Genome editing and Stem Cell Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
[Mark Herbert. **Genome editing and Stem Cell Research Literatures.** *Stem Cell* 2019;10(1):115-119]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 8. doi:10.7537/marsscj100119.08.

Key words: stem cell; life; research; literature

Introduction

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

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

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

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

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

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

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.

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

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

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

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

Ding, Q., et al. (2013). "Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs." Cell Stem Cell **12**(4): 393-394.

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.

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.

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

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

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

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

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.

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

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

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

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

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

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

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

Efficient strategies for precise genome editing in human-induced pluripotent cells (hiPSCs) will enable sophisticated genome engineering for research and clinical purposes. The development of programmable sequence-specific nucleases such as Transcription Activator-Like Effectors Nucleases (TALENs) and Cas9-gRNA allows genetic modifications to be made more efficiently at targeted sites of interest. However,

many opportunities remain to optimize these tools and to enlarge their spheres of application. We present several improvements: First, we developed functional re-coded TALEs (reTALEs), which not only enable simple one-pot TALE synthesis but also allow TALE-based applications to be performed using lentiviral vectors. We then compared genome-editing efficiencies in hiPSCs mediated by 15 pairs of reTALENs and Cas9-gRNA targeting CCR5 and optimized ssODN design in conjunction with both methods for introducing specific mutations. We found Cas9-gRNA achieved 7-8x higher non-homologous end joining efficiencies (3%) than reTALENs (0.4%) and moderately superior homology-directed repair efficiencies (1.0 versus 0.6%) when combined with ssODN donors in hiPSCs. Using the optimal design, we demonstrated a streamlined process to generated seamlessly genome corrected hiPSCs within 3 weeks.

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.

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3/25/2019