expression of MECOM in LUSC cell lines promoted cancer stem cell (CSC) properties, and its knockout inhibited CSC phenotypes. Furthermore, systemic delivery of CRISPR-mediated MECOM depletion cassette using adenovirus with an adaptor, which is composed of a single-chain fragment variable (scFv) against epithelial cell adhesion molecules (EpCAM) fused to the ectodomain of coxsackievirus and adenovirus receptor, and a protector, which consists of the scFv connected to the hexon symmetry of the adenovirus, could specifically target subcutaneous and orthotopic LUSC and retard tumor growth. This study could provide a novel therapeutic strategy for LUSC with high efficacy and specificity.

Malerba, N., et al. (2019). "Generation of the induced human pluripotent stem cell lines CSSi009-A from a patient with a GNB5 pathogenic variant, and CSSi010-A from a CRISPR/Cas9 engineered GNB5 knock-out human cell line." Stem Cell Res **40**: 101547.

GNB5 loss-of-function pathogenic variants cause IDDCA, a rare autosomal recessive human genetic disease characterized by infantile onset of intellectual disability, sinus bradycardia, hypotonia, visual abnormalities, and epilepsy. We generated human induced pluripotent stem cells (hiPSCs) from skin fibroblasts of a patient with the homozygous c.136delG frameshift variant, and a GNB5 knock-out (KO) line by CRISPR/Cas9 editing. hiPSCs express common pluripotency markers and differentiate into the three germ layers. These lines represent a powerful cellular model to study the molecular basis of GNB5-related disorders as well as offer an in vitro model for drug screening.

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.

Mehravar, M., et al. (2019). "Efficient Production of Biallelic RAG1 Knockout Mouse Embryonic Stem Cell Using CRISPR/Cas9." Iran J Biotechnol **17**(1): e2205.

BACKGROUND: Recombination Activating Genes (RAG) mutated embryonic stem cells are (ES) cells which are unable to perform V (D) J recombination. These cells can be used for generation of immunodeficient mouse. Creating biallelic mutations by CRISPR/Cas9 genome editing has emerged as a powerful technique to generate site-specific mutations in different sequences. OBJECTIVES: The main purposes of this study were to achieve complete knock-out of RAG1 gene by investigating the nature of mutations in mutant mESC and to generate RAG1 knock-out mESCs containing homozygous indels with the aim of creating desired and specific RAG-1 -/- mutant mouse in a shorter period of time. MATERIALS AND METHODS: Here, we first utilized CRISPR/Cas9 system to target RAG1/RAG2 genes in NIH3T3 cells to test the activity and efficiency of our CRISPR system. Then we used the system for targeting RAG1 gene in mouse embryonic stem cell (mESCs) to generate knock-out embryonic stem cells. This method combined with highly active single guide RNA (sgRNA) is an efficient way to produce new RAG1-knockout mESCs in the selected regions of early coding DNA sequence, approximately between nucleotide c. 512-c. 513 and nucleotide c. 725-c. 726 of RAG1 coding sequence that had not been targeted previously. RESULTS: CRISPR gene editing resulted in a multitude of engineered homozygous and compound heterozygous mutations, including both in-frame and out-of-frame indels in 92% of mES cell clones. Most of the mutations generated by CRISPR/Cas9 system were out-of-frame, resulting in a complete gene knockout. In addition, 59% of the mutant ES cell clones carried out-of-frame homozygous indel mutations. The RAG1-knockout mESC clones retained normal morphology and pluripotent gene expression. CONCLUSIONS: Our study demonstrated that CRISPR/Cas9 system can efficiently create biallelic indels containing both homozygous and compound heterozygous RAG1 mutations in about 92% of the mutant mESC clones. The 59% of mutant ES cell clones carried out-of-frame homozygous indel mutations.

Morera, C., et al. (2022). "CRISPR-mediated correction of skeletal muscle Ca(2+) handling in a novel DMD patient-derived pluripotent stem cell model." Neuromuscul Disord **32**(11-12): 908-922.

Mutations in the dystrophin gene cause the most common and currently incurable Duchenne muscular dystrophy (DMD) characterized by progressive muscle wasting. Although abnormal Ca(2+) handling is a pathological feature of DMD, mechanisms underlying defective Ca(2+) homeostasis remain unclear. Here we generate a novel DMD patient-derived pluripotent stem cell (PSC) model of skeletal muscle with an isogenic control using clustered regularly interspaced short palindromic repeat (CRISPR)-mediated precise gene correction. Transcriptome analysis identifies dysregulated gene sets in the absence of dystrophin, including genes involved in Ca(2+) handling, excitation-contraction coupling and muscle contraction. Specifically, analysis of intracellular Ca(2+) transients and mathematical modeling of Ca(2+) dynamics reveal significantly reduced cytosolic Ca(2+) clearance rates in DMD-PSC derived myotubes. Pharmacological assays demonstrate Ca(2+) flux in myotubes is determined by both intracellular and extracellular sources. DMD-PSC derived myotubes display significantly reduced velocity of contractility. Compared with a non-isogenic wildtype PSC line, these pathophysiological defects could be rescued by CRISPR-mediated precise gene correction. Our study provides new insights into abnormal Ca(2+) homeostasis in DMD and suggests that Ca(2+) signaling pathways amenable to pharmacological modulation are potential therapeutic targets. Importantly, we have established a human physiology-relevant in vitro model enabling rapid pre-clinical testing of potential therapies for DMD.

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

AIMS: Sarcomeric gene mutations frequently underlie hypertrophic cardiomyopathy (HCM), a prevalent and complex condition leading to left ventricle thickening and heart dysfunction. We evaluated isogenic genome-edited human pluripotent stem cell-cardiomyocytes (hPSC-CM) for their validity

to model, and add clarity to, HCM. METHODS AND RESULTS: CRISPR/Cas9 editing produced 11 variants of the HCM-causing mutation c.C9123T-MYH7 [(p.R453C-beta-myosin heavy chain (MHC)] in 3 independent hPSC lines. Isogenic sets were differentiated to hPSC-CMs for high-throughput, non-subjective molecular and functional assessment using 12 approaches in 2D monolayers and/or 3D engineered heart tissues. Although immature, edited hPSC-CMs exhibited the main hallmarks of HCM (hypertrophy, multi-nucleation, hypertrophic marker expression, sarcomeric disarray). Functional evaluation supported the energy depletion model due to higher metabolic respiration activity, accompanied by abnormalities in calcium handling, arrhythmias, and contraction force. Partial phenotypic rescue was achieved with ranolazine but not omecamtiv mecarbil, while RNAseq highlighted potentially novel molecular targets. CONCLUSION: Our holistic and comprehensive approach showed that energy depletion affected core cardiomyocyte functionality. The engineered R453C-betaMHC-mutation triggered compensatory responses in hPSC-CMs, causing increased ATP production and alphaMHC to energy-efficient betaMHC switching. We showed that pharmacological rescue of arrhythmias was possible, while MHY7: MYH6 and mutant: wild-type MYH7 ratios may be diagnostic, and previously undescribed lncRNAs and gene modifiers are suggestive of new mechanisms.

Mun, D., et al. (2022). "Generation of two PITX2 knock-out human induced pluripotent stem cell lines using CRISPR/Cas9 system." Stem Cell Res **65**: 102940.

PITX2 is a homeobox gene located in the human 4q25 locus and is commonly associated with atrial fibrillation (AF). Here, we generated two PITX2 knock-out human induced pluripotent stem cell (iPSC) lines using CRISPR/Cas9 genome editing. The edited iPSCs maintained fullpluripotency, normal karyotype and spontaneousdifferentiation capability. This cell line provides a suitable model for investigating the physiopathologyof PITX2 mutation in atrial fibrillation.

Navarro-Guerrero, E., et al. (2021). "Genome-wide CRISPR/Cas9-knockout in human induced Pluripotent Stem Cell (iPSC)-derived macrophages." Sci Rep **11**(1): 4245.

Genome engineering using CRISPR/Cas9 technology enables simple, efficient and precise genomic modifications in human cells. Conventional immortalized cell lines can be easily edited or screened using genome-wide libraries with lentiviral transduction. However, cell types derived from the differentiation of induced Pluripotent Stem Cells (iPSC), which often represent more relevant, patient-derived models for human pathology, are much more difficult to engineer as CRISPR/Cas9 delivery to these differentiated cells can be inefficient and toxic. Here, we present an efficient, lentiviral transduction protocol for delivery of CRISPR/Cas9 to macrophages derived from human iPSC with efficiencies close to 100%. We demonstrate CRISPR/Cas9 knockouts for three nonessential proof-of-concept genes-HPRT1, PPIB and CDK4. We then scale the protocol and validate for a genome-wide pooled CRISPR/Cas9 loss-of-function screen. This methodology enables, for the first time, systematic exploration of macrophage involvement in immune responses, chronic inflammation, neurodegenerative diseases and cancer progression, using efficient genome editing techniques.

Pan, A., et al. (2014). "Enhancing stem cell survival in an ischemic heart by CRISPR-dCas9-based gene regulation." Med Hypotheses **83**(6): 702-705.

Ischemic heart disease has remained the number one killer around the world for over the past 20 years. While stem cell therapy has become a promising new frontier to repair the damaged heart, limited stem cell survivability post-transplantation has precluded widespread use of this therapy. Strategies to genetically modify stem cells to activate pro-survival and anti-apoptotic and anti-inflammatory pathways, such as Akt and heme oxygenase-1, have been shown to improve the lifespan of transplanted stem cells within the ischemic myocardium, but constitutive overexpression of these pathways at high levels has been shown to have side effects. Therefore, more specific and controlled gene activation would be necessary. Current techniques used for gene regulation include zinc finger and TALE proteins, but there are still disadvantages to each of these methods, such as ease and cost of use. Also, those methods use synthesized promoters to express synthesized cDNA, which lack regulatory elements, including introns and 3' untranslated regions for microRNA mediated post-transcriptional regulation. A new novel technique, the CRISPR/dCas9 system, was recently developed as a simple and efficient method for endogenous gene regulation. With its use of single guide chimeric RNA's (sgRNA's), this system has been shown to provide a high level of specificity and efficiency. When targeting different loci, past studies have found that the CRISPR/dCas9 system can activate gene expression at varying levels. In addition, this system makes use of the genome's endogenous regulatory elements, such as the aforementioned introns and 3' UTR's, which can help provide a safer method of gene activation. If targeted to a gene promoting cellular survival or decreasing cell death, it could potentially improve stem cell longevity in a more efficient and controllable manner. As a result, our hypothesis is to use the CRISPR/dCas9 system to

activate expression of an anti-inflammatory and anti-apoptotic gene, such as heme oxygenase-1 (HO-1), to an optimal level to increase transplanted stem cell survival while also mitigating its cytotoxic effects due to lack of internal regulation, thus prolonging its effects within the ischemic myocardium leading to greater therapeutic benefit.

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.

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.

Schmidt, J. K., et al. (2022). "CRISPR/Cas9 genome editing to create nonhuman primate models for studying stem cell therapies for HIV infection." Retrovirology **19**(1): 17.

Nonhuman primates (NHPs) are well-established basic and translational research models for human immunodeficiency virus (HIV) infections and pathophysiology, hematopoietic stem cell (HSC) transplantation, and assisted reproductive technologies. Recent advances in CRISPR/Cas9 gene editing technologies present opportunities to refine NHP HIV models for investigating genetic factors that affect HIV replication and designing cellular therapies that exploit genetic barriers to HIV infections, including engineering mutations into CCR5 and conferring resistance to HIV/simian immunodeficiency virus (SIV) infections. In this report, we provide an overview of recent advances and challenges in gene editing NHP embryos and discuss the value of genetically engineered animal models for developing novel stem cell-based therapies for curing HIV.

Schneider, Y., et al. (2022). "Generation of a homozygous and a heterozygous SNCA gene knockout human-induced pluripotent stem cell line by CRISPR/Cas9 mediated allele-specific tuning of SNCA expression." Stem Cell Res **65**: 102952.

Aggregation of alpha-synuclein (aSyn) is closely linked to Parkinson's disease, probably due to the loss of physiological functions and/or gain of toxic functions of aggregated aSyn. Significant efforts have been made elucidating the physiological structure and function of aSyn, however, with limited success thus far in human-derived cells, partly because of restricted resources. Here, we developed two human-induced pluripotent stem cell lines using CRISPR/Cas9-mediated allele-specific frame-shift deletion of the aSyn encoding gene SNCA, resulting in homo- and heterozygous SNCA knockout. The generated cell lines are promising cellular tools for studying aSyn dosage-dependent functions and structural alterations in human neural cells.

Schoger, E., et al. (2021). "Establishment of two homozygous CRISPR interference (CRISPRi) knock-in human induced pluripotent stem cell (hiPSC) lines for titratable endogenous gene repression." Stem Cell Res **55**: 102473.

Using nuclease-deficient dead (d)Cas9 without enzymatic activity fused to transcriptional inhibitors (CRISPRi) allows for transcriptional interference and results in a powerful tool for the elucidation of developmental, homeostatic and disease

mechanisms. We inserted dCas9KRAB (CRISPRi) cassette into the AAVS1 locus of hiPSC lines, which resulted in homozygous knock-in with an otherwise unaltered genome. Expression of dCas9KRAB protein, pluripotency and the ability to differentiate into all three embryonic germ layers were validated. Furthermore, functional cardiomyocyte generation was tested. The hiPSC-CRISPRi cell lines offer a valuable tool for studying endogenous transcriptional repression with single and multiplexed possibilities in all human cell types.

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.

Shahabipour, F., et al. (2020). "CRISPR/Cas9 mediated GFP-human dentin matrix protein 1 (DMP1) promoter knock-in at the ROSA26 locus in mesenchymal stem cell for monitoring osteoblast differentiation." J Gene Med **22**(12): e3288.

BACKGROUND: Dentin matrix protein 1 (DMP1) is highly expressed in mineralized tooth and bone, playing a critical role in mineralization and phosphate metabolism. One important role for the expression of DMP1 in the nucleus of preosteoblasts is the up-regulation of osteoblast-specific genes such as osteocalcin and alkaline phosphatase(1) . The present study aimed to investigate the potential application of human DMP1 promoter as an indicator marker of osteoblastic differentiation. METHODS: In the present study, we developed DMP1 promoter-DsRed-GFP knock-in mesenchymal stem cell (MSCs) via the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system that enabled automatic detection of osteoblast differentiation. With the application of a homology-directed knock-in strategy, a 2-kb fragment of DMP1 promoter, which was inserted upstream of the GFP and DsRed reporter cassette, was integrated into the human ROSA locus to generate double fluorescent cells. We further differentiated MSCs under osteogenic media to monitor the fate of MSCs. First, cells were transfected using CRISPR/Cas9 plasmids, which culminated in MSCs with a green fluorescence intensity, then GFP-positive cells were selected using puromycin. Second, the GFP-positive MSCs were differentiated toward osteoblasts, which demonstrated an increased red fluorescence intensity. The osteoblast differentiation of MSCs was also verified by performing alkaline phosphatase and Alizarin Red assays. RESULTS: We have exploited the DMP1 promoter as a predictive marker of MSC differentiation toward osteoblasts. Using the CRISPR/Cas9 technology, we have identified a distinctive change in the fluorescence intensities of GFP knock-in (green) and osteoblast differentiated MSCs (2) . CONCLUSIONS: The data show that DMP1-DsRed-GFP knock-in MSCs through CRISPR/Cas9 technology provide a valuable indicator for osteoblast differentiation. Moreover, The DMP1 promoter might be used as a predictive marker of MSCs differentiated toward osteoblasts.

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.

Smith, R. H., et al. (2020). "Genome-Wide Analysis of Off-Target CRISPR/Cas9 Activity in Single-Cell-Derived Human Hematopoietic Stem and Progenitor Cell Clones." Genes (Basel) **11**(12).

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9)-mediated genome editing holds remarkable promise for the treatment of human genetic diseases. However, the possibility of off-target Cas9 activity remains a concern. To address this issue using clinically relevant target cells, we electroporated Cas9 ribonucleoprotein (RNP) complexes (independently targeted to two different genomic loci, the CXCR4 locus on chromosome 2 and the AAVS1 locus on chromosome 19) into human mobilized peripheral blood-derived hematopoietic stem and progenitor cells (HSPCs) and assessed the acquisition of somatic mutations in an unbiased, genome-wide manner via whole genome sequencing (WGS) of single-cell-derived HSPC clones. Bioinformatic analysis identified >20,000 total somatic variants (indels, single nucleotide variants, and structural variants) distributed among Cas9-treated and non-Cas9-treated control HSPC clones. Statistical analysis revealed no significant difference in the number of novel non-targeted indels among the samples. Moreover, data analysis showed no evidence of Cas9-mediated indel formation at 623 predicted off-target sites. The median number of novel single nucleotide variants was slightly elevated in Cas9 RNP-recipient sample groups compared to baseline, but did not reach statistical significance. Structural variants were rare and demonstrated no clear causal connection to Cas9-mediated gene editing procedures. We find that the collective somatic mutational burden observed within Cas9 RNP-edited human HSPC clones is indistinguishable from naturally occurring levels of background genetic heterogeneity.

Song, Y., et al. (2022). "Deciphering Common Long QT Syndrome Using CRISPR/Cas9 in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes." Front Cardiovasc Med **9**: 889519.

From carrying potentially pathogenic genes to severe clinical phenotypes, the basic research in the inherited cardiac ion channel disease such as long QT syndrome (LQTS) has been a significant challenge in explaining gene-phenotype heterogeneity. These have opened up new pathways following the parallel development and successful application of stem cell and genome editing technologies. Stem cell-derived cardiomyocytes and subsequent genome editing have allowed researchers to introduce desired genes into cells in a dish to replicate the disease features of LQTS or replace causative genes to normalize the cellular phenotype. Importantly, this has made it possible to elucidate potential genetic modifiers contributing to clinical heterogeneity and hierarchically manage newly identified variants of uncertain significance (VUS) and more therapeutic options to be tested in vitro. In this paper, we focus on and summarize the recent advanced application of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) combined with clustered regularly interspaced short palindromic repeats/CRISPR-associated system 9 (CRISPR/Cas9) in the interpretation for the gene-phenotype relationship of the common LQTS and presence challenges, increasing our understanding of the effects of mutations and the physiopathological mechanisms in the field of cardiac arrhythmias.

Sun, C., et al. (2021). "Generation of GPAM knockout human embryonic stem cell line SYSUe-008-A using CRISPR/Cas9." Stem Cell Res **53**: 102303.

GPAM (glycerol-3-phosphateacyltransferase1) is a mitochondrial enzyme that catalyze an essential step in glycerolphospholipids and triacylglycerol biosynthesis process. Loss-of-function mutation of GPAM has been shown to lead to hypomyelination of corticospinal tract in cerebral palsy patient. To model this rare disease with human brain organoid, we generated a GPAM knockout human embryonic stem cell line SYSUe-008-A by CRISPR/cas9. The GPAM knockout cell line maintains a normal karyotype and shows comparable level of pluripotent stem cell marker expression and differentiation potential as wild-type human embryonic stem cells.

Sun, Y. H., et al. (2020). "Human induced pluripotent stem cell line with genetically encoded fluorescent voltage indicator generated via CRISPR for action potential assessment post-cardiogenesis." Stem Cells **38**(1): 90-101.

Genetically encoded fluorescent voltage indicators, such as ArcLight, have been used to report action potentials (APs) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). However, the ArcLight expression, in all cases, relied on a high number of lentiviral vector-mediated random genome integrations (8-12 copy/cell), raising concerns such as gene disruption and alteration of global and local gene expression, as well as loss or silencing of reporter genes after differentiation. Here, we report the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nuclease technique to develop a hiPSC line stably expressing ArcLight from the AAVS1 safe harbor locus. The hiPSC line retained proliferative ability with a growth rate similar to its parental strain. Optical recording with conventional epifluorescence microscopy allowed the detection of

APs as early as 21 days postdifferentiation, and could be repeatedly monitored for at least 5 months. Moreover, quantification and analysis of the APs of ArcLight-CMs identified two distinctive subtypes: a group with high frequency of spontaneous APs of small amplitudes that were pacemaker-like CMs and a group with low frequency of automaticity and large amplitudes that resembled the working CMs. Compared with FluoVolt voltage-sensitive dye, although dimmer, the ArcLight reporter exhibited better optical performance in terms of phototoxicity and photostability with comparable sensitivities and signal-to-noise ratios. The hiPSC line with targeted ArcLight engineering design represents a useful tool for studying cardiac development or hiPSC-derived cardiac disease models and drug testing.

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.

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.

Tian, L., et al. (2021). "Generation of a COL4A5 heterozygous mutation human embryonic stem cell line (WAe009-A-58) using an episomal vector-based CRISPR/Cas9 system." Stem Cell Res **55**: 102481.

X-linked Alport syndrome (XLAS) is the second most common inherited kidney disease which pathogenic variants related to a mutation in the COL4A5 gene encoding the type IV collagen alpha5 chain. Here, we have generated a COL4A5 heterozygous mutant human embryonic stem cell (hESC) line (H9-COL4A5(+/-)) by an episomal vector-based CRISPR/Cas9 system. The generated H9-COL4A5(+/-) maintained a normal stem cell morphology, stably expressed pluripotent markers, and could differentiate into all three germ layers in vivo. This cell line offers an in vitro efficient platform to explore pathogenic mechanisms in XLAS and provides a cell-based disease model for drug testing.

Tsukamoto, S., et al. (2021). "Generation of two ISL1-tdTomato reporter human induced pluripotent stem cell lines using CRISPR-Cas9 genome editing." Stem Cell Res **53**: 102363.

ISL1 encodes a member of the LIM/homeodomain family of transcription factors. This encoded protein plays central roles in the development of motor neuron, pancreas, and secondary heart field. Here we generated heterozygous fluorescent reporters of the ISL1 gene in human induced pluripotent stem cells (hiPSCs). CRISPR/Cas9 genome editing technology was employed to knock-in 2A-tdTomato and EF1 alpha promoter-driven Bleomycin resistance gene to the translational ISL1 C-terminal region. The resulting ISL1-TEZ lines showed tdTomato fluorescence upon motor neuron differentiation. These

reporter iPSC lines provide opportunity for monitoring and purifying these related cell lineages.

Urrutia-Cabrera, D., et al. (2022). "Combinatorial Approach of Binary Colloidal Crystals and CRISPR Activation to Improve Induced Pluripotent Stem Cell Differentiation into Neurons." ACS Appl Mater Interfaces **14**(7): 8669-8679.

Conventional methods of neuronal differentiation in human induced pluripotent stem cells (iPSCs) are tedious and complicated, involving multistage protocols with complex cocktails of growth factors and small molecules. Artificial extracellular matrices with a defined surface topography and chemistry represent a promising venue to improve neuronal differentiation in vitro. In the present study, we test the impact of a type of colloidal self-assembled patterns (cSAPs) called binary colloidal crystals (BCCs) on neuronal differentiation. We developed a CRISPR activation (CRISPRa) iPSC platform that constitutively expresses the dCas9-VPR system, which allows robust activation of the proneural transcription factor NEUROD1 to rapidly induce neuronal differentiation within 7 days. We show that the combinatorial use of BCCs can further improve this neuronal differentiation system. In particular, our results indicate that fine tuning of silica (Si) and polystyrene (PS) particle size is critical to generate specific topographies to improve neuronal differentiation and branching. BCCs with 5 mum silica and 100 nm carboxylated PS (PSC) have the most prominent effect on increasing neurite outgrowth and more complex ramification, while BCCs with 2 mum Si and 65 nm PSC particles are better at promoting neuronal enrichment. These results indicate that biophysical cues can support rapid differentiation and improve neuronal maturation. In summary, our combinatorial approach of CRISPRa and BCCs provides a robust and rapid pipeline for the in vitro production of human neurons. Specific BCCs can be adapted to the late stages of neuronal differentiation protocols to improve neuronal maturation, which has important implications in tissue engineering, in vitro biological studies, and disease modeling.

Valenti, M. T., et al. (2019). "CRISPR/Cas system: An emerging technology in stem cell research." World J Stem Cells **11**(11): 937-956.

The identification of new and even more precise technologies for modifying and manipulating the genome has been a challenge since the discovery of the DNA double helix. The ability to modify selectively specific genes provides a powerful tool for characterizing gene functions, performing gene therapy, correcting specific genetic mutations, eradicating diseases, engineering cells and organisms to achieve new and different functions and obtaining transgenic animals as models for studying specific diseases. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology has recently revolutionized genome engineering. The application of this new technology to stem cell research allows disease models to be developed to explore new therapeutic tools. The possibility of translating new systems of molecular knowledge to clinical research is particularly appealing for addressing degenerative diseases. In this review, we describe several applications of CRISPR/Cas9 to stem cells related to degenerative diseases. In addition, we address the challenges and future perspectives regarding the use of CRISPR/Cas9 as an important technology in the medical sciences.

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

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

Vitale, M. R., et al. (2021). "Generation of induced pluripotent stem cell (iPSC) lines carrying a heterozygous (UKWMPi002-A-1) and null mutant knockout (UKWMPi002-A-2) of Cadherin 13 associated with neurodevelopmental disorders using CRISPR/Cas9." Stem Cell Res **51**: 102169.

Fibroblasts isolated from a skin biopsy of a healthy 46-year-old female were infected with Sendai virus containing the Yamanaka factors to produce transgene-free human induced pluripotent stem cells (iPSCs). CRISPR/Cas9 was used to generate isogenic cell lines with a gene dose-dependent deficiency of CDH13, a risk gene associated with neurodevelopmental and psychiatric disorders. Thereby, a heterozygous CDH13 knockout (CDH13(+/-)) and a CDH13 null mutant (CDH13(-/-)) iPSC line was

obtained. All three lines showed expression of pluripotency-associated markers, the ability to differentiate into cells of the three germ layers in vitro, and a normal female karyotype.

Vojnits, K., et al. (2022). "Developing CRISPR/Cas9-Mediated Fluorescent Reporter Human Pluripotent Stem-Cell Lines for High-Content Screening." Molecules **27**(8).

Application of the CRISPR/Cas9 system to knock in fluorescent proteins to endogenous genes of interest in human pluripotent stem cells (hPSCs) has the potential to facilitate hPSC-based disease modeling, drug screening, and optimization of transplantation therapy. To evaluate the capability of fluorescent reporter hPSC lines for high-content screening approaches, we targeted EGFP to the endogenous OCT4 locus. Resulting hPSC-OCT4-EGFP lines generated expressed EGFP coincident with pluripotency markers and could be adapted to multi-well formats for high-content screening (HCS) campaigns. However, after long-term culture, hPSCs transiently lost their EGFP expression. Alternatively, through EGFP knock-in to the AAVS1 locus, we established a stable and consistent EGFP-expressing hPSC-AAVS1-EGFP line that maintained EGFP expression during in vitro hematopoietic and neural differentiation. Thus, hPSC-AAVS1-EGFP-derived sensory neurons could be adapted to a high-content screening platform that can be applied to high-throughput small-molecule screening and drug discovery campaigns. Our observations are consistent with recent findings indicating that high-frequency on-target complexities appear following CRISPR/Cas9 genome editing at the OCT4 locus. In contrast, we demonstrate that the AAVS1 locus is a safe genomic location in hPSCs with high gene expression that does not impact hPSC quality and differentiation. Our findings suggest that the CRISPR/Cas9-integrated AAVS1 system should be applied for generating stable reporter hPSC lines for long-term HCS approaches, and they underscore the importance of careful evaluation and selection of the applied reporter cell lines for HCS purposes.

Wang, B., et al. (2022). "Spinal cord injury target-immunotherapy with TNF-alpha autoregulated and feedback-controlled human umbilical cord mesenchymal stem cell derived exosomes remodelled by CRISPR/Cas9 plasmid." Biomater Adv **133**: 112624.

Human umbilical cord mesenchymal stem cell (hucMSC) derived exosomes (EXOs) have been investigated as a new treatment for spinal cord injury (SCI) because of their anti-inflammatory, anti-apoptotic, angiogenesis-promoting, and axonal regeneration properties. The CAQK peptide found in the brains of mice and humans after trauma has recently been found to specifically bind to the injured site after SCI. Thus, we developed a nanocarrier system called EXO-C@P based on hucMSC exosomes remodelled by the CRISPR/Cas9 plasmid to control inflammation and modified by the CAQK peptide. EXO-C@P was shown to effectively accumulate at the injury site and saturate the macrophages to significantly reduce the expression of inflammatory cytokines in a mouse model of SCI. Moreover, EXO-C@P treatment improved the performance of mice in behavioural assessments and upregulated soluble tumour necrosis factor receptor-1 (sTNFR1) in serum and at the trauma site after SCI surgery, but lowered the proportion of iNOS(+) cells and the concentration of proinflammatory factors. In conclusion, EXO-C@P provides an effective alternative to multiple topical administration and drug delivery approaches for the treatment of SCI. STATEMENT OF SIGNIFICANCE: SCI is a serious disease characterised by a high incidence, high disability rate, and high medical costs, and has become a global medical problem. Several studies have shown that the inflammatory response is the critical inducer of secondary injury after SCI. The inflammatory cytokine TNF-alpha is considered to be one of the most significant therapeutic targets for autoimmune diseases. Antibodies targeting TNF-alpha and sTNFR1 are capable of neutralising free TNF-alpha. In this study, exosomes in the CRISPR/Cas9 system were used to establish stem cells with an autoregulated and feedback-controlled TNF-alpha response, with these cells secreting sTNFR1, which neutralised TNF-alpha and antagonised the inflammation stimulated by TNF-alpha. Moreover, the plasmid was combined with CAQK, which targeted the injury site and promoted the recovery of SCI function.

Wang, B., et al. (2021). "Establishment of a CRISPR/Cas9-mediated GATAD2B homozygous knockout human embryonic stem cell line." Stem Cell Res **57**: 102590.

As a subunit of the nucleosome remodeling and histone deacetylation (NuRD) complex, GATA zinc finger domain containing 2B (GATAD2B) plays a vital role in chromatin modification and transcriptional regulation. To further investigate the role of GATAD2B in cell fate determination of human ESCs, we generated two GATAD2B homozygous knockout human ESC lines by CRISPR/Cas9 technology. The cell line exhibits normal karyotype and typical stem cell morphology, following the high expression of pluripotent genes and differentiation potential in vitro. These cell lines will provide cell resources to investigate epigenetic regulation in extensive biological processes as menthioned above.

Wang, D., et al. (2021). "CRISPR Screening of CAR T Cells and Cancer Stem Cells Reveals Critical Dependencies for Cell-Based Therapies." Cancer Discov **11**(5): 1192-1211.

Glioblastoma (GBM) contains self-renewing GBM stem cells (GSC) potentially amenable to immunologic targeting, but chimeric antigen receptor (CAR) T-cell therapy has demonstrated limited clinical responses in GBM. Here, we interrogated molecular determinants of CAR-mediated GBM killing through whole-genome CRISPR screens in both CAR T cells and patient-derived GSCs. Screening of CAR T cells identified dependencies for effector functions, including TLE4 and IKZF2. Targeted knockout of these genes enhanced CAR antitumor efficacy. Bulk and single-cell RNA sequencing of edited CAR T cells revealed transcriptional profiles of superior effector function and inhibited exhaustion responses. Reciprocal screening of GSCs identified genes essential for susceptibility to CAR-mediated killing, including RELA and NPLOC4, the knockout of which altered tumor-immune signaling and increased responsiveness of CAR therapy. Overall, CRISPR screening of CAR T cells and GSCs discovered avenues for enhancing CAR therapeutic efficacy against GBM, with the potential to be extended to other solid tumors. SIGNIFICANCE: Reciprocal CRISPR screening identified genes in both CAR T cells and tumor cells regulating the potency of CAR T-cell cytotoxicity, informing molecular targeting strategies to potentiate CAR T-cell antitumor efficacy and elucidate genetic modifications of tumor cells in combination with CAR T cells to advance immuno-oncotherapy.This article is highlighted in the In This Issue feature, p. 995.

Wang, M., et al. (2022). "Generation of a homozygous FIS1 knockout human embryonic stem cell line GIBHe015-B by CRISPR/Cas9 system." Stem Cell Res **65**: 102980.

The mitochondrial fission protein 1 (FIS1) is essential for mitochondrial division or fission and has been determined to mediate mitochondrial and peroxisomal fission. Other studies also found that FIS1 functions as an essential component of the mitophagy and apoptosis pathways in mammalian cells, suggesting that FIS1 has multiple important roles. Here, we generated homozygous FIS1 knockout human embryonic stem cells (hESCs) using the CRISPR/Cas9 system. This cell line exhibits normal karyotype, pluripotency, and trilineage differentiation potential, which could provide a useful cellular resource for exploring the functions of FIS1 and their implications in human health and diseases.

Wang, T., et al. (2021). "Generation of a heterozygous SOX10 knockout human embryonic stem cell line using CRISPR/Cas9 technology." Stem Cell Res **57**: 102567.

SOX10 is one of the master transcription factors in neural crest development. Human SOX10 mutations are associated with Waardenburg syndrome type 4 (Waardenburg-Shah, WS4), which can be inherited in both autosomal dominant and recessive patterns. Here, the human embryonic stem cell (hESC) line, H9, was used to generate a heterozygous SOX10 knockout cell line as the in vitro model of WS4 by CRISPR/Cas9-mediated gene targeting. This cell line may represent a valuable tool for uncovering the pathogenesis of WS4.

Wang, W., et al. (2021). "Generating dHAND homozygous knockout human embryonic stem cell line (WAe009-A-59) by episomal vector-based CRISPR/Cas9 system." Stem Cell Res **55**: 102471.

In order to determine the function of dHAND in a specific subset of cardiomyocyte progenitor cells responsible for the heart and its function in the cellular mechanism of Hand2-/- mouse ventricular hypoplasia, we established an allelic knockout model of dHAND in human embryonic stem cells (hESCs-H9) by an episomal vector-based CRISPR/Cas9 system. This dHAND KO hESC line maintained normal karyotype and typical primed pluripotent human stem cell morphology, and maintained pluripotency, could differentiate into all three germ layers in vivo.

Wang, Z., et al. (2020). "Generation of a MCPH1 knockout human embryonic stem cell line by CRISPR/Cas9 technology." Stem Cell Res **49**: 102105.

Human MCPH1 (Microcephalin 1) encodes a DNA damage response protein. Mutations in this gene have been associated with Primary Autosomal Recessive Microcephaly and premature chromosome condensation syndrome. To further understand the roles of MCPH1 in neural differentiation and brain development, here we generated a MCPH1 knockout human embryonic stem cell line by CRISPR/Cas9 genome editing technology. This cell line maintained a normal karyotype and typical undifferentiated state in terms of morphology, pluripotent gene expression, and had differentiation potential in vitro. This cell line provides a good resource to study the role of MCPH1 gene in neurogenesis and regulation of the size of the cerebral cortex in vitro.

Wei, C., et al. (2021). "Generation of a FTO gene knockout human embryonic stem cell line using CRISPR/Cas9 editing." Stem Cell Res **53**: 102362.

Fat mass and obesity-associated protein (FTO) is the first protein found to have the activity of N6-

methyladenosine (m6A) demethylation. It has been reported that FTO was involved in different physiological and pathological processes, including stem cell differentiation, sex determination, tumorigenesis, and progression. To further understand the exact role of FTO in these processes, we generated a FTO knockout human embryonic stem cell (hESC) line by CRISPR/Cas9 mediated gene editing method. This cell line maintained normal karyotype, pluripotency, and trilineage differentiation potential, which are considered as a model for function studies of the FTO protein in hESC self-renewal and differentiation.

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. (2022). "CRISPR-targeted genome editing of human induced pluripotent stem cell-derived hepatocytes for the treatment of Wilson's disease." JHEP Rep **4**(1): 100389.

BACKGROUND & AIMS: Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism caused by loss-of-function mutations in ATP7B, which encodes a copper-transporting protein. It is characterized by excessive copper deposition in tissues, predominantly in the liver and brain. We sought to investigate whether gene-corrected patient-specific induced pluripotent stem cell (iPSC)-derived hepatocytes (iHeps) could serve as an autologous cell source for cellular transplantation therapy in WD. METHODS: We first compared the in vitro phenotype and cellular function of ATP7B before and after gene correction using CRISPR/Cas9 and single-stranded oligodeoxynucleotides (ssODNs) in iHeps (derived from patients with WD) which were homozygous for the ATP7B R778L mutation (ATP7B(R778L/R778L)). Next, we evaluated the in vivo therapeutic potential of cellular transplantation of WD gene-corrected iHeps in an immunodeficient WD mouse model (Atp7b (-/-) / Rag2 (-/-) / Il2rg (-/-) ; ARG). RESULTS: We successfully created iPSCs with heterozygous gene correction carrying 1 allele of the wild-type ATP7B gene (ATP7B(WT/-)) using CRISPR/Cas9 and ssODNs. Compared with ATP7B(R778L/R778L) iHeps, gene-corrected ATP7B(WT/-) iHeps restored i n vitro ATP7B subcellular localization, its subcellular trafficking in response to copper overload and its copper exportation function. Moreover, in vivo cellular transplantation of ATP7B(WT/-) iHeps into ARG mice via intra-splenic injection significantly attenuated the hepatic manifestations of WD. Liver function improved and liver fibrosis decreased due to reductions in hepatic copper accumulation and consequently copper-induced hepatocyte toxicity. CONCLUSIONS: Our findings demonstrate that gene-corrected patient-specific iPSC-derived iHeps can rescue the in vitro and in vivo disease phenotypes of WD. These proof-of-principle data suggest that iHeps derived from gene-corrected WD iPSCs have potential use as an autologous ex vivo cell source for in vivo therapy of WD as well as other inherited liver disorders. LAY SUMMARY: Gene correction restored ATP7B function in hepatocytes derived from induced pluripotent stem cells that originated from a patient with Wilson's disease. These gene-corrected hepatocytes are potential cell sources for autologous cell therapy in patients with Wilson's disease.

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.

Widjaya, M. A., et al. (2022). "CRISPR-Edited Stem Cell Transplantation for HIV-Related Gene Modification In Vivo: A Systematic Review." Stem Cell Rev Rep **18**(5): 1743-1755.

BACKGROUND: CRISPR is a novel genomic editing technology which can be useful for the treatment of immune diseases such as HIV. However, the application of CRISPR in stem cells for HIV-related research was not effective, and most of the research was done in vivo. This systematic review is to identify a new research idea about increase CRISPR-editing efficiencies in stem cell transplantation for HIV treatment, as well as its future perspective. METHOD: Four databases were searched for articles published during 1952 to 2020. PRISMA method was used to select appropriate research papers. CAMARADES was used to identify the paper quality. The outcome was engraftment efficiency, gene disruption percentage, differentiation ability, HIV-resistant efficiency. RESULT: Screening method showed 196 papers mentioned the topic. However, only 5 studies were reliable with the research objective. We found that (1) Two research ideas which was double gene knockout and knockout-knockin method to provide HIV-resistant cells, engraftment support and avoid cardiac disease as an HIV disease side effect. (2) Ribonucleoprotein (RNP) delivery was the best way to deliver the CRISPR/Cas9 and Adeno-Associated Virus (AAV) would be effective for knockin purpose. (3) CRISPR/SaCas9 could replace CRISPR/Cas9 role in editing HIV-related gene. CONCLUSION: Potential genes to increase HIV resistance and stem cell engraftment should be explored more in the future. Double knockout and knock-in procedures should be applied to set up a better engraftment for improving HIV treatment or resistance of patients. CRISPR/SaCas9 and RNP delivery should be explored more in the future. SYSTEMATIC REVIEW REGISTRATION: PROSPERO CRD42020203312.

Widjaya, M. A., et al. (2021). "Impactful factors and research design in CRISPR-edited stem cell research from top 10 highly cited articles." Stem Cell Res Ther **12**(1): 411.

Our objective in this review was to determine (1) impactful research articles about CRISPR-edited stem cells, (2) factors that affected CRISPR method performance in stem cell, and (3) research design related to CRISPR-edited stem cells. Screening research papers of related topic was carried out by using the Science Citation Index Expanded (SCIE) database of the Clarivate Analytics Web of Science Core Collection updated. We screened impactful CRISPR/Cas9-edited stem cells based on total citation until 2020. The result showed the title "RNA-guided human genome engineering via Cas9" was the highest citation in stem cell research using the CRISPR method with total citation 4789 from Web of Science Core Collection until 2020. It became the most influenced paper because this was the first research using CRISPR method for modifying human cells. On the other hand, cell type, CRISPR/Cas9 delivery, and gene target affected CRISPR/Cas9 performance in stem cells. The more complex the cell structure, the more difficult for CRISPR/Cas9 to mutate the host cells. This problem could be solved by modifying the CRISPR/Cas9 delivery by liposome and SaCas9 modification. Another way was using ribonucleoprotein (RNP) as a delivery method. Then, double gene target was more difficult to execute than single gene target. Although it is difficult, CRISPR/Cas9 had the capability to target any genome region from promoter until intron. Research design used a combination of dry lab and wet lab. The dry lab is usually used for sequence analysis and gRNA design. The wet lab which consisted of in vitro and in vivo was used for gene characterization. In particular, colony selection, DNA analysis, and sequencing were important parts for in vitro research design, while DNA analysis and sequencing were crucial parts for in vivo research design. We hoped these findings could give researchers, investor, and students a guideline to conduct CRISPR-edited stem cells in the future.

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.

Wu, F., et al. (2020). "Generation of a Junctophilin-2 homozygous knockout human embryonic stem cell line (WAe009-A-36) by an episomal vector-based CRISPR/Cas9 system." Stem Cell Res **48**: 101930.

Mutations in Junctophilin-2(JPH2) gene is the cause of hypertrophic cardiomyopathy (HCM) and leading inherited cause of left ventricular hypertrophy and myofilaments disarray. JPH2 protein, a member of the Junctophilin family, is mainly expressed in heart and plays an important role in E-C coupling. We have generated a homozygous JPH2 knockout (JPH2-KO) human embryonic stem cell (hESC) line using an episomal vector-based CRISPR/Cas9 system. This JPH2-KO hESC line maintained stem cell like morphology, pluripotency, normal karyotype and could differentiate into all three germ layers in vivo.

Wu, S., et al. (2022). "Generation of CD16A gene knockout human embryonic stem cell line using CRISPR/Cas9." Stem Cell Res **64**: 102935.

CD16A is a receptor for the Fc portion of immunoglobulin G, and is involved in the antibody dependent cellular cytotoxicity (ADCC) of nature killer cells(Zhu et al., 2020) and antibody dependent enhancement (ADE) of virus infections(Wan et al., 2020). However, the role of CD16A in human embryonic stem cell modeled development has been merely documented. Hence, to illustrate the role of CD16A in the human cell development, we reported a CD16A knockout human embryonic stem(hESC) cell line via CRISPR/Cas9 mediated gene knockout. The CD16A mutated cell line displayed normal karyotype, pluripotent stem cell marker gene expression and differentiation potential.

Xiao, X., et al. (2021). "Generation of a homozygous ALX1 knockout human embryonic stem cell line (WAe001-A-060) by a CRISPR/Cas9 system." Stem Cell Res **53**: 102309.

Human ALX1 gene (ALX Homeobox 1) is a protein coding gene and gene ontology annotations related to this gene include DNA-binding transcription factor activity and protein heterdimerization activity. It is necessary for survival of forebrain mesenchyme and may be involved in development of cervix. However, the function of the gene has yet to be determined in humans. Here we generated an ALX1 homozygous human embryonic stem cell line (WAe001-A-060) by a CRISPR/Cas9 system. The WAe001-A-060 has a normal undifferentiated morphology and karyotype, pluripotency and three germ layers differentiation potential in vivo.

Xie, L., et al. (2019). "Generation of a homozygous HDAC6 knockout human embryonic stem cell line by CRISPR/Cas9 editing." Stem Cell Res **41**: 101610.

Histone deacetylase 6 (HDAC6) is a unique cytoplasmic enzyme in the HDAC family. The HDAC6 has been shown to play important roles in several biological processes. Meanwhile, it is also an attractive therapeutic target for a variety of diseases. However, the mechanism of HDAC6 function is not fully understood yet, and it is still lacking highly specific targeted drugs. Here, we generated a homozygous HDAC6 knockout human embryonic stem cell (hESC) line, WAe009-A-21 by the CRISPR/Cas9-based gene editing method. The WAe009-A-21 cell line does not express HDAC6 protein, while maintaining normal 46, XX karyotype, pluripotency, and trilineage differentiation potential.

Xu, C., et al. (2020). "Generation of a DAPK1 knockout first (conditional ready) human embryonic stem cell line (ZSSYe001-A) by CRISPR-Cas9 technology." Stem Cell Res **43**: 101693.

Death-associated protein kinase 1 (DAPK1) is a Ca(2+)/calmodulin regulated Ser/Thr kinase involved in various cellular processes including cell death, autophagy and inflammation. Its dysregulation has been linked to tumour metastasis, anti-viral responses, Alzheimer's disease and other neurological disorders. To further investigate the role of DAPK1 in these processes, we generated a DAPK1 knockout first (conditional ready) human embryonic stem (hES) cell line in which the endogenous DAPK1 can be easily restored with expression of FLPe. This cell line provides an ideal model to study the role of DAPK1 in human development and various pathologies related to DAPK1 dysregulation in vitro.

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.

Xue, Y., et al. (2019). "Establishment of an ectodermal dysplasia related gene EDA Knockout human embryonic stem cell line (WAe001-A-22) by CRISPR-Cas9 technology." Stem Cell Res **34**: 101379.

EDA is a gene located at Xq13.1. It encodes different isoforms of tumor necrosis factor (TNF) superfamily member ectodysplasin A. Ectodysplasin A is a transmembrane protein which can be cleaved to form a secreted form and interact with EDA receptor to mediate the development of ectoderm. Mutations of the EDA gene are related to ectodermal dysplasia and tooth agenesis. Here, we report the establishment of the EDA gene knockout human embryonic stem (hES) cell line by CRISPR-Cas9 technology. This cell line provides good materials for further studies of the roles ectodysplasin A plays in ectoderm differentiation and tooth development.

Xue, Y., et al. (2017). "Establishment of a congenital tooth agenesis related gene MSX1 knockout human embryonic stem cell lines by CRISPR-Cas9 technology." Stem Cell Res **24**: 151-154.

Human MSX1 gene is mapped to chromosome 4 and encodes a 303aa homeobox protein MSX1. MSX1 expression appears during early tooth development of vertebrate embryogenesis. Mutations in this protein are related to human tooth anomalie, cleft lip and palate and congenital ectodermal dysplasia syndrome. Most of the confirmed pathogenic mutations are located in exon2 encoded homeobox domain. Here, we report the establishment of MSX1 gene knockout human embryonic stem (hES) cell lines by CRISPR-Cas9 technology. These cell lines provide good materials for further studies of the roles MSX1 plays in human tooth development and congenital tooth agenesis.

Yang, Z., et al. (2021). "Generation of a DKK1 homozygous knockout human embryonic stem cell line WAe001-A-21 using CRISPR/Cas9." Stem Cell Res **51**: 102161.

Dickkopf1 (DKK1) is a secreted inhibitor for the Wnt signalling, which is involved in cell proliferation, tissue regeneration and embryonic development. Using CRISPR/Cas9 editing, we established a homozygous mutant DKK1 human embryonic stem cell line (WAe001-A-21). It has a 41 bp deletion in exon 2 of DKK1, leading to its coding frame shift. The WAe001-A-21 cell line maintains a normal karyotype, pluripotency markers, typical stem cell morphology and the ability to differentiate into three germ layers.

Yi, T., et al. (2020). "Generation of a NONO homozygous knockout human induced pluripotent stem cell line by CRISPR/Cas9 editing." Stem Cell Res **47**: 101893.

The non-POU domain containing octamer-binding gene (NONO) encodes a member of a small family of RNA-binding and DNA-binding proteins, whose variants can cause intellectual disability and congenital heart defects. In this study, we generated a homozygous NONO knockout (NONO-KO) induced pluripotent stem cell (iPSC) line (CMUi002-A-1) using the CRISPR/Cas9-based genome editing system. The gene-edited line had a normal karyotype, expressed pluripotency markers, and was able to differentiate into all three germ layers in vivo. This cell line will provide a platform to study the pathogenic mechanisms of noncompaction cardiomyopathy and neurocyte dysfunction related to NONO mutations.

Yi, T., et al. (2023). "Generation of a TIMP3 knockout stem cell line via CRISPR/Cas9 system." Stem Cell Res **67**: 103034.

The tissue inhibitors of metalloproteinases 3 (TIMP3) play an essential role in the tumorigenesis of human pancreatic endocrine tumors and Sorsby fundus dystrophy. To further investigate the significance of TIMP3 in disease, we used CRISPR/Cas9 to create a TIMP3 knock out human embryonic stem cell line

(WAe009-A-89) that can differentiate into any desired cell type. Our results show that the WAe009-A-89 cell line retains the typical colony form and normal karyotype of stem cells. The cells strongly expressed pluripotency markers and could differentiate into tissues of all three germ layers in vivo. This cell line allowed exploring the role of the TIMP3 gene in related diseases.

Yoshimatsu, S., et al. (2020). "Generation of a male common marmoset embryonic stem cell line DSY127-BV8VT1 carrying double reporters specific for the germ cell linage using the CRISPR-Cas9 and PiggyBac transposase systems." Stem Cell Res **44**: 101740.

BLIMP1 (PRDM1) and VASA (DDX4) play pivotal roles in the development of the germ cell linage. Importantly, these genes are specifically expressed in germ cells; BLIMP1 in primordial germ cells (PGCs) to early-stage gonocytes, and VASA in migration-stage PGCs to mature gametes. The high reproductive efficiency of common marmosets (marmosets; Callithrix jacchus) makes them advantageous for use in germ cell research. We herein report the generation of a male marmoset embryonic stem cell (ESC) line harboring BLIMP1 and DDX4 double reporters. This ESC line will be a useful tool for investigating male gametogenesis in non-human primates.

You, J., et al. (2021). "Generation of a homozygous LRP2 knockout human embryonic stem cell line (FDCHDPe010-A-56) by CRISPR/Cas9 system." Stem Cell Res **53**: 102342.

LRP2 is mainly expressed in the cell membrane of epithelia, maintaining normal endocytosis of nutrients from the extracellular microenvironment and mediating growth factor signals. The deficiency of LRP2 can result in abnormal lysosomal and mitochondrial function as well as insufficient resistance to oxidative stress. LRP2-KO animals show enlarged eyes and malfunction of the retinal pigment epithelium (RPE). We were able to generate an LRP2-KO human embryonic stem (ES) cell line using CRISPR/Cas9 gene editing and differentiate the mutant ES cells into RPE cells. Thus, this LRP2-KO human ES line will facilitate studying cellular mechanisms of eye disease due to LRP2 deficiency.

You, J., et al. (2022). "Generation of a homozygous P4HA2 knockout human embryonic stem cell line (FDCHDPe012-A) by CRISPR/Cas9 system." Stem Cell Res **64**: 102930.

Prolyl 4-hydroxylase subunit alpha-2(P4HA2) is associated with autosomal dominant high myopia. A significant reduction of P4HA2 protein expression has been observed in fibroblast cells of high myopia patients with inherited P4HA2 mutations. To explore the function of P4HA2 in cases of high myopia, we generated a P4HA2-KO hES cell line (FDCHDPe012-A) efficiently through CRISPR/Cas9 system. We confirmed the presence of a 5-bp biallelic deletion, causing a frameshift and premature P4HA2 translation termination. The FDCHDPe012-A presented morphology of typical stem cells expressed pluripotent genes, possessed a normal parental karyotype, and could differentiate into three germ layers.

You, J., et al. (2021). "Generation of a homozygous LRPAP1 knockout human embryonic stem cell line (FDCHDPe009-B) by CRISPR/Cas9 system." Stem Cell Res **56**: 102516.

The homozygous autosomal recessive truncating mutations of LDL receptor related protein associated protein 1 (LRPAP1) is a possible reason for Nonsyndromic Extreme Myopia, patients with which show typical chorioretinal degeneration. We generated an LRPAP1 knockout FDCHDPe009-B embryonic stem cell line to study mechanisms of retinal degeneration underlying LRPAP1 deficiency with the help of the CRISPR/Cas9 system. Two distinct biallelic deletions in the cell line have been confirmed, which causing a frameshift and premature stop codons thus influence the translation of LRPAP1. FDCHDPe009-B has maintained normal stem cell morphology, pluripotent gene expression, parental karyotype, and ability to differentiate into three germ layers.

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.

Zhang, C., et al. (2021). "Establishment of a CRISPR/Cas9-mediated ANP32A homozygous knockout human embryonic stem cell line." Stem Cell Res **52**: 102234.

ANP32A is a member of acidic leucine-rich nuclear phosphoprotein 32 family, which is involved in diverse biochemical processes, including chromatin modification and remodeling. Here, we established the CRISPR/Cas9-mediated ANP32A homozygous knockout human embryonic stem cell (ESC) line to investigate the roles of ANP32A in pluripotency maintenance and differentiation process of human ESCs. This cell line shows the normal karyotype and typical stem cell morphology, in accordance with high expression of pluripotent genes and the differentiation potential in vitro. Consequently, the ANP32A knockout cell line provides a promising approach for investigating the roles of ANP32A in human ESC cell fate decisions.

Zhang, C., et al. (2020). "Generating an MEIS1 homozygous knockout human embryonic stem cell line using the CRISPR/Cas9 system." Stem Cell Res **49**: 102069.

Myeloid ecotropic viral integration site 1 (MEIS1) plays an essential role in the development of several embryonic organs, such as the central nervous system and eyes. To further investigate the role of MEIS1 in embryonic development, herein, we generated a MEIS1 homozygous knockout human embryonic stem cell (hESC) line using the CRISPR/Cas9 genome-editing technology. We believe that this cell line will be a good resource for exploring the function of the MEIS1 gene in embryonic development in vitro. Furthermore, the gene-knockout method reported in this study is efficient and labor-saving, which may provide an effective strategy for hESC gene deletion.

Zhang, D., et al. (2022). "Generation of an RNF1-deficient human pluripotent stem cell line using CRISPR/Cas9 technology." Stem Cell Res **62**: 102809.

RNF1 (RING1A) is a catalytic component of the polycomb repressive complex 1 (PRC1) involved in regulation of, among others, embryonic development and disease progression. However, the exact role of RNF1 in self-renewal and differentiation of human embryonic stem cells (ESCs) remains unknown. Here, we derive one RNF1 knockout human ESC line using CRISPR/Cas9 system. The cell line retains the canonical stem cell morphology and normal karyotype. Moreover, the cell line highly expresses pluripotency genes and has three germ-layer differentiation potential. The RNF1 -/- cell line will be useful for studies on the function and role of RNF1 in human embryonic stem cell fate decisions.

Zhang, J., et al. (2022). "Generation of RYBP FLAG-HA knock-in human embryonic stem cell line through CRISPR/Cas9-mediated homologous recombination." Stem Cell Res **62**: 102803.

RYBP, a critical component of polycomb repressive complex1 (PRC1), is required for the pluripotency and differentiation of mouse embryonic stem cells(mESCs). However, its function and mechanism to regulate human embryonic stem cells(hESCs) remain unknown. Here, to investigate the role of RYBP in hESCs, we generate an hESC line with FLAG-HA tag knock-in to RYBP locus through CRISPR/Cas9-mediated homologous recombination. hESC with RYBP_FLAG-HA knock-in maintains normal morphology and karyotype, while it maintains pluripotency to differentiate into three germ layers.

Zhang, P., et al. (2022). "Generation of an S100B homozygous knockout pluripotent stem cell line (WAe009-A-94) by the CRISPR/Cas9 system." Stem Cell Res **64**: 102924.

S100 calcium binding protein beta (S100B) is an S-100 low molecular weight binding protein that regulates intracellular processes. This protein is involved in myocardial contractility and calcium handling capacity. In this study, a human embryonic stem cell (hESC) line with homozygous S100B knockout (S100B-KO) was generated using the CRISPR/Cas9 editing system. This S100B-KO hESC line maintained normal cell morphology and karyotype, expressed pluripotency markers, and could differentiate into cells of all three germ layers.

Zhang, S., et al. (2021). "Generation of a homozygous S100A1 knockout human embryonic stem cell line (WAe009-A-73) by the CRISPR/Cas9 editing system." Stem Cell Res **59**: 102631.

S100A1 is a calcium-binding protein involved in myocardial contractility,which possesses a high affinity for calcium. Several studies have demonstrated that S100A1 is a protector against myocardial injury. In this study, we have generated a homozygous S100A1 knockout (S100A1-KO) human embryonic stem cell (hESC) line by the CRISPR/Cas9 editing system. This S100A1-KO hESC line maintained normal morphology, pluripotency and karyotype, which can differentiate into three germ layers in vivo.

Zhang, T., et al. (2020). "Generation of a FOXH1 homozygous knockout human embryonic stem cell line by CRISPR/Cas9 system." Stem Cell Res **50**: 102121.

Human FOXH1 (Forkhead Box H1) gene encodes a human homolog of Xenopus forkhead activing signal transducer-1 and has been shown to play an important role in mesendoderm formation in X. tropicalis and mice. However, little is known about the function of FOXH1 in human development. Here we generated a FOXH1 homozygous knockout human embryonic stem cell (hESC), WAe009-A-42 by CRISPR/Cas9 mediated gene targeting. The WAe009-A-42 retained a typical undifferentiated morphology and normal karyotype, pluripotenty, and trilineage differentiation potential in vivo and vitro.

Zhang, W., et al. (2021). "Generation of a DKK2 homozygous knockout human embryonic stem cell line using the CRISPR/Cas9 system." Stem Cell Res **57**: 102611.

Dickkopf-2 (DKK2) is an antagonist of canonical Wnt signaling, which is involved invarious biological processes of development, such as epidermal appendage formation andeye development. To identify underlying effects of DKK2 during embryonic development, we generated a DKK2 homozygous knockout human embryonic stem cell (hESC) line through the CRISPR/Cas9 genome-editing technology. This cell line, which maintains normal stem cell morphology and stably expresses pluripotent markers, could provide an ideal platform for exploring the role of DKK2 in embryonic development. In addition, Zeocin selection combined with tiny clone picking might be a highly efficient way to generate gene-knockout hESC lines.

Zhang, X. H., et al. (2021). "Calcium signaling consequences of RyR2 mutations associated with CPVT1 introduced via CRISPR/Cas9 gene editing in human-induced pluripotent stem cell-derived cardiomyocytes: Comparison of RyR2-R420Q, F2483I, and Q4201R." Heart Rhythm **18**(2): 250-260.

BACKGROUND: Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) created from patients with catecholaminergic polymorphic ventricular tachycardia 1 (CPVT1) have been used to study CPVT1 arrhythmia. OBJECTIVE: The purpose of this study was to evaluate the Ca(2+) signaling aberrancies and pharmacological sensitivities of 3 CRISPR/Cas9-introduced CPVT1 mutations located in different molecular domains of ryanodine receptor 2 (RyR2). METHODS: CRISPR/Cas9-engineered hiPSC-CMs carrying RyR2 mutations-R420Q, Q4201R, and F2483I-were voltage clamped, and their electrophysiology, pharmacology, and Ca(2+) signaling phenotypes measured using total internal reflection fluorescence microscopy. RESULTS: R420Q and Q4201R mutant hiPSC-CMs exhibit irregular, long-lasting, spatially wandering Ca(2+) sparks and aberrant Ca(2+) releases similar to F2483I unlike the wild-type myocytes. Large sarcoplasmic reticulum (SR) Ca(2+) leaks and smaller SR Ca(2+) contents were detected in cells expressing Q4201R and F2483I, but not R420Q. Fractional Ca(2+) release and calcium-induced calcium release gain were higher in Q4201R than in R420Q and F2483I hiPSC-CMs. JTV519 was equally effective in suppressing Ca(2+) sparks, waves, and SR Ca(2+) leaks in hiPSC-CMs derived from all 3 mutant lines. Flecainide and dantrolene similarly suppressed SR Ca(2+) leaks, but were less effective in decreasing spark frequency and durations. CONCLUSION: CRISPR/Cas9 gene editing of hiPSCs provides a novel approach in studying CPVT1-associated RyR2 mutations and suggests that Ca(2+)-signaling aberrancies and drug sensitivities may vary depending on the mutation site.

Zhang, Y., et al. (2016). "Generation of a human induced pluripotent stem cell line via CRISPR-Cas9 mediated integration of a site-specific heterozygous mutation in CHMP2B." Stem Cell Res **17**(1): 148-150.

Frontotemporal dementia (FTD) is an early onset neurodegenerative disease. Mutations in several genes cause familial FTD and one of them is charged multivesicular body protein 2B (CHMP2B) on chromosome 3 (FTD3), a component of the endosomal sorting complex required for transport III (ESCRT-III). We have generated an induced pluripotent stem cell (iPSC) line of a healthy individual and inserted the CHMP2B IVS5AS G-C gene mutation into one of the alleles, resulting in aberrant splicing. This human iPSC line provides an ideal model to study CHMP2B-dependent phenotypes of FTD3.

Zhang, Y., et al. (2016). "Generation of a human induced pluripotent stem cell line via CRISPR-Cas9 mediated integration of a site-specific homozygous mutation in CHMP2B." Stem Cell Res **17**(1): 151-153.

Frontotemporal dementia (FTD) is an early onset neurodegenerative disease. Mutations in several genes cause familial FTD and one of them is charged multivesicular body protein 2B (CHMP2B) on chromosome 3 (FTD3), a component of the endosomal sorting complex required for transport III (ESCRT-III). We have generated an induced pluripotent stem cell (iPSC) line of a healthy individual and inserted the CHMP2B IVS5AS G-C gene mutation into both alleles, resulting in aberrant splicing. This human iPSC line provides an ideal model to study CHMP2B-dependent phenotypes of FTD3.

Zhang, Y., et al. (2022). "Generation of a heterozygous FUS-Q290X knock in human embryonic stem cell line (WAe009-A-83) using CRISPR/Cas9 system." Stem Cell Res **60**: 102734.

Fused in Sarcoma (FUS) gene encodes FUS RNA binding protein, a multifunctional protein component of the heterogeneous nuclear ribonucleoprotein complex, which is involved in pre-mRNA splicing and the export of fully processed mRNA to the cytoplasm, and it has been implicated in regulation of gene expression, maintenance of genomic integrity and mRNA/microRNA processing. FUS gene mutations result in amyotrophic lateral sclerosis and Liposarcoma. This heterozygous FUS-Q290X knock in hESC line will be a valuable tool to investigate the disease mechanisms of amyotrophic lateral sclerosis and Liposarcoma.

Zhao, H., et al. (2020). "Generation of a tdTomato-GAD67 reporter human epilepsia mutation induced pluripotent stem cell line, USTCi001-A-2, using CRISPR/Cas9 editing." Stem Cell Res **48**: 102003.

Dravet syndrome is an epileptic encephalopathy largely due to haploinsufficiency of the voltage-gated sodium channel Nav1.1 that is expressed primarily in GABAergic neurons. In order to distinguish the different subtypes, we used gene editing to introduce tdTomato gene into the genome of iPSCs to label the GABAergic neurons in the differentiated neuronal networks. The gene-edited cell line demonstrates normal karyotype, expresses the main pluripotency markers, and shows the presence of differentiation into the three embryonic germ layers in teratomas.

Zhao, T., et al. (2021). "Generation of a TBX5 homozygous knockout embryonic stem cell line (WAe009-A-45) by CRISPR/Cas9 genome editing." Stem Cell Res **51**: 102156.

Holt-Oram syndrome (HOS), which is caused by genetic changes in the TBX5 gene, affects the hands and heart. HOS patients have heart defects, including atrial septal defects (ASD), ventricular septal defects (VSD) and heart conduction disease. Here, we generated a homozygous TBX5 knockout human embryonic stem cell (hESC) line (TBX5-KO) using a CRISPR/Cas9 system. The TBX5-KO maintained stem cell like morphology, pluripotency markers, normal karyotype, and could differentiate into all three germ layers in vivo. This cell line can provide an in vitro platform for studying the pathogenic mechanisms and biological function of TBX5 in the heart development.

Zheng, W., et al. (2022). "Generation of a human embryonic stem cell line (SMUDHe010-A-82) carrying a homozygous c.1538G > A (p.G513D) mutation in the OSMR gene by CRISPR/Cas9-mediated homologous recombination." Stem Cell Res **63**: 102842.

Mutations in the tumor suppressor M receptor (OSMR) gene are associated with primary localized cutaneous amyloidosis (PLCA). Recently, we confirmed that OSMR loss-of-function mutations enhance epidermal keratinocyte differentiation via inactivation of the STAT5/KLF7 signaling. However, no disease model was available for PLCA. Accordingly, we generated an OSMR c.1538G > A mutant human embryonic stem cell line (SMUDHe010-A-82) using CRISPR/Cas9-mediated homologous recombination. The cell line preserves normal karyotype, pluripotency and the ability to differentiate into all three germ layers. Moreover, the cell line can be used to prepare human skin organoid, which may provide a disease model for PLCA.

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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