Stem Cell and Genome editing 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. Genome editing, or genome engineering is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. In 2018, the common methods for such editing use engineered nucleases, or "molecular scissors". These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations. As of 2015 four families of engineered nucleases were used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system.
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Key words: stem cell; genome editing; life; research; literature

Introduction

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. 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. **Genome editing**, or **genome engineering** is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. In 2018, the common methods for such editing use engineered nucleases, or "molecular scissors". These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations. As of 2015 four families of engineered nucleases were used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system. Nine genome editors were available as of 2017.

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

Benakanakere, M. R., et al. (2016). "Investigation of the functional role of human Interleukin-8 gene haplotypes by CRISPR/Cas9 mediated genome editing." Sci Rep **6**: 31180.

Interleukin-8 (IL-8) gene polymorphisms have been considered as susceptibility factors in periodontal disease. However, the functional roles of IL-8 gene haplotypes have not been investigated. Here, we demonstrate for the first time the use of the CRISPR/Cas9 system to engineer the IL-8 gene, and tested the functionality of different haplotypes. Two sgRNAs vectors targeting the IL-8 gene and the naked homologous repair DNA carrying different haplotypes were used to successfully generate HEK293T cells carrying the AT genotype at the first SNP - rs4073 (alias -251), TT genotype at the second SNP - rs2227307 (alias +396), TC or CC genotypes at the third SNP - rs2227306 (alias +781) at the IL-8 locus. When stimulated with Poly I:C, ATC/TTC haplotype, cells significantly up-regulated the IL-8 at both transcriptional and translational levels. To test whether ATC/TTC haplotype is functional, we used a trans-well assay to measure the transmigration of primary neutrophils incubated with supernatants from the Poly I:C stimulation experiment. ATC/TTC haplotype cells significantly increased transmigration of neutrophils confirming the functional role for this IL-8 haplotype. Taken together, our data provides evidence that carriage of the ATC/TTC haplotype in itself may

increase the influx of neutrophils in inflammatory lesions and influence disease susceptibility.

Boulad, F., et al. (2018). "Gene Therapy and Genome Editing." Hematol Oncol Clin North Am **32**(2): 329-342.

The beta-thalassemias are inherited blood disorders that result from insufficient production of the beta-chain of hemoglobin. More than 200 different mutations have been identified. beta-Thalassemia major requires life-long transfusions. The only cure for severe beta-thalassemia is to provide patients with hematopoietic stem cells. Globin gene therapy promises a curative autologous stem cell transplantation without the immunologic complications of allogeneic transplantation. The future directions of gene therapy include enhancement of lentiviral vector-based approaches, fine tuning of the conditioning regimen, and the design of safer vectors. Progress in genetic engineering bodes well for finding a cure for severe globin disorders.

Brown, A. J., et al. (2013). "Whole-rat conditional gene knockout via genome editing." Nat Methods **10**(7): 638-640.

Animal models with genetic modifications under temporal and/or spatial control are invaluable to functional genomics and medical research. Here we report the generation of tissue-specific knockout rats via microinjection of zinc-finger nucleases (ZFNs) into fertilized eggs. We generated rats with loxP-flanked (floxed) alleles and a tyrosine hydroxylase promoter-driven cre allele and demonstrated Cre-dependent gene disruption in vivo. Pronuclear microinjection of ZFNs, shown by our data to be an efficient and rapid method for creating conditional knockout rats, should also be applicable in other species.

Burle-Caldas, G. A., et al. (2018). "Assessment of two CRISPR-Cas9 genome editing protocols for rapid generation of Trypanosoma cruzi gene knockout mutants." Int J Parasitol.

CRISPR/Cas9 technology has been used to edit genomes in a variety of organisms. Using the GP72 gene as a target sequence, we tested two distinct approaches to generate Trypanosoma cruzi knockout mutants using the Cas9 nuclease and in vitro transcribed single guide RNA. Highly efficient rates of disruption of GP72 were achieved either by transfecting parasites stably expressing Streptococcus pyogenes Cas9 with single guide RNA or by transfecting wild type parasites with recombinant Staphylococcus aureus Cas9 previously associated with single guide RNA. In both protocols, we used single-stranded oligonucleotides as a repair template for homologous recombination and insertion of stop codons in the target gene.

Ma, S., et al. (2014). "Genome editing of BmFib-H gene provides an empty Bombyx mori silk gland for a highly efficient bioreactor." Sci Rep **4**: 6867.

Evolution has produced some remarkable creatures, of which silk gland is a fascinating organ that exists in a variety of insects and almost half of the 34,000 spider species. The impressive ability to secrete huge amount of pure silk protein, and to store proteins at an extremely high concentration (up to 25%) make the silk gland of Bombyx mori hold great promise to be a cost-effective platform for production of recombinant proteins. However, the extremely low production yields of the numerous reported expression systems greatly hindered the exploration and application of silk gland bioreactors. Using customized zinc finger nucleases (ZFN), we successfully performed genome editing of Bmfib-H gene, which encodes the largest and most abundant silk protein, in B. mori with efficiency higher than any previously reported. The resulted Bmfib-H knocked-out B. mori showed a smaller and empty silk gland, abnormally developed posterior silk gland cells, an extremely thin cocoon that contain only sericin proteins, and a slightly heavier pupae. We also showed that removal of endogenous Bmfib-H protein could significantly increase the expression level of exogenous protein. Furthermore, we demonstrated that the bioreactor is suitable for large scale production of protein-based materials.

Maeder, M. L. and C. A. Gersbach (2016). "Genome-editing Technologies for Gene and Cell Therapy." Mol Ther **24**(3): 430-446.

Gene therapy has historically been defined as the addition of new genes to human cells. However, the recent advent of genome-editing technologies has enabled a new paradigm in which the sequence of the human genome can be precisely manipulated to achieve a therapeutic effect. This includes the correction of mutations that cause disease, the addition of therapeutic genes to specific sites in the genome, and the removal of deleterious genes or genome sequences. This review presents the mechanisms of different genome-editing strategies and describes each of the common nuclease-based platforms, including zinc finger nucleases, transcription activator-like effector nucleases (TALENs), meganucleases, and the CRISPR/Cas9 system. We then summarize the progress made in applying genome editing to various areas of gene and cell therapy, including antiviral strategies, immunotherapies, and the treatment of monogenic hereditary disorders. The current challenges and future prospects for genome editing as

a transformative technology for gene and cell therapy are also discussed.

Makai, S., et al. (2016). "A Catalog of Regulatory Sequences for Trait Gene for the Genome Editing of Wheat." Front Plant Sci **7**: 1504.

Wheat has been cultivated for 10000 years and ever since the origin of hexaploid wheat it has been exempt from natural selection. Instead, it was under the constant selective pressure of human agriculture from harvest to sowing during every year, producing a vast array of varieties. Wheat has been adopted globally, accumulating variation for genes involved in yield traits, environmental adaptation and resistance. However, one small but important part of the wheat genome has hardly changed: the regulatory regions of both the x- and y-type high molecular weight glutenin subunit (HMW-GS) genes, which are alone responsible for approximately 12% of the grain protein content. The phylogeny of the HMW-GS regulatory regions of the Triticeae demonstrates that a genetic bottleneck may have led to its decreased diversity during domestication and the subsequent cultivation. It has also highlighted the fact that the wild relatives of wheat may offer an unexploited genetic resource for the regulatory region of these genes. Significant research efforts have been made in the public sector and by international agencies, using wild crosses to exploit the available genetic variation, and as a result synthetic hexaploids are now being utilized by a number of breeding companies. However, a newly emerging tool of genome editing provides significantly improved efficiency in exploiting the natural variation in HMW-GS genes and incorporating this into elite cultivars and breeding lines. Recent advancement in the understanding of the regulation of these genes underlines the needs for an overview of the regulatory elements for genome editing purposes.

Yusa, K. (2013). "Seamless genome editing in human pluripotent stem cells using custom endonuclease-based gene targeting and the piggyBac transposon." Nat Protoc **8**(10): 2061-2078.

I report here a detailed protocol for seamless genome editing using the piggyBac transposon in human pluripotent stem cells (hPSCs). Recent advances in custom endonucleases have enabled us to routinely perform genome editing in hPSCs. Conventional approaches use the Cre/loxP system that leaves behind residual sequences in the targeted genome. I used the piggyBac transposon to seamlessly remove a drug selection cassette and demonstrated safe genetic correction of a mutation causing alpha-1 antitrypsin deficiency in patient-derived hPSCs. An alternative approach to using the piggyBac transposon to correct mutations involves using single-stranded oligonucleotides, which is a faster process to complete. However, this experimental procedure is rather complicated and it may be hard to achieve homozygous modifications. In contrast, using the piggyBac transposon with drug selection-based enrichment of genetic modifications, as described here, is simple and can yield multiple correctly targeted clones, including homozygotes. Although two rounds of genetic manipulation are required to achieve homozygote modifications, the entire process takes approximately 3 months to complete.

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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