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Cancer Biology

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Cancer Research Literatures (2)

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.
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Key words: cancer; life; research; literature; cell

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.

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

Annunziato, S., et al. (2016). "Modeling invasive lobular breast carcinoma by CRISPR/Cas9-mediated somatic genome editing of the mammary gland." Genes Dev **30**(12): 1470-1480.

Large-scale sequencing studies are rapidly identifying putative oncogenic mutations in human tumors. However, discrimination between passenger and driver events in tumorigenesis remains challenging and requires in vivo validation studies in reliable animal models of human cancer. In this study, we describe a novel strategy for in vivo validation of candidate tumor suppressors implicated in invasive lobular breast carcinoma (ILC), which is hallmarked by loss of the cell-cell adhesion molecule E-cadherin. We describe an approach to model ILC by intraductal injection of lentiviral vectors encoding Cre recombinase, the CRISPR/Cas9 system, or both in female mice carrying conditional alleles of the Cdh1 gene, encoding for E-cadherin. Using this approach, we were able to target ILC-initiating cells and induce specific gene disruption of Pten by CRISPR/Cas9-mediated somatic gene editing. Whereas intraductal injection of Cas9-encoding lentiviruses induced Cas9-specific immune responses and development of tumors that did not resemble ILC, lentiviral delivery of a Pten targeting single-guide RNA (sgRNA) in mice with mammary gland-specific loss of E-cadherin and expression of Cas9 efficiently induced ILC development. This versatile platform can be used for rapid in vivo testing of putative tumor suppressor genes implicated in ILC, providing new opportunities for modeling invasive lobular breast carcinoma in mice.

Benahmed-Miniuk, F., et al. (2017). "Genome-editing technologies and patent landscape overview." Pharm Pat Anal **6**(3): 115-134.

Unlike with zinc finger nuclease and transcriptional activator-like effector nuclease DNA modification technologies that rely on lead proteins, developed through expensive and time-consuming processes, the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system has rapidly emerged as the most promising gene-editing technology to date for the modification of any selected DNA sequence. CRISPR is receiving tremendous fanfare due, in part, to its potential to provide a means to fundamentally alter medical genetics and especially cancer medicine. In this review, we compare key technologies of genome-editing zinc finger nucleases, transcriptional activator-like effector nucleases and CRISPR, with a focus on the race to acquire lucrative intellectual property rights, the current CRISPR patent dispute and potential repercussions on innovation and the adoption of this promising technology by the medical community.

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.

Brinkman, E. K., et al. (2014). "Easy quantitative assessment of genome editing by sequence trace decomposition." Nucleic Acids Res **42**(22): e168.

The efficacy and the mutation spectrum of genome editing methods can vary substantially depending on the targeted sequence. A simple, quick assay to accurately characterize and quantify the induced mutations is therefore needed. Here we present TIDE, a method for this purpose that requires only a pair of PCR reactions and two standard capillary sequencing runs. The sequence traces are then analyzed by a specially developed decomposition algorithm that identifies the major induced mutations in the projected editing site and accurately determines their frequency in a cell population. This method is cost-effective and quick, and it provides much more detailed information than current enzyme-based assays. An interactive web tool for automated decomposition of the sequence traces is available. TIDE greatly facilitates the testing and rational design of genome editing strategies.

Buechele, C., et al. (2015). "MLL leukemia induction by genome editing of human CD34+ hematopoietic cells." Blood **126**(14): 1683-1694.

Chromosomal rearrangements involving the mixed-lineage leukemia (MLL) gene occur in primary and treatment-related leukemias and confer a poor prognosis. Studies based primarily on mouse models have substantially advanced our understanding of MLL leukemia pathogenesis, but often use supraphysiological oncogene expression with uncertain implications for human leukemia. Genome editing using site-specific nucleases provides a powerful new technology for gene modification to potentially model human disease, however, this approach has not been used to re-create acute leukemia in human cells of origin comparable to disease observed in patients. We applied transcription activator-like effector nuclease-mediated genome editing to generate endogenous MLL-AF9 and MLL-ENL oncogenes through insertional mutagenesis in primary human hematopoietic stem and progenitor cells (HSPCs) derived from human umbilical cord blood. Engineered HSPCs displayed altered in vitro growth potentials and induced acute leukemias following transplantation in immunocompromised mice at a mean latency of 16 weeks. The leukemias displayed phenotypic and morphologic similarities with patient leukemia blasts including a subset with mixed phenotype, a distinctive feature seen in clinical disease. The leukemic blasts expressed an MLL-associated transcriptional program with elevated levels of crucial MLL target genes, displayed heightened sensitivity to DOT1L inhibition, and demonstrated increased oncogenic potential ex vivo and in secondary transplant assays. Thus, genome editing to create endogenous MLL oncogenes in primary human HSPCs faithfully models acute MLL-rearranged leukemia and provides an experimental platform for prospective studies of leukemia initiation and stem cell biology in a genetic subtype of poor prognosis leukemia.

Calos, M. P. (2017). "Genome Editing Techniques and Their Therapeutic Applications." Clin Pharmacol Ther **101**(1): 42-51.

Fueled by advances in the field of genetics, the methods available to edit DNA sequences in living cells have continued to develop steadily. These technologies directly impact the fields of gene and cell therapy, where changes in the DNA sequence of target cells offer a route to correct genetic diseases and manipulate disorders like cancer. We review here the expanding menu of genome editing techniques and how they are being applied to therapeutic targets. The methods encompass a myriad of approaches to modify the covalent structure of DNA, including the targeted creation of double-strand breaks that can catalyze genomic changes, as well as the use of retroviruses and transposons to mediate gene addition, recombinases for sequence-specific gene addition and deletion, and base repair for direct sequence changes. The continued growth of the exciting field of genome editing is opening new possibilities for therapeutic intervention.

Canver, M. C., et al. (2017). "Functional interrogation of non-coding DNA through CRISPR genome editing." Methods **121-122**: 118-129.

Methodologies to interrogate non-coding regions have lagged behind coding regions despite comprising the vast majority of the genome. However, the rapid evolution of clustered regularly interspaced short palindromic repeats (CRISPR)-based genome editing has provided a multitude of novel techniques

for laboratory investigation including significant contributions to the toolbox for studying non-coding DNA. CRISPR-mediated loss-of-function strategies rely on direct disruption of the underlying sequence or repression of transcription without modifying the targeted DNA sequence. CRISPR-mediated gain-of-function approaches similarly benefit from methods to alter the targeted sequence through integration of customized sequence into the genome as well as methods to activate transcription. Here we review CRISPR-based loss- and gain-of-function techniques for the interrogation of non-coding DNA.

Ceasar, S. A., et al. (2016). "Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9." Biochim Biophys Acta **1863**(9): 2333-2344.

The clustered, regularly interspaced, short palindromic repeat (CRISPR) and CRISPR associated protein 9 (Cas9) system discovered as an adaptive immunity mechanism in prokaryotes has emerged as the most popular tool for the precise alterations of the genomes of diverse species. CRISPR/Cas9 system has taken the world of genome editing by storm in recent years. Its popularity as a tool for altering genomes is due to the ability of Cas9 protein to cause double-stranded breaks in DNA after binding with short guide RNA molecules, which can be produced with dramatically less effort and expense than required for production of transcription-activator like effector nucleases (TALEN) and zinc-finger nucleases (ZFN). This system has been exploited in many species from prokaryotes to higher animals including human cells as evidenced by the literature showing increasing sophistication and ease of CRISPR/Cas9 as well as increasing species variety where it is applicable. This technology is poised to solve several complex molecular biology problems faced in life science research including cancer research. In this review, we highlight the recent advancements in CRISPR/Cas9 system in editing genomes of prokaryotes, fungi, plants and animals and provide details on software tools available for convenient design of CRISPR/Cas9 targeting plasmids. We also discuss the future prospects of this advanced molecular technology.

Chakravarti, D., et al. (2016). "Synthetic biology approaches in cancer immunotherapy, genetic network engineering, and genome editing." Integr Biol (Camb) **8**(4): 504-517.

Investigations into cells and their contents have provided evolving insight into the emergence of complex biological behaviors. Capitalizing on this knowledge, synthetic biology seeks to manipulate the cellular machinery towards novel purposes, extending discoveries from basic science to new applications. While these developments have demonstrated the potential of building with biological parts, the complexity of cells can pose numerous challenges. In this review, we will highlight the broad and vital role that the synthetic biology approach has played in applying fundamental biological discoveries in receptors, genetic circuits, and genome-editing systems towards translation in the fields of immunotherapy, biosensors, disease models and gene therapy. These examples are evidence of the strength of synthetic approaches, while also illustrating considerations that must be addressed when developing systems around living cells.

Chen, S., et al. (2016). "CRISPR-Cas9: from Genome Editing to Cancer Research." Int J Biol Sci **12**(12): 1427-1436.

Cancer development is a multistep process triggered by innate and acquired mutations, which cause the functional abnormality and determine the initiation and progression of tumorigenesis. Gene editing is a widely used engineering tool for generating mutations that enhance tumorigenesis. The recent developed clustered regularly interspaced short palindromic repeats-CRISPR-associated 9 (CRISPR-Cas9) system renews the genome editing approach into a more convenient and efficient way. By rapidly introducing genetic modifications in cell lines, organs and animals, CRISPR-Cas9 system extends the gene editing into whole genome screening, both in loss-of-function and gain-of-function manners. Meanwhile, the system accelerates the establishment of animal cancer models, promoting in vivo studies for cancer research. Furthermore, CRISPR-Cas9 system is modified into diverse innovative tools for observing the dynamic bioprocesses in cancer studies, such as image tracing for targeted DNA, regulation of transcription activation or repression. Here, we view recent technical advances in the application of CRISPR-Cas9 system in cancer genetics, large-scale cancer driver gene hunting, animal cancer modeling and functional studies.

Chiang, T. W., et al. (2016). "CRISPR-Cas9(D10A) nickase-based genotypic and phenotypic screening to enhance genome editing." Sci Rep **6**: 24356.

The RNA-guided Cas9 nuclease is being widely employed to engineer the genomes of various cells and organisms. Despite the efficient mutagenesis induced by Cas9, off-target effects have raised concerns over the system's specificity. Recently a "double-nicking" strategy using catalytic mutant Cas9(D10A) nickase has been developed to minimise off-target effects. Here, we describe a Cas9(D10A)-based screening approach that combines an All-in-One Cas9(D10A) nickase vector with fluorescence-activated cell sorting enrichment followed by high-

throughput genotypic and phenotypic clonal screening strategies to generate isogenic knockouts and knock-ins highly efficiently, with minimal off-target effects. We validated this approach by targeting genes for the DNA-damage response (DDR) proteins MDC1, 53BP1, RIF1 and P53, plus the nuclear architecture proteins Lamin A/C, in three different human cell lines. We also efficiently obtained biallelic knock-in clones, using single-stranded oligodeoxynucleotides as homologous templates, for insertion of an EcoRI recognition site at the RIF1 locus and introduction of a point mutation at the histone H2AFX locus to abolish assembly of DDR factors at sites of DNA double-strand breaks. This versatile screening approach should facilitate research aimed at defining gene functions, modelling of cancers and other diseases underpinned by genetic factors, and exploring new therapeutic opportunities.

Chiou, S. H., et al. (2015). "Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing." Genes Dev **29**(14): 1576-1585.

Pancreatic ductal adenocarcinoma (PDAC) is a genomically diverse, prevalent, and almost invariably fatal malignancy. Although conventional genetically engineered mouse models of human PDAC have been instrumental in understanding pancreatic cancer development, these models are much too labor-intensive, expensive, and slow to perform the extensive molecular analyses needed to adequately understand this disease. Here we demonstrate that retrograde pancreatic ductal injection of either adenoviral-Cre or lentiviral-Cre vectors allows titratable initiation of pancreatic neoplasias that progress into invasive and metastatic PDAC. To enable in vivo CRISPR/Cas9-mediated gene inactivation in the pancreas, we generated a Cre-regulated Cas9 allele and lentiviral vectors that express Cre and a single-guide RNA. CRISPR-mediated targeting of Lkb1 in combination with oncogenic Kras expression led to selection for inactivating genomic alterations, absence of Lkb1 protein, and rapid tumor growth that phenocopied Cre-mediated genetic deletion of Lkb1. This method will transform our ability to rapidly interrogate gene function during the development of this recalcitrant cancer.

Chira, S., et al. (2017). "CRISPR/Cas9: Transcending the Reality of Genome Editing." Mol Ther Nucleic Acids **7**: 211-222.

With the expansion of the microbiology field of research, a new genome editing tool arises from the biology of bacteria that holds the promise of achieving precise modifications in the genome with a simplicity and versatility that surpasses previous genome editing methods. This new technique, commonly named CRISPR/Cas9, led to a rapid expansion of the biomedical field; more specifically, cancer characterization and modeling have benefitted greatly from the genome editing capabilities of CRISPR/Cas9. In this paper, we briefly summarize recent improvements in CRISPR/Cas9 design meant to overcome the limitations that have arisen from the nuclease activity of Cas9 and the influence of this technology in cancer research. In addition, we present challenges that might impede the clinical applicability of CRISPR/Cas9 for cancer therapy and highlight future directions for designing CRISPR/Cas9 delivery systems that might prove useful for cancer therapeutics.

Cornu, T. I., et al. (2017). "Refining strategies to translate genome editing to the clinic." Nat Med **23**(4): 415-423.

Recent progress in developing programmable nucleases, such as zinc-finger nucleases, transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas nucleases, have paved the way for gene editing to enter clinical practice. This translation is a result of combining high nuclease activity with high specificity and successfully applying this technology in various preclinical disease models, including infectious disease, primary immunodeficiencies, hemoglobinopathies, hemophilia and muscular dystrophy. Several clinical gene-editing trials, both ex vivo and in vivo, have been initiated in the past 2 years, including studies that aim to knockout genes as well as to add therapeutic transgenes. Here we discuss the advances made in the gene-editing field in recent years, and specify priorities that need to be addressed to expand therapeutic genome editing to further disease entities.

Cortina, C., et al. (2017). "A genome editing approach to study cancer stem cells in human tumors." EMBO Mol Med **9**(7): 869-879.

The analysis of stem cell hierarchies in human cancers has been hampered by the impossibility of identifying or tracking tumor cell populations in an intact environment. To overcome this limitation, we devised a strategy based on editing the genomes of patient-derived tumor organoids using CRISPR/Cas9 technology to integrate reporter cassettes at desired marker genes. As proof of concept, we engineered human colorectal cancer (CRC) organoids that carry EGFP and lineage-tracing cassettes knocked in the LGR5 locus. Analysis of LGR5-EGFP(+) cells isolated from organoid-derived xenografts demonstrated that these cells express a gene program similar to that of normal intestinal stem cells and that they propagate the disease to recipient mice very efficiently. Lineage-tracing experiments showed that LGR5(+) CRC cells

self-renew and generate progeny over long time periods that undergo differentiation toward mucosecreting- and absorptive-like phenotypes. These genetic experiments confirm that human CRCs adopt a hierarchical organization reminiscent of that of the normal colonic epithelium. The strategy described herein may have broad applications to study cell heterogeneity in human tumors.

Cui, Z., et al. (2017). "Genome editing reveals dmrt1 as an essential male sex-determining gene in Chinese tongue sole (Cynoglossus semilaevis)." Sci Rep **7**: 42213.

Chinese tongue sole is a marine fish with ZW sex determination. Genome sequencing suggested that the Z-linked dmrt1 is a putative male determination gene, but direct genetic evidence is still lacking. Here we show that TALEN of dmrt1 efficiently induced mutations of this gene. The ZZ dmrt1 mutant fish developed ovary-like testis, and the spermatogenesis was disrupted. The female-related genes foxl2 and cyp19a1a were significantly increased in the gonad of the ZZ dmrt1 mutant. Conversely, the male-related genes Sox9a and Amh were significantly decreased. The dmrt1 deficient ZZ fish grew much faster than ZZ male control. Notably, we obtained an intersex ZW fish with a testis on one side and an ovary on the other side. This fish was chimeric for a dmrt1 mutation in the ovary, and wild-type dmrt1 in the testis. Our data provide the first functional evidence that dmrt1 is a male determining gene in tongue sole.

Dalvai, M., et al. (2015). "A Scalable Genome-Editing-Based Approach for Mapping Multiprotein Complexes in Human Cells." Cell Rep **13**(3): 621-633.

Conventional affinity purification followed by mass spectrometry (AP-MS) analysis is a broadly applicable method used to decipher molecular interaction networks and infer protein function. However, it is sensitive to perturbations induced by ectopically overexpressed target proteins and does not reflect multilevel physiological regulation in response to diverse stimuli. Here, we developed an interface between genome editing and proteomics to isolate native protein complexes produced from their natural genomic contexts. We used CRISPR/Cas9 and TAL effector nucleases (TALENs) to tag endogenous genes and purified several DNA repair and chromatin-modifying holoenzymes to near homogeneity. We uncovered subunits and interactions among well-characterized complexes and report the isolation of MCM8/9, highlighting the efficiency and robustness of the approach. These methods improve and simplify both small- and large-scale explorations of protein interactions as well as the study of biochemical activities and structure-function relationships.

Daly, O. M., et al. (2016). "CEP164-null cells generated by genome editing show a ciliation defect with intact DNA repair capacity." J Cell Sci **129**(9): 1769-1774.

Primary cilia are microtubule structures that extend from the distal end of the mature, mother centriole. CEP164 is a component of the distal appendages carried by the mother centriole that is required for primary cilium formation. Recent data have implicated CEP164 as a ciliopathy gene and suggest that CEP164 plays some roles in the DNA damage response (DDR). We used reverse genetics to test the role of CEP164 in the DDR. We found that conditional depletion of CEP164 in chicken DT40 cells using an auxin-inducible degron led to no increase in sensitivity to DNA damage induced by ionising or ultraviolet irradiation. Disruption of CEP164 in human retinal pigmented epithelial cells blocked primary cilium formation but did not affect cellular proliferation or cellular responses to ionising or ultraviolet irradiation. Furthermore, we observed no localisation of CEP164 to the nucleus using immunofluorescence microscopy and analysis of multiple tagged forms of CEP164. Our data suggest that CEP164 is not required in the DDR.

De Silva Feelixge, H. S., et al. (2018). "CRISPR/Cas9 and Genome Editing for Viral Disease-Is Resistance Futile?" ACS Infect Dis.

Chronic viral infections remain a major public health issue affecting millions of people worldwide. Highly active antiviral treatments have significantly improved prognosis and infection-related morbidity and mortality but have failed to eliminate persistent viral forms. Therefore, new strategies to either eradicate or control these viral reservoirs are paramount to allow patients to stop antiretroviral therapy and realize a cure. Viral genome disruption based on gene editing by programmable endonucleases is one promising curative gene therapy approach. Recent findings on RNA-guided human immunodeficiency virus 1 (HIV-1) genome cleavage by Cas9 and other gene-editing enzymes in latently infected cells have shown high levels of site-specific genome disruption and potent inhibition of virus replication. However, HIV-1 can readily develop resistance to genome editing at a single antiviral target site. Current data suggest that cellular repair associated with DNA double-strand breaks can accelerate the emergence of resistance. On the other hand, a combination antiviral target strategy can exploit the same repair mechanism to functionally cure HIV-1 infection in vitro while avoiding the development of resistance. This perspective summarizes recent findings on the biology of resistance to genome editing and

discusses the significance of viral genetic diversity on the application of gene editing strategies toward cure.

DeNicola, G. M., et al. (2015). "The utility of transposon mutagenesis for cancer studies in the era of genome editing." Genome Biol **16**: 229.

The use of transposons as insertional mutagens to identify cancer genes in mice has generated a wealth of information over the past decade. Here, we discuss recent major advances in transposon-mediated insertional mutagenesis screens and compare this technology with other screening strategies.

Dow, L. E., et al. (2015). "Inducible in vivo genome editing with CRISPR-Cas9." Nat Biotechnol **33**(4): 390-394.

CRISPR-Cas9-based genome editing enables the rapid genetic manipulation of any genomic locus without the need for gene targeting by homologous recombination. Here we describe a conditional transgenic approach that allows temporal control of CRISPR-Cas9 activity for inducible genome editing in adult mice. We show that doxycycline-regulated Cas9 induction enables widespread gene disruption in multiple tissues and that limiting the duration of Cas9 expression or using a Cas9(D10A) (Cas9n) variant can regulate the frequency and size of target gene modifications, respectively. Further, we show that this inducible CRISPR (iCRISPR) system can be used effectively to create biallelic mutation in multiple target loci and, thus, provides a flexible and fast platform to study loss-of-function phenotypes in vivo.

El Fatimy, R., et al. (2017). "Genome Editing Reveals Glioblastoma Addiction to MicroRNA-10b." Mol Ther **25**(2): 368-378.

Glioblastoma (GBM) brain tumor remains among the most lethal and incurable human diseases. Oncogenic microRNA-10b (miR-10b) is strongly and universally upregulated in GBM, and its inhibition by antisense oligonucleotides (ASOs) reduces the growth of heterogeneous glioma cells; therefore, miR-10b represents a unique therapeutic target for GBM. Here we explored the effects of miR-10b gene editing on GBM. Using the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system, we investigated effects of miR-10b gene editing on the growth of cultured human glioma cells, tumor-initiating stem-like cells, and mouse GBM xenografts, as well as the oncogene-induced transformation of normal astrocytes. We show that GBM is strictly "addicted" to miR-10b and that miR-10b gene ablation is lethal for glioma cell cultures and established intracranial tumors. miR-10b loss-of-function mutations lead to the death of glioma, but not other cancer cell lines. We have not detected escaped proliferative clones of GBM cells edited in the miR-10b locus. Finally, neoplastic transformation of normal astrocytes was abolished by the miR-10b-editing vectors. This study demonstrates the feasibility of gene editing for brain tumors in vivo and suggests virus-mediated miR-10b gene ablation as a promising therapeutic approach that permanently eliminates the key regulator essential for tumor growth and survival.

El Refaey, M., et al. (2017). "In Vivo Genome Editing Restores Dystrophin Expression and Cardiac Function in Dystrophic Mice." Circ Res **121**(8): 923-929.

RATIONALE: Duchenne muscular dystrophy is a severe inherited form of muscular dystrophy caused by mutations in the reading frame of the dystrophin gene disrupting its protein expression. Dystrophic cardiomyopathy is a leading cause of death in Duchenne muscular dystrophy patients, and currently no effective treatment exists to halt its progression. Recent advancement in genome editing technologies offers a promising therapeutic approach in restoring dystrophin protein expression. However, the impact of this approach on Duchenne muscular dystrophy cardiac function has yet to be evaluated. Therefore, we assessed the therapeutic efficacy of CRISPR (clustered regularly interspaced short palindromic repeats)-mediated genome editing on dystrophin expression and cardiac function in mdx/Utr(+/-) mice after a single systemic delivery of recombinant adeno-associated virus. OBJECTIVE: To examine the efficiency and physiological impact of CRISPR-mediated genome editing on cardiac dystrophin expression and function in dystrophic mice. METHODS AND RESULTS: Here, we packaged SaCas9 (clustered regularly interspaced short palindromic repeat-associated 9 from Staphylococcus aureus) and guide RNA constructs into an adeno-associated virus vector and systemically delivered them to mdx/Utr(+/-) neonates. We showed that CRIPSR-mediated genome editing efficiently excised the mutant exon 23 in dystrophic mice, and immunofluorescence data supported the restoration of dystrophin protein expression in dystrophic cardiac muscles to a level approaching 40%. Moreover, there was a noted restoration in the architecture of cardiac muscle fibers and a reduction in the extent of fibrosis in dystrophin-deficient hearts. The contractility of cardiac papillary muscles was also restored in CRISPR-edited cardiac muscles compared with untreated controls. Furthermore, our targeted deep sequencing results confirmed that our adeno-associated virus-CRISPR/Cas9 strategy was very efficient in deleting the approximately 23 kb of intervening genomic sequences. CONCLUSIONS: This study provides evidence for using CRISPR-based genome editing as a

potential therapeutic approach for restoring dystrophic cardiomyopathy structurally and functionally.

El-Sayed, A. S. A., et al. (2017). "Genome editing approaches: manipulating of lovastatin and taxol synthesis of filamentous fungi by CRISPR/Cas9 system." Appl Microbiol Biotechnol **101**(10): 3953-3976.

Filamentous fungi are prolific repertoire of structurally diverse secondary metabolites of remarkable biological activities such as lovastatin and paclitaxel that have been approved by FDA as drugs for hypercholesterolemia and cancer treatment. The clusters of genes encoding lovastatin and paclitaxel are cryptic at standard laboratory cultural conditions (Kennedy et al. Science 284:1368-1372, 1999; Bergmann et al. Nature Chem Biol 3:213-217, 2007). The expression of these genes might be triggered in response to nutritional and physical conditions; nevertheless, the overall yield of these metabolites does not match the global need. Consequently, overexpression of the downstream limiting enzymes and/or blocking the competing metabolic pathways of these metabolites could be the most successful technologies to enhance their yield. This is the first review summarizing the different strategies implemented for fungal genome editing, molecular regulatory mechanisms, and prospective of clustered regulatory interspaced short palindromic repeat/Cas9 system in metabolic engineering of fungi to improve their yield of lovastatin and taxol to industrial scale. Thus, elucidating the putative metabolic pathways in fungi for overproduction of lovastatin and taxol was the ultimate objective of this review.

Feng, W., et al. (2018). "CRISPR-engineered genome editing for the next generation neurological disease modeling." Prog Neuropsychopharmacol Biol Psychiatry **81**: 459-467.

Neurological disorders often occur because of failure of proper brain development and/or appropriate maintenance of neuronal circuits. In order to understand roles of causative factors (e.g. genes, cell types) in disease development, generation of solid animal models has been one of straight-forward approaches. Recent next generation sequencing studies on human patient-derived clinical samples have identified various types of recurrent mutations in individual neurological diseases. While these discoveries have prompted us to evaluate impact of mutated genes on these neurological diseases, a feasible but flexible genome editing tool had remained to be developed. An advance of genome editing technology using the clustered regularly interspaced short palindromic repeats (CRISPR) with the CRISPR-associated protein (Cas) offers us a tremendous potential to create a variety of mutations in the cell, leading to "next generation" disease models carrying disease-associated mutations. We will here review recent progress of CRISPR-based brain disease modeling studies and discuss future requirement to tackle current difficulties in usage of these technologies.

Feng, Y., et al. (2014). "A robust TALENs system for highly efficient mammalian genome editing." Sci Rep **4**: 3632.

Recently, transcription activator-like effector nucleases (TALENs) have emerged as a highly effective tool for genomic editing. A pair of TALENs binds to two DNA recognition sites separated by a spacer sequence, and the dimerized FokI nucleases at the C terminal then cleave DNA in the spacer. Because of its modular design and capacity to precisely target almost any desired genomic locus, TALEN is a technology that can revolutionize the entire biomedical research field. Currently, for genomic editing in cultured cells, two plasmids encoding a pair of TALENs are co-transfected, followed by limited dilution to isolate cell colonies with the intended genomic manipulation. However, uncertain transfection efficiency becomes a bottleneck, especially in hard-to-transfect cells, reducing the overall efficiency of genome editing. We have developed a robust TALENs system in which each TALEN plasmid also encodes a fluorescence protein. Thus, cells transfected with both TALEN plasmids, a prerequisite for genomic editing, can be isolated by fluorescence-activated cell sorting. Our improved TALENs system can be applied to all cultured cells to achieve highly efficient genomic editing. Furthermore, an optimized procedure for genomic editing using TALENs is also presented. We expect our system to be widely adopted by the scientific community.

Ferreccio, A., et al. (2018). "Inducible CRISPR genome editing platform in naive human embryonic stem cells reveals JARID2 function in self-renewal." Cell Cycle: 1-15.

To easily edit the genome of naive human embryonic stem cells (hESC), we introduced a dual cassette encoding an inducible Cas9 into the AAVS1 site of naive hESC (iCas9). The iCas9 line retained karyotypic stability, expression of pluripotency markers, differentiation potential, and stability in 5iLA and EPS pluripotency conditions. The iCas9 line induced efficient homology-directed repair (HDR) and non-homologous end joining (NHEJ) based mutations through CRISPR-Cas9 system. We utilized the iCas9 line to study the epigenetic regulator, PRC2 in early human pluripotency. The PRC2 requirement distinguishes between early pluripotency stages, however, what regulates PRC2 activity in these stages

is not understood. We show reduced H3K27me3 and pluripotency markers in JARID2 2iL-I-F hESC mutants, indicating JARID2 requirement in maintenance of hESC 2iL-I-F state. These data suggest that JARID2 regulates PRC2 in 2iL-I-F state and the lack of PRC2 function in 5iLA state may be due to lack of sufficient JARID2 protein.

Fogarty, N. M. E., et al. (2017). "Genome editing reveals a role for OCT4 in human embryogenesis." Nature **550**(7674): 67-73.

Despite their fundamental biological and clinical importance, the molecular mechanisms that regulate the first cell fate decisions in the human embryo are not well understood. Here we use CRISPR-Cas9-mediated genome editing to investigate the function of the pluripotency transcription factor OCT4 during human embryogenesis. We identified an efficient OCT4-targeting guide RNA using an inducible human embryonic stem cell-based system and microinjection of mouse zygotes. Using these refined methods, we efficiently and specifically targeted the gene encoding OCT4 (POU5F1) in diploid human zygotes and found that blastocyst development was compromised. Transcriptomics analysis revealed that, in POU5F1-null cells, gene expression was downregulated not only for extra-embryonic trophectoderm genes, such as CDX2, but also for regulators of the pluripotent epiblast, including NANOG. By contrast, Pou5f1-null mouse embryos maintained the expression of orthologous genes, and blastocyst development was established, but maintenance was compromised. We conclude that CRISPR-Cas9-mediated genome editing is a powerful method for investigating gene function in the context of human development.

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.

Germini, D., et al. (2018). "A Comparison of Techniques to Evaluate the Effectiveness of Genome Editing." Trends Biotechnol **36**(2): 147-159.

Genome editing using engineered nucleases (meganucleases, zinc finger nucleases, transcription activator-like effector nucleases) has created many recent breakthroughs. Prescreening for efficiency and specificity is a critical step prior to using any newly designed genome editing tool for experimental purposes. The current standard screening methods of evaluation are based on DNA sequencing or use mismatch-sensitive endonucleases. They can be time-consuming and costly or lack reproducibility. Here, we review and critically compare standard techniques with those more recently developed in terms of reliability, time, cost, and ease of use.

Gonzalez, F., et al. (2014). "An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells." Cell Stem Cell **15**(2): 215-226.

Human pluripotent stem cells (hPSCs) offer a unique platform for elucidating the genes and molecular pathways that underlie complex traits and diseases. To realize this promise, methods for rapid and controllable genetic manipulations are urgently needed. By combining two newly developed gene-editing tools, the TALEN and CRISPR/Cas systems, we have developed a genome-engineering platform in hPSCs, which we named iCRISPR. iCRISPR enabled rapid and highly efficient generation of biallelic knockout hPSCs for loss-of-function studies, as well as homozygous knockin hPSCs with specific nucleotide alterations for precise modeling of disease conditions. We further demonstrate efficient one-step generation of double- and triple-gene knockout hPSC lines, as well as stage-specific inducible gene knockout during hPSC differentiation. Thus the iCRISPR platform is uniquely suited for dissection of complex genetic interactions and pleiotropic gene functions in human disease studies and has the potential to support high-throughput genetic analysis in hPSCs.

Grenier, A., et al. (2018). "Knockdown of Human AMPK Using the CRISPR/Cas9 Genome-Editing System." Methods Mol Biol **1732**: 171-194.

AMP-activated protein kinase (AMPK) is a critical energy sensor, regulating signaling networks involved in pathology including metabolic diseases and cancer. This increasingly recognized role of AMPK has prompted tremendous research efforts to develop new pharmacological AMPK activators. To precisely study the role of AMPK, and the specificity and activity of AMPK activators in cellular models, genetic AMPK inactivating tools are required. We report here methods for genetic inactivation of AMPK alpha1/alpha2 catalytic subunits in human cell lines by the CRISPR/Cas9 technology, a recent breakthrough technique for genome editing.

Gutschner, T. (2015). "Silencing long noncoding RNAs with genome-editing tools." Methods Mol Biol **1239**: 241-250.

Long noncoding RNAs (lncRNAs) are a functional and structural diverse class of cellular transcripts that comprise the largest fraction of the human transcriptome. However, detailed functional analysis lags behind their rapid discovery. This might be partially due to the lack of loss-of-function approaches that efficiently reduce the expression of these transcripts. Here, I describe a method that allows a specific and efficient targeting of the highly abundant lncRNA MALAT1 in human (lung) cancer cells. The method relies on the site-specific integration of RNA-destabilizing elements mediated by Zinc Finger Nucleases (ZFNs).

Haas, S. A., et al. (2017). "Therapeutic genome editing with engineered nucleases." Hamostaseologie **37**(1): 45-52.

Targeted genome editing with designer nucleases, such as zinc finger nucleases, TALE nucleases, and CRISPR-Cas nucleases, has heralded a new era in gene therapy. Genetic disorders, which have not been amenable to conventional gene-addition-type gene therapy approaches, such as disorders with dominant inheritance or diseases caused by mutations in tightly regulated genes, can now be treated by precise genome surgery. Moreover, engineered nucleases enable novel genetic interventions to fight infectious diseases or to improve cancer immunotherapies. Here, we review the development of the different classes of programmable nucleases, discuss the challenges and improvements in translating gene editing into clinical use, and give an outlook on what applications can expect to enter the clinic in the near future.

Heckl, D., et al. (2014). "Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing." Nat Biotechnol **32**(9): 941-946.

Genome sequencing studies have shown that human malignancies often bear mutations in four or more driver genes, but it is difficult to recapitulate this degree of genetic complexity in mouse models using conventional breeding. Here we use the CRISPR-Cas9 system of genome editing to overcome this limitation. By delivering combinations of small guide RNAs (sgRNAs) and Cas9 with a lentiviral vector, we modified up to five genes in a single mouse hematopoietic stem cell (HSC), leading to clonal outgrowth and myeloid malignancy. We thereby generated models of acute myeloid leukemia (AML) with cooperating mutations in genes encoding epigenetic modifiers, transcription factors and mediators of cytokine signaling, recapitulating the combinations of mutations observed in patients. Our results suggest that lentivirus-delivered sgRNA:Cas9 genome editing should be useful to engineer a broad array of in vivo cancer models that better reflect the complexity of human disease.

Hindriksen, S., et al. (2017). "Baculoviral delivery of CRISPR/Cas9 facilitates efficient genome editing in human cells." PLoS One **12**(6): e0179514.

The CRISPR/Cas9 system is a highly effective tool for genome editing. Key to robust genome editing is the efficient delivery of the CRISPR/Cas9 machinery. Viral delivery systems are efficient vehicles for the transduction of foreign genes but commonly used viral vectors suffer from a limited capacity in the genetic information they can carry. Baculovirus however is capable of carrying large exogenous DNA fragments. Here we investigate the use of baculoviral vectors as a delivery vehicle for CRISPR/Cas9 based genome-editing tools. We demonstrate transduction of a panel of cell lines with Cas9 and an sgRNA sequence, which results in efficient knockout of all four targeted subunits of the chromosomal passenger complex (CPC). We further show that introduction of a homology directed repair template into the same CRISPR/Cas9 baculovirus facilitates introduction of specific point mutations and endogenous gene tags. Tagging of the CPC recruitment factor Haspin with the fluorescent reporter YFP allowed us to study its native localization as well as recruitment to the cohesin subunit Pds5B.

Ho, P. and Y. Y. Chen (2017). "Mammalian synthetic biology in the age of genome editing and personalized medicine." Curr Opin Chem Biol **40**: 57-64.

The recent expansion of molecular tool kits has propelled synthetic biology toward the design of increasingly sophisticated mammalian systems. Specifically, advances in genome editing, protein engineering, and circuitry design have enabled the programming of cells for diverse applications, including regenerative medicine and cancer

immunotherapy. The ease with which molecular and cellular interactions can be harnessed promises to yield novel approaches to elucidate genetic interactions, program cellular functions, and design therapeutic interventions. Here, we review recent advancements in the development of enabling technologies and the practical applications of mammalian synthetic biology.

Hoban, M. D. and D. E. Bauer (2016). "A genome editing primer for the hematologist." Blood **127**(21): 2525-2535.

Gene editing enables the site-specific modification of the genome. These technologies have rapidly advanced such that they have entered common use in experimental hematology to investigate genetic function. In addition, genome editing is becoming increasingly plausible as a treatment modality to rectify genetic blood disorders and improve cellular therapies. Genome modification typically ensues from site-specific double-strand breaks and may result in a myriad of outcomes. Even single-strand nicks and targeted biochemical modifications that do not permanently alter the DNA sequence (epigenome editing) may be powerful instruments. In this review, we examine the various technologies, describe their advantages and shortcomings for engendering useful genetic alterations, and consider future prospects for genome editing to impact hematology.

Hossain, M. A. and J. Bungert (2017). "Genome Editing for Sickle Cell Disease: A Little BCL11A Goes a Long Way." Mol Ther **25**(3): 561-562.

Hu, Z., et al. (2017). "Genome editing of factor X in zebrafish reveals unexpected tolerance of severe defects in the common pathway." Blood **130**(5): 666-676.

Deficiency of factor X (F10) in humans is a rare bleeding disorder with a heterogeneous phenotype and limited therapeutic options. Targeted disruption of F10 and other common pathway factors in mice results in embryonic/neonatal lethality with rapid resorption of homozygous mutants, hampering additional studies. Several of these mutants also display yolk sac vascular defects, suggesting a role for thrombin signaling in vessel development. The zebrafish is a vertebrate model that demonstrates conservation of the mammalian hemostatic and vascular systems. We have leveraged these advantages for in-depth study of the role of the coagulation cascade in the developmental regulation of hemostasis and vasculogenesis. In this article, we show that ablation of zebrafish f10 by using genome editing with transcription activator-like effector nucleases results in a major embryonic hemostatic defect. However, widespread hemorrhage and subsequent lethality does not occur until later stages, with absence of any detectable defect in vascular development. We also use f10(-/-) zebrafish to confirm 5 novel human F10 variants as causative mutations in affected patients, providing a rapid and reliable in vivo model for testing the severity of F10 variants. These findings as well as the prolonged survival of f10(-/-) mutants will enable us to expand our understanding of the molecular mechanisms of hemostasis, including a platform for screening variants of uncertain significance in patients with F10 deficiency and other coagulation disorders. Further study as to how fish tolerate what is an early lethal mutation in mammals could facilitate improvement of diagnostics and therapeutics for affected patients with bleeding disorders.

Joung, J. K. and J. D. Sander (2013). "TALENs: a widely applicable technology for targeted genome editing." Nat Rev Mol Cell Biol **14**(1): 49-55.

Engineered nucleases enable the targeted alteration of nearly any gene in a wide range of cell types and organisms. The newly-developed transcription activator-like effector nucleases (TALENs) comprise a nonspecific DNA-cleaving nuclease fused to a DNA-binding domain that can be easily engineered so that TALENs can target essentially any sequence. The capability to quickly and efficiently alter genes using TALENs promises to have profound impacts on biological research and to yield potential therapeutic strategies for genetic diseases.

Li, B., et al. (2017). "Engineering CRISPR-Cpf1 crRNAs and mRNAs to maximize genome editing efficiency." Nat Biomed Eng **1**(5).

Cpf1, a type-V CRISPR-Cas effector endonuclease, exhibits gene-editing activity in human cells through a single RNA-guided approach. Here, we report the design and assessment of an array of 42 types of engineered Acidaminococcus sp. Cpf1 (AsCpf1) CRISPR RNAs (crRNAs) and 5 types of AsCpf1 mRNAs, and show that the top-performing modified crRNA (cr3'5F, containing five 2'-fluoro ribose at the 3' termini) and AsCpf1 mRNA (full psi-modification) improved gene-cutting efficiency by, respectively, 127% and 177%, with respect to unmodified crRNA and plasmid-encoding AsCpf1. We also show that the combination of cr3'5F and psi-modified AsCpf1 or Lachnospiraceae bacterium Cpf1 (LbCpf1) mRNAs augmented gene-cutting efficiency by over 300% with respect to the same control, and discovered that 11 out of 16 crRNAs from Cpf1 orthologs enabled genome editing in the presence of AsCpf1. Engineered CRISPR-Cpf1 systems should facilitate a broad range of genome editing applications.

Li, C. X. and H. L. Qian (2015). "A double-edged sword: CRISPR-Cas9 is emerging as a revolutionary technique for genome editing." Mil Med Res **2**: 25.

In May 2015, Professor Xiao Yang authored a review on the development of CRISPR-Cas9 techniques in the journal of Military Medical Research. This review provided a valuable overview of this major scientific advance. It has been four years since the first publication of the CRISPR-Cas9 breakthrough (Science. 2012; 337: 816-21). The use of this technique has expanded into various scientific areas and is being developed into a systematic technical platform that may contribute to many bioengineering fields involving DNA sequence editing.

Li, L., et al. (2017). "Artificial Virus Delivers CRISPR-Cas9 System for Genome Editing of Cells in Mice." ACS Nano **11**(1): 95-111.

CRISPR-Cas9 has emerged as a versatile genome-editing platform. However, due to the large size of the commonly used CRISPR-Cas9 system, its effective delivery has been a challenge and limits its utility for basic research and therapeutic applications. Herein, a multifunctional nucleus-targeting "core-shell" artificial virus (RRPHC) was constructed for the delivery of CRISPR-Cas9 system. The artificial virus could efficiently load with the CRISPR-Cas9 system, accelerate the endosomal escape, and promote the penetration into the nucleus without additional nuclear-localization signal, thus enabling targeted gene disruption. Notably, the artificial virus is more efficient than SuperFect, Lipofectamine 2000, and Lipofectamine 3000. When loaded with a CRISPR-Cas9 plasmid, it induced higher targeted gene disruption efficacy than that of Lipofectamine 3000. Furthermore, the artificial virus effectively targets the ovarian cancer via dual-receptor-mediated endocytosis and had minimum side effects. When loaded with the Cas9-hMTH1 system targeting MTH1 gene, RRPHC showed effective disruption of MTH1 in vivo. This strategy could be adapted for delivering CRISPR-Cas9 plasmid or other functional nucleic acids in vivo.

Li, R., et al. (2017). "Functional dissection of NEAT1 using genome editing reveals substantial localization of the NEAT1_1 isoform outside paraspeckles." RNA **23**(6): 872-881.

Large numbers of long noncoding RNAs have been discovered in recent years, but only a few have been characterized. NEAT1 (nuclear paraspeckle assembly transcript 1) is a mammalian long noncoding RNA that is important for the reproductive physiology of mice, cancer development, and the formation of subnuclear bodies termed paraspeckles. The two major isoforms of NEAT1 (3.7 kb NEAT1_1 and 23 kb NEAT1_2 in human) are generated from a common promoter and are produced through the use of alternative transcription termination sites. This gene structure has made the functional relationship between the two isoforms difficult to dissect. Here we used CRISPR-Cas9 genome editing to create several different cell lines: total NEAT1 knockout cells, cells that only express the short form NEAT1_1, and cells with twofold more NEAT1_2. Using these reagents, we obtained evidence that NEAT1_1 is not a major component of paraspeckles. In addition, our data suggest NEAT1_1 localizes in numerous nonparaspeckle foci we termed "microspeckles," which may carry paraspeckle-independent functions. This study highlights the complexity of lncRNA and showcases how genome editing tools are useful in dissecting the structural and functional roles of overlapping transcripts.

Li, X., et al. (2016). "The present and future of genome editing in cancer research." Hum Genet **135**(9): 1083-1092.

The widespread use of high-throughput genome sequencing methods is profoundly changing the way we understand, classify, and treat human cancers. To make sense of the deluge of sequencing data generated in the clinic, more effective and rapid assessments of the functional relevance of newly discovered cancer-associated mutations are urgently needed. In this review, we discuss how genome editing technologies are responding to this major challenge. Largely focusing on CRISPR-based methods, we will highlight their potential to accelerate discovery, discuss their current limitations, and speculate about future applications.

Liang, C., et al. (2017). "Tumor cell-targeted delivery of CRISPR/Cas9 by aptamer-functionalized lipopolymer for therapeutic genome editing of VEGFA in osteosarcoma." Biomaterials **147**: 68-85.

Osteosarcoma (OS) is a highly aggressive pediatric cancer, characterized by frequent lung metastasis and pathologic bone destruction. Vascular endothelial growth factor A (VEGFA), highly expressed in OS, not only contributes to angiogenesis within the tumor microenvironment via paracrine stimulation of vascular endothelial cells, but also acts as an autocrine survival factor for tumor cell themselves, thus making it a promising therapeutic target for OS. CRISPR/Cas9 is a versatile genome editing technology and holds tremendous promise for cancer treatment. However, a major bottleneck to achieve the therapeutic potential of the CRISPR/Cas9 is the lack of in vivo tumor-targeted delivery systems. Here, we screened an OS cell-specific aptamer (LC09) and developed a LC09-functionalized PEG-PEI-Cholesterol (PPC) lipopolymer encapsulating

CRISPR/Cas9 plasmids encoding VEGFA gRNA and Cas9. Our results demonstrated that LC09 facilitated selective distribution of CRISPR/Cas9 in both orthotopic OS and lung metastasis, leading to effective VEGFA genome editing in tumor, decreased VEGFA expression and secretion, inhibited orthotopic OS malignancy and lung metastasis, as well as reduced angiogenesis and bone lesion with no detectable toxicity. The delivery system simultaneously restrained autocrine and paracrine VEGFA signaling in tumor cells and could facilitate translating CRISPR-Cas9 into clinical cancer treatment.

Lin, C., et al. (2016). "Increasing the Efficiency of CRISPR/Cas9-mediated Precise Genome Editing of HSV-1 Virus in Human Cells." Sci Rep **6**: 34531.

Genetically modified HSV-1 viruses serve as promising vectors for tumour therapy and vaccine development. The CRISPR/Cas9 system is one of the most powerful tools for precise gene editing of the genomes of organisms. However, whether the CRISPR/Cas9 system can precisely and efficiently make gene replacements in the genome of HSV-1 remains essentially unknown. Here, we reported CRISPR/Cas9-mediated editing of the HSV-1 genome in human cells, including the knockout and replacement of large genes. In established cells stably expressing CRISPR/Cas9, gRNA in coordination with Cas9 could direct a precise cleavage within a pre-defined target region, and foreign genes were successfully used to replace the target gene seamlessly by HDR-mediated gene replacement. Introducing the NHEJ inhibitor SCR7 to the CRISPR/Cas9 system greatly facilitated HDR-mediated gene replacement in the HSV-1 genome. We provided the first genetic evidence that two copies of the ICP0 gene in different locations on the same HSV-1 genome could be simultaneously modified with high efficiency and with no off-target modifications. We also developed a revolutionized isolation platform for desired recombinant viruses using single-cell sorting. Together, our work provides a significantly improved method for targeted editing of DNA viruses, which will facilitate the development of anti-cancer oncolytic viruses and vaccines.

Lin, S., et al. (2016). "Multi-OMICs and Genome Editing Perspectives on Liver Cancer Signaling Networks." Biomed Res Int **2016**: 6186281.

The advent of the human genome sequence and the resulting ~20,000 genes provide a crucial framework for a transition from traditional biology to an integrative "OMICs" arena (Lander et al., 2001; Venter et al., 2001; Kitano, 2002). This brings in a revolution for cancer research, which now enters a big data era. In the past decade, with the facilitation by next-generation sequencing, there have been a huge number of large-scale sequencing efforts, such as The Cancer Genome Atlas (TCGA), the HapMap, and the 1000 genomes project. As a result, a deluge of genomic information becomes available from patients stricken by a variety of cancer types. The list of cancer-associated genes is ever expanding. New discoveries are made on how frequent and highly penetrant mutations, such as those in the telomerase reverse transcriptase (TERT) and TP53, function in cancer initiation, progression, and metastasis. Most genes with relatively frequent but weakly penetrant cancer mutations still remain to be characterized. In addition, genes that harbor rare but highly penetrant cancer-associated mutations continue to emerge. Here, we review recent advances related to cancer genomics, proteomics, and systems biology and suggest new perspectives in targeted therapy and precision medicine.

Liu, T., et al. (2016). "Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma." Cancer Lett **373**(1): 109-118.

Sarcomas include some of the most aggressive tumors and typically respond poorly to chemotherapy. In recent years, specific gene fusion/mutations and gene over-expression/activation have been shown to drive sarcoma pathogenesis and development. These emerging genomic alterations may provide targets for novel therapeutic strategies and have the potential to transform sarcoma patient care. The RNA-guided nuclease CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein-9 nuclease) is a convenient and versatile platform for site-specific genome editing and epigenome targeted modulation. Given that sarcoma is believed to develop as a result of genetic alterations in mesenchymal progenitor/stem cells, CRISPR-Cas9 genome editing technologies hold extensive application potentials in sarcoma models and therapies. We review the development and mechanisms of the CRISPR-Cas9 system in genome editing and introduce its application in sarcoma research and potential therapy in clinic. Additionally, we propose future directions and discuss the challenges faced with these applications, providing concise and enlightening information for readers interested in this area.

Liu, Z., et al. (2016). "Efficient genome editing of genes involved in neural crest development using the CRISPR/Cas9 system in Xenopus embryos." Cell Biosci **6**: 22.

BACKGROUND: The RNA guided CRISPR/Cas9 nucleases have been proven to be effective for gene disruption in various animal models including Xenopus tropicalis. The neural crest (NC) is a transient cell population during embryonic

development and contributes to a large variety of tissues. Currently, loss-of-function studies on NC development in X. tropicalis are largely based on morpholino antisense oligonucleotide. It is worthwhile establishing targeted gene knockout X. tropicails line using CRISPR/Cas9 system to study NC development. METHODS: We utilized CRISPR/Cas9 to disrupt genes that are involved in NC formation in X. tropicalis embryos. A single sgRNA and Cas9 mRNA synthesized in vitro, were co-injected into X. tropicalis embryos at one-cell stage to induce single gene disruption. We also induced duplex mutations, large segmental deletions and inversions in X. tropicalis by injecting Cas9 and a pair of sgRNAs. The specificity of CRISPR/Cas9 was assessed in X. tropicalis embryos and the Cas9 nickase was used to reduce the off-target cleavages. Finally, we crossed the G0 mosaic frogs with targeted mutations to wild type frogs and obtained the germline transmission. RESULTS: Total 16 target sites in 15 genes were targeted by CRISPR/Cas9 and resulted in successful indel mutations at 14 loci with disruption efficiencies in a range from 9.3 to 57.8 %. Furthermore, we demonstrated the feasibility of generation of duplex mutations, large segmental deletions and inversions by using Cas9 and a pair of sgRNAs. We observed that CRISPR/Cas9 displays obvious off-target effects at some loci in X. tropicalis embryos. Such off-target cleavages was reduced by using the D10A Cas9 nickase. Finally, the Cas9 induced indel mutations were efficiently passed to G1 offspring. CONCLUSION: Our study proved that CRISPR/Cas9 could mediate targeted gene mutation in X. tropicalis with high efficiency. This study expands the application of CRISPR/Cas9 platform in X. tropicalis and set a basis for studying NC development using genetic approach.

Luo, Y., et al. (2012). "Targeted genome editing by recombinant adeno-associated virus (rAAV) vectors for generating genetically modified pigs." J Genet Genomics **39**(6): 269-274.

Recombinant adeno-associated virus (rAAV) vectors have been extensively used for experimental gene therapy of inherited human diseases. Several advantages, such as simple vector construction, high targeting frequency by homologous recombination, and applicability to many cell types, make rAAV an attractive approach for targeted genome editing. Combined with cloning by somatic cell nuclear transfer (SCNT), this technology has recently been successfully adapted to generate gene-targeted pigs as models for cystic fibrosis, hereditary tyrosinemia type 1, and breast cancer. This review summarizes the development of rAAV for targeted genome editing in mammalian cells and provides strategies for enhancing the rAAV-mediated targeting frequency by homologous recombination. We discuss current development and application of the rAAV vectors for targeted genome editing in porcine primary fibroblasts, which are subsequently used as donor cells for SCNT to generate cloned genetically designed pigs and provide positive perspectives for the generation of gene-targeted pigs with rAAV in the future.

Ma, Y., et al. (2016). "Increasing the efficiency of CRISPR/Cas9-mediated precise genome editing in rats by inhibiting NHEJ and using Cas9 protein." RNA Biol **13**(7): 605-612.

Precise modifications such as site mutation, codon replacement, insertion or precise targeted deletion are needed for studies of accurate gene function. The CRISPR/Cas9 system has been proved as a powerful tool to generate gene knockout and knockin animals. But the homologous recombination (HR)-directed precise genetic modification mediated by CRISPR/Cas9 is relatively lower compared with nonhomologous end-joining (NHEJ) pathway and extremely expected to be improved. Here, in this study 2 strategies were used to increase the precise genetic modification in rats. Scr7, a DNA ligase IV inhibitor, first identified as an anti-cancer compound, and considered as a potential NHEJ inhibitor, was used to increase the HR-mediated precise genetic modification. Meanwhile, the Cas9 protein instead of mRNA was used to save the mRNA to protein translation step to improve the precise modification efficiency. The Fabp2 and Dbndd1 loci were selected to knockin Cre and CreER(T2), respectively. Our result showed that both Scr7 and Cas9 protein can increase the precise modification.

Meissner, T. B., et al. (2014). "Genome editing for human gene therapy." Methods Enzymol **546**: 273-295.

The rapid advancement of genome-editing techniques holds much promise for the field of human gene therapy. From bacteria to model organisms and human cells, genome editing tools such as zinc-finger nucleases (ZNFs), TALENs, and CRISPR/Cas9 have been successfully used to manipulate the respective genomes with unprecedented precision. With regard to human gene therapy, it is of great interest to test the feasibility of genome editing in primary human hematopoietic cells that could potentially be used to treat a variety of human genetic disorders such as hemoglobinopathies, primary immunodeficiencies, and cancer. In this chapter, we explore the use of the CRISPR/Cas9 system for the efficient ablation of genes in two clinically relevant primary human cell types, CD4+ T cells and CD34+ hematopoietic stem and progenitor cells. By using two guide RNAs directed at a single locus, we achieve highly efficient and predictable deletions that ablate gene function. The use

of a Cas9-2A-GFP fusion protein allows FACS-based enrichment of the transfected cells. The ease of designing, constructing, and testing guide RNAs makes this dual guide strategy an attractive approach for the efficient deletion of clinically relevant genes in primary human hematopoietic stem and effector cells and enables the use of CRISPR/Cas9 for gene therapy.

Moreno-Mateos, M. A., et al. (2017). "CRISPR-Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing." Nat Commun **8**(1): 2024.

Cpf1 is a novel class of CRISPR-Cas DNA endonucleases, with a wide range of activity across different eukaryotic systems. Yet, the underlying determinants of this variability are poorly understood. Here, we demonstrate that LbCpf1, but not AsCpf1, ribonucleoprotein complexes allow efficient mutagenesis in zebrafish and Xenopus. We show that temperature modulates Cpf1 activity by controlling its ability to access genomic DNA. This effect is stronger on AsCpf1, explaining its lower efficiency in ectothermic organisms. We capitalize on this property to show that temporal control of the temperature allows post-translational modulation of Cpf1-mediated genome editing. Finally, we determine that LbCpf1 significantly increases homology-directed repair in zebrafish, improving current approaches for targeted DNA integration in the genome. Together, we provide a molecular understanding of Cpf1 activity in vivo and establish Cpf1 as an efficient and inducible genome engineering tool across ectothermic species.

Morsy, S. G., et al. (2017). "Divergent susceptibilities to AAV-SaCas9-gRNA vector-mediated genome-editing in a single-cell-derived cell population." BMC Res Notes **10**(1): 720.

OBJECTIVE: Recombinant adeno-associated virus (AAV)-based vectors are characterized by their robust and safe transgene delivery. The CRISPR/Cas9 and guide RNA (gRNA) system present a promising genome-editing platform, and a recent development of a shorter Cas9 enzyme from Staphylococcus aureus (SaCas9) allows generation of high titer single AAV vectors which carry both saCas9- and gRNA-expression cassettes. Here, we used two AAV-SaCas9 vectors with distinct GFP-targeted gRNA sequences and determined the impact of AAV-SaCas9-gRNA vector treatment in a single cell clone carrying a GFP-expression cassette. RESULTS: Our results showed comparable GFP knockout efficiencies (40-50%) upon a single low-dose infection. Three consecutive transductions of 25-fold higher doses of vectors showed 80% GFP knockout efficiency. To analyze the "AAV-SaCas9-resistant cell population", we sorted the residual GFP-positive cells and assessed their permissiveness to super-infection with two AAV-Cas9-GFP vectors. We found the sorted cells were significantly more resistant to the GFP knockout mediated by the same AAV vector, but not by the other GFP-targeted AAV vector. Our data therefore demonstrate highly efficient genome-editing by the AAV-SaCas9-gRNA vector system. Differential susceptibilities of single cell-derived cells to the AAV-SaCas9-gRNA-mediated genome editing may represent a formidable barrier to achieve 100% genome editing efficiency by this vector system.

Mou, H., et al. (2015). "Precision cancer mouse models through genome editing with CRISPR-Cas9." Genome Med **7**(1): 53.

The cancer genome is highly complex, with hundreds of point mutations, translocations, and chromosome gains and losses per tumor. To understand the effects of these alterations, precise models are needed. Traditional approaches to the construction of mouse models are time-consuming and laborious, requiring manipulation of embryonic stem cells and multiple steps. The recent development of the clustered regularly interspersed short palindromic repeats (CRISPR)-Cas9 system, a powerful genome-editing tool for efficient and precise genome engineering in cultured mammalian cells and animals, is transforming mouse-model generation. Here, we review how CRISPR-Cas9 has been used to create germline and somatic mouse models with point mutations, deletions and complex chromosomal rearrangements. We highlight the progress and challenges of such approaches, and how these models can be used to understand the evolution and progression of individual tumors and identify new strategies for cancer treatment. The generation of precision cancer mouse models through genome editing will provide a rapid avenue for functional cancer genomics and pave the way for precision cancer medicine.

Mou, H., et al. (2017). "CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion." Genome Biol **18**(1): 108.

CRISPR is widely used to disrupt gene function by inducing small insertions and deletions. Here, we show that some single-guide RNAs (sgRNAs) can induce exon skipping or large genomic deletions that delete exons. For example, CRISPR-mediated editing of beta-catenin exon 3, which encodes an autoinhibitory domain, induces partial skipping of the in-frame exon and nuclear accumulation of beta-catenin. A single sgRNA can induce small insertions or deletions that partially alter splicing or unexpected larger deletions that remove exons. Exon skipping adds to the unexpected outcomes that must be accounted for,

and perhaps taken advantage of, in CRISPR experiments.

Munoz, N. M., et al. (2012). "Novel reporter systems for facile evaluation of I-SceI-mediated genome editing." Nucleic Acids Res **40**(2): e14.

Two major limitations to achieve efficient homing endonuclease-stimulated gene correction using retroviral vectors are low frequency of gene targeting and random integration of the targeting vectors. To overcome these issues, we developed a reporter system for quick and facile testing of novel strategies to promote the selection of cells that undergo targeted gene repair and to minimize the persistence of random integrations and non-homologous end-joining events. In this system, the gene target has an I-SceI site upstream of an EGFP reporter; and the repair template includes a non-functional EGFP gene, the positive selection transgene MGMTP140K tagged with mCherry, and the inducible Caspase-9 suicide gene. Using this dual fluorescent reporter system it is possible to detect properly targeted integration. Furthermore, this reporter system provides an efficient approach to enrich for gene correction events and to deplete events produced by random integration. We have also developed a second reporter system containing MGMTP140K in the integrated target locus, which allows for selection of primary cells with the integrated gene target after transplantation. This system is particularly useful for testing repair strategies in primary hematopoietic stem cells. Thus, our reporter systems should allow for more efficient gene correction with less unwanted off target effects.

Neggers, J. E., et al. (2015). "Identifying drug-target selectivity of small-molecule CRM1/XPO1 inhibitors by CRISPR/Cas9 genome editing." Chem Biol **22**(1): 107-116.

Validation of drug-target interaction is essential in drug discovery and development. The ultimate proof for drug-target validation requires the introduction of mutations that confer resistance in cells, an approach that is not straightforward in mammalian cells. Using CRISPR/Cas9 genome editing, we show that a homozygous genomic C528S mutation in the XPO1 gene confers cells with resistance to selinexor (KPT-330). Selinexor is an orally bioavailable inhibitor of exportin-1 (CRM1/XPO1) with potent anticancer activity and is currently under evaluation in human clinical trials. Mutant cells were resistant to the induction of cytotoxicity, apoptosis, cell cycle arrest, and inhibition of XPO1 function, including direct binding of the drug to XPO1. These results validate XPO1 as the prime target of selinexor in cells and identify the selectivity of this drug toward the cysteine 528 residue of XPO1. Our findings demonstrate that CRISPR/Cas9 genome editing enables drug-target validation and drug-target selectivity studies in cancer cells.

O'Duibhir, E., et al. (2017). "Accelerating glioblastoma drug discovery: Convergence of patient-derived models, genome editing and phenotypic screening." Mol Cell Neurosci **80**: 198-207.

Patients diagnosed with glioblastoma (GBM) continue to face a bleak prognosis. It is critical that new effective therapeutic strategies are developed. GBM stem cells have molecular hallmarks of neural stem and progenitor cells and it is possible to propagate both non-transformed normal neural stem cells and GBM stem cells, in defined, feeder-free, adherent culture. These primary stem cell lines provide an experimental model that is ideally suited to cell-based drug discovery or genetic screens in order to identify tumour-specific vulnerabilities. For many solid tumours, including GBM, the genetic disruptions that drive tumour initiation and growth have now been catalogued. CRISPR/Cas-based genome editing technologies have recently emerged, transforming our ability to functionally annotate the human genome. Genome editing opens prospects for engineering precise genetic changes in normal and GBM-derived neural stem cells, which will provide more defined and reliable genetic models, with critical matched pairs of isogenic cell lines. Generation of more complex alleles such as knock in tags or fluorescent reporters is also now possible. These new cellular models can be deployed in cell-based phenotypic drug discovery (PDD). Here we discuss the convergence of these advanced technologies (iPS cells, neural stem cell culture, genome editing and high content phenotypic screening) and how they herald a new era in human cellular genetics that should have a major impact in accelerating glioblastoma drug discovery.

Ouchi, Y., et al. (2018). "Generation of tumor antigen-specific murine CD8+ T cells with enhanced anti-tumor activity via highly efficient CRISPR/Cas9 genome editing." Int Immunol **30**(4): 141-154.

Immunotherapies have led to the successful development of novel therapies for cancer. However, there is increasing concern regarding the adverse effects caused by non-tumor-specific immune responses. Here, we report an effective strategy to generate high-avidity tumor-antigen-specific CTLs, using Cas9/single-guide RNA (sgRNA) ribonucleoprotein (RNP) delivery. As a proof-of-principle demonstration, we selected the gp100 melanoma-associated tumor antigen, and cloned the gp100-specific high-avidity TCR from gp100-immunized mice. To enable rapid structural dissection of the TCR, we developed a 3D protein structure

modeling system for the TCR/antigen-major histocompatibility complex (pMHC) interaction. Combining these technologies, we efficiently generated gp100-specific PD-1(-) CD8+ T cells, and demonstrated that the genetically engineered CD8+ T cells have high avidity against melanoma cells both in vitro and in vivo. Our methodology offers computational prediction of the TCR response, and enables efficient generation of tumor antigen-specific CD8+ T cells that can neutralize tumor-induced immune suppression leading to a potentially powerful cancer therapeutic.

Pankowicz, F. P., et al. (2016). "Reprogramming metabolic pathways in vivo with CRISPR/Cas9 genome editing to treat hereditary tyrosinaemia." Nat Commun **7**: 12642.

Many metabolic liver disorders are refractory to drug therapy and require orthotopic liver transplantation. Here we demonstrate a new strategy, which we call metabolic pathway reprogramming, to treat hereditary tyrosinaemia type I in mice; rather than edit the disease-causing gene, we delete a gene in a disease-associated pathway to render the phenotype benign. Using CRISPR/Cas9 in vivo, we convert hepatocytes from tyrosinaemia type I into the benign tyrosinaemia type III by deleting Hpd (hydroxyphenylpyruvate dioxigenase). Edited hepatocytes (Fah(-/-)/Hpd(-/-)) display a growth advantage over non-edited hepatocytes (Fah(-/-)/Hpd(+/+)) and, in some mice, almost completely replace them within 8 weeks. Hpd excision successfully reroutes tyrosine catabolism, leaving treated mice healthy and asymptomatic. Metabolic pathway reprogramming sidesteps potential difficulties associated with editing a critical disease-causing gene and can be explored as an option for treating other diseases.

Park, M. Y., et al. (2017). "Generation of lung cancer cell lines harboring EGFR T790M mutation by CRISPR/Cas9-mediated genome editing." Oncotarget **8**(22): 36331-36338.

Tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib are effective against lung adenocarcinomas harboring epidermal growth factor receptor (EGFR) mutations. However, cancer cells can develop resistance to these agents with prolonged exposure; in over 50% of cases, this is attributable to the EGFR T790M mutation. Moreover, additional resistance mutations can arise with the use of new drugs. Cancer cell lines with specific mutations can enable the study of resistance mechanisms. In this study, we introduced the EGFR T790M mutation into the PC9 human lung cancer cell line-which has a deletion in exon 19 of the EGFR gene-by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)9-mediated genome editing. EGFR pyrosequencing and peptide nucleic acid clamping revealed that PC9 cells with EGFR T790M generated by CRISPR/Cas 9 had a higher T790M mutation rate than those with the same mutation generated by long-term exposure to gefitinib (PC9-G); moreover, resistance to gefitinib in these clones was higher than that in PC9-G cells. The clones were also highly sensitive to the 3rd-generation EGFR TKI AZD9291, which is cytotoxic to lung cancer cells with EGFR T790M. The CRISPR/Cas9 programmable nuclease system can be used to generate various cancer cell lines with specific mutations that can facilitate studies on resistance mechanisms and drug efficacy.

Perez, E. E., et al. (2008). "Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases." Nat Biotechnol **26**(7): 808-816.

Homozygosity for the naturally occurring Delta32 deletion in the HIV co-receptor CCR5 confers resistance to HIV-1 infection. We generated an HIV-resistant genotype de novo using engineered zinc-finger nucleases (ZFNs) to disrupt endogenous CCR5. Transient expression of CCR5 ZFNs permanently and specifically disrupted approximately 50% of CCR5 alleles in a pool of primary human CD4(+) T cells. Genetic disruption of CCR5 provided robust, stable and heritable protection against HIV-1 infection in vitro and in vivo in a NOG model of HIV infection. HIV-1-infected mice engrafted with ZFN-modified CD4(+) T cells had lower viral loads and higher CD4(+) T-cell counts than mice engrafted with wild-type CD4(+) T cells, consistent with the potential to reconstitute immune function in individuals with HIV/AIDS by maintenance of an HIV-resistant CD4(+) T-cell population. Thus adoptive transfer of ex vivo expanded CCR5 ZFN-modified autologous CD4(+) T cells in HIV patients is an attractive approach for the treatment of HIV-1 infection.

Pinder, J., et al. (2015). "Nuclear domain 'knock-in' screen for the evaluation and identification of small molecule enhancers of CRISPR-based genome editing." Nucleic Acids Res **43**(19): 9379-9392.

CRISPR is a genome-editing platform that makes use of the bacterially-derived endonuclease Cas9 to introduce DNA double-strand breaks at precise locations in the genome using complementary guide RNAs. We developed a nuclear domain knock-in screen, whereby the insertion of a gene encoding the green fluorescent protein variant Clover is inserted by Cas9-mediated homology directed repair (HDR) within the first exon of genes that are required for the structural integrity of subnuclear domains such as the nuclear lamina and promyelocytic leukemia nuclear

bodies (PML NBs). Using this approach, we compared strategies for enhancing CRISPR-mediated HDR, focusing on known genes and small molecules that impact non-homologous end joining (NHEJ) and homologous recombination (HR). Ultimately, we identified the small molecule RS-1 as a potent enhancer of CRISPR-based genome editing, enhancing HDR 3- to 6-fold depending on the locus and transfection method. We also characterized U2OS human osteosarcoma cells expressing Clover-tagged PML and demonstrate that this strategy generates cell lines with PML NBs that are structurally and functionally similar to bodies in the parental cell line. Thus, the nuclear domain knock-in screen that we describe provides a simple means of rapidly evaluating methods and small molecules that have the potential to enhance Cas9-mediated HDR.

Platt, R. J., et al. (2014). "CRISPR-Cas9 knockin mice for genome editing and cancer modeling." Cell **159**(2): 440-455.

CRISPR-Cas9 is a versatile genome editing technology for studying the functions of genetic elements. To broadly enable the application of Cas9 in vivo, we established a Cre-dependent Cas9 knockin mouse. We demonstrated in vivo as well as ex vivo genome editing using adeno-associated virus (AAV)-, lentivirus-, or particle-mediated delivery of guide RNA in neurons, immune cells, and endothelial cells. Using these mice, we simultaneously modeled the dynamics of KRAS, p53, and LKB1, the top three significantly mutated genes in lung adenocarcinoma. Delivery of a single AAV vector in the lung generated loss-of-function mutations in p53 and Lkb1, as well as homology-directed repair-mediated Kras(G12D) mutations, leading to macroscopic tumors of adenocarcinoma pathology. Together, these results suggest that Cas9 mice empower a wide range of biological and disease modeling applications.

Poppe, D., et al. (2018). "Genome Editing in Neuroepithelial Stem Cells to Generate Human Neurons with High Adenosine-Releasing Capacity." Stem Cells Transl Med.

As a powerful regulator of cellular homeostasis and metabolism, adenosine is involved in diverse neurological processes including pain, cognition, and memory. Altered adenosine homeostasis has also been associated with several diseases such as depression, schizophrenia, or epilepsy. Based on its protective properties, adenosine has been considered as a potential therapeutic agent for various brain disorders. Since systemic application of adenosine is hampered by serious side effects such as vasodilatation and cardiac suppression, recent studies aim at improving local delivery by depots, pumps, or cell-based applications. Here, we report on the characterization of adenosine-releasing human embryonic stem cell-derived neuroepithelial stem cells (long-term self-renewing neuroepithelial stem [lt-NES] cells) generated by zinc finger nuclease (ZFN)-mediated knockout of the adenosine kinase (ADK) gene. ADK-deficient lt-NES cells and their differentiated neuronal and astroglial progeny exhibit substantially elevated release of adenosine compared to control cells. Importantly, extensive adenosine release could be triggered by excitation of differentiated neuronal cultures, suggesting a potential activity-dependent regulation of adenosine supply. Thus, ZFN-modified neural stem cells might serve as a useful vehicle for the activity-dependent local therapeutic delivery of adenosine into the central nervous system. Stem Cells Translational Medicine 2018.

Porteus, M. (2015). "Strategies to increase genome editing frequencies and to facilitate the identification of edited cells." Methods Mol Biol **1239**: 281-289.

The power of genome editing is increasingly recognized as it has become more accessible to a wide range of scientists and a wider range of uses has been reported. Nonetheless, an important practical aspect of the strategy is develop methods to increase the frequency of genome editing or methods that enrich for genome-edited cells such that they can be more easily identified. This chapter discusses several different approaches including the use of cold-shock, exonucleases, surrogate markers, specialized donor vectors, and oligonucleotides to enhance the frequency of genome editing or to facilitate the identification of genome-edited cells.

Rahdar, M., et al. (2015). "Synthetic CRISPR RNA-Cas9-guided genome editing in human cells." Proc Natl Acad Sci U S A **112**(51): E7110-7117.

Genome editing with the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 nuclease system is a powerful technology for manipulating genomes, including introduction of gene disruptions or corrections. Here we develop a chemically modified, 29-nucleotide synthetic CRISPR RNA (scrRNA), which in combination with unmodified transactivating crRNA (tracrRNA) is shown to functionally replace the natural guide RNA in the CRISPR-Cas9 nuclease system and to mediate efficient genome editing in human cells. Incorporation of rational chemical modifications known to protect against nuclease digestion and stabilize RNA-RNA interactions in the tracrRNA hybridization region of CRISPR RNA (crRNA) yields a scrRNA with enhanced activity compared with the unmodified crRNA and comparable gene disruption activity to the previously published single guide RNA. Taken

together, these findings provide a platform for therapeutic applications, especially for nervous system disease, using successive application of cell-permeable, synthetic CRISPR RNAs to activate and then silence Cas9 nuclease activity.

Rahman, S. H., et al. (2015). "Rescue of DNA-PK Signaling and T-Cell Differentiation by Targeted Genome Editing in a prkdc Deficient iPSC Disease Model." PLoS Genet **11**(5): e1005239.

In vitro disease modeling based on induced pluripotent stem cells (iPSCs) provides a powerful system to study cellular pathophysiology, especially in combination with targeted genome editing and protocols to differentiate iPSCs into affected cell types. In this study, we established zinc-finger nuclease-mediated genome editing in primary fibroblasts and iPSCs generated from a mouse model for radiosensitive severe combined immunodeficiency (RS-SCID), a rare disorder characterized by cellular sensitivity to radiation and the absence of lymphocytes due to impaired DNA-dependent protein kinase (DNA-PK) activity. Our results demonstrate that gene editing in RS-SCID fibroblasts rescued DNA-PK dependent signaling to overcome radiosensitivity. Furthermore, in vitro T-cell differentiation from iPSCs was employed to model the stage-specific T-cell maturation block induced by the disease causing mutation. Genetic correction of the RS-SCID iPSCs restored T-lymphocyte maturation, polyclonal V(D)J recombination of the T-cell receptor followed by successful beta-selection. In conclusion, we provide proof that iPSC-based in vitro T-cell differentiation is a valuable paradigm for SCID disease modeling, which can be utilized to investigate disorders of T-cell development and to validate gene therapy strategies for T-cell deficiencies. Moreover, this study emphasizes the significance of designer nucleases as a tool for generating isogenic disease models and their future role in producing autologous, genetically corrected transplants for various clinical applications.

Ran, F. A., et al. (2015). "In vivo genome editing using Staphylococcus aureus Cas9." Nature **520**(7546): 186-191.

The RNA-guided endonuclease Cas9 has emerged as a versatile genome-editing platform. However, the size of the commonly used Cas9 from Streptococcus pyogenes (SpCas9) limits its utility for basic research and therapeutic applications that use the highly versatile adeno-associated virus (AAV) delivery vehicle. Here, we characterize six smaller Cas9 orthologues and show that Cas9 from Staphylococcus aureus (SaCas9) can edit the genome with efficiencies similar to those of SpCas9, while being more than 1 kilobase shorter. We packaged SaCas9 and its single guide RNA expression cassette into a single AAV vector and targeted the cholesterol regulatory gene Pcsk9 in the mouse liver. Within one week of injection, we observed >40% gene modification, accompanied by significant reductions in serum Pcsk9 and total cholesterol levels. We further assess the genome-wide targeting specificity of SaCas9 and SpCas9 using BLESS, and demonstrate that SaCas9-mediated in vivo genome editing has the potential to be efficient and specific.

Ren, J., et al. (2017). "Multiplex Genome Editing to Generate Universal CAR T Cells Resistant to PD1 Inhibition." Clin Cancer Res **23**(9): 2255-2266.

Purpose: Using gene-disrupted allogeneic T cells as universal effector cells provides an alternative and potentially improves current chimeric antigen receptor (CAR) T-cell therapy against cancers and infectious diseases.Experimental Design: The CRISPR/Cas9 system has recently emerged as a simple and efficient way for multiplex genome engineering. By combining lentiviral delivery of CAR and electro-transfer of Cas9 mRNA and gRNAs targeting endogenous TCR, beta-2 microglobulin (B2M) and PD1 simultaneously, to generate gene-disrupted allogeneic CAR T cells deficient of TCR, HLA class I molecule and PD1.Results: The CRISPR gene-edited CAR T cells showed potent antitumor activities, both in vitro and in animal models and were as potent as non-gene-edited CAR T cells. In addition, the TCR and HLA class I double deficient T cells had reduced alloreactivity and did not cause graft-versus-host disease. Finally, simultaneous triple genome editing by adding the disruption of PD1 led to enhanced in vivo antitumor activity of the gene-disrupted CAR T cells.Conclusions: Gene-disrupted allogeneic CAR and TCR T cells could provide an alternative as a universal donor to autologous T cells, which carry difficulties and high production costs. Gene-disrupted CAR and TCR T cells with disabled checkpoint molecules may be potent effector cells against cancers and infectious diseases. Clin Cancer Res; 23(9); 2255-66. (c)2016 AACR.

Reyon, D., et al. (2012). "FLASH assembly of TALENs for high-throughput genome editing." Nat Biotechnol **30**(5): 460-465.

Engineered transcription activator-like effector nucleases (TALENs) have shown promise as facile and broadly applicable genome editing tools. However, no publicly available high-throughput method for constructing TALENs has been published, and large-scale assessments of the success rate and targeting range of the technology remain lacking. Here we describe the fast ligation-based automatable solid-phase high-throughput (FLASH) system, a rapid and

cost-effective method for large-scale assembly of TALENs. We tested 48 FLASH-assembled TALEN pairs in a human cell-based EGFP reporter system and found that all 48 possessed efficient gene-modification activities. We also used FLASH to assemble TALENs for 96 endogenous human genes implicated in cancer and/or epigenetic regulation and found that 84 pairs were able to efficiently introduce targeted alterations. Our results establish the robustness of TALEN technology and demonstrate that FLASH facilitates high-throughput genome editing at a scale not currently possible with other genome modification technologies.

Robbez-Masson, L. J., et al. (2013). "Functional analysis of a breast cancer-associated FGFR2 single nucleotide polymorphism using zinc finger mediated genome editing." PLoS One **8**(11): e78839.

Genome wide association studies have identified single nucleotide polymorphisms (SNP) within fibroblast growth factor receptor 2 (FGFR2) as one of the highest ranking risk alleles in terms of development of breast cancer. The potential effect of these SNPs, in intron two, was postulated to be due to the differential binding of cis-regulatory elements, such as transcription factors, since all the SNPs in linkage disequilibrium were located in a regulatory DNA region. A Runx2 binding site was reported to be functional only in the minor, disease associated allele of rs2981578, resulting in increased expression of FGFR2 in cancers from patients homozygous for that allele. Moreover, the increased risk conferred by the minor FGFR2 allele associates most strongly in oestrogen receptor alpha positive (ERalpha) breast tumours, suggesting a potential interaction between ERalpha and FGFR signalling. Here, we have developed a human cell line model system to study the effect of the putative functional SNP, rs2981578, on cell behaviour. MCF7 cells, an ERalpha positive breast cancer cell line homozygous for the wild-type allele were edited using a Zinc Finger Nuclease approach. Unexpectedly, the acquisition of a single risk allele in MCF7 clones failed to affect proliferation or cell cycle progression. Binding of Runx2 to the risk allele was not observed. However FOXA1 binding, an important ERalpha partner, appeared decreased at the rs2981578 locus in the risk allele cells. Differences in allele specific expression (ASE) of FGFR2 were not observed in a panel of 72 ERalpha positive breast cancer samples. Thus, the apparent increased risk of developing ERalpha positive breast cancer seems not to be caused by rs2981578 alone. Rather, the observed increased risk of developing breast cancer might be the result of a coordinated effect of multiple SNPs forming a risk haplotype in the second intron of FGFR2.

Robert, F., et al. (2015). "Pharmacological inhibition of DNA-PK stimulates Cas9-mediated genome editing." Genome Med **7**: 93.

BACKGROUND: The ability to modify the genome of any cell at a precise location has drastically improved with the recent discovery and implementation of CRISPR/Cas9 editing technology. However, the capacity to introduce specific directed changes at given loci is hampered by the fact that the major cellular repair pathway that occurs following Cas9-mediated DNA cleavage is the erroneous non-homologous end joining (NHEJ) pathway. Homology-directed recombination (HDR) is far less efficient than NHEJ and makes screening of clones containing directed changes time-consuming and labor-intensive. METHODS: We investigated the possibility of pharmacologically inhibiting DNA-PKcs, a key player in NHEJ, using small molecule inhibitors (NU7441 and KU-0060648), to ameliorate the rates of HDR repair events. These compounds were tested in a sensitive reporter assay capable of simultaneously informing on NHEJ and HDR, as well as on an endogenous gene targeted by Cas9. RESULTS: We find that NU7441 and KU-0060648 reduce the frequency of NHEJ while increasing the rate of HDR following Cas9-mediated DNA cleavage. CONCLUSIONS: Our results identify two small molecules compatible for use with Cas9-editing technology to improve the frequency of HDR.

Roper, J., et al. (2018). "Colonoscopy-based colorectal cancer modeling in mice with CRISPR-Cas9 genome editing and organoid transplantation." Nat Protoc **13**(2): 217-234.

Most genetically engineered mouse models (GEMMs) of colorectal cancer are limited by tumor formation in the small intestine, a high tumor burden that limits metastasis, and the need to generate and cross mutant mice. Cell line or organoid transplantation models generally produce tumors in ectopic locations-such as the subcutaneous space, kidney capsule, or cecal wall-that do not reflect the native stromal environment of the colon mucosa. Here, we describe detailed protocols to rapidly and efficiently induce site-directed tumors in the distal colon of mice that are based on colonoscopy-guided mucosal injection. These techniques can be adapted to deliver viral vectors carrying Cre recombinase, CRISPR-Cas9 components, CRISPR-engineered mouse tumor organoids, or human cancer organoids to mice to model the adenoma-carcinoma-metastasis sequence of tumor progression. The colonoscopy injection procedure takes approximately 15 min, including preparation. In our experience, anyone with reasonable hand-eye coordination can become proficient with mouse colonoscopy and mucosal injection with a few hours of practice. These approaches are ideal for a wide range of

applications, including assessment of gene function in tumorigenesis, examination of tumor-stroma interactions, studies of cancer metastasis, and translational research with patient-derived cancers.

Roper, J., et al. (2017). "In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis." Nat Biotechnol **35**(6): 569-576.

In vivo interrogation of the function of genes implicated in tumorigenesis is limited by the need to generate and cross germline mutant mice. Here we describe approaches to model colorectal cancer (CRC) and metastasis, which rely on in situ gene editing and orthotopic organoid transplantation in mice without cancer-predisposing mutations. Autochthonous tumor formation is induced by CRISPR-Cas9-based editing of the Apc and Trp53 tumor suppressor genes in colon epithelial cells and by orthotopic transplantation of Apc-edited colon organoids. ApcDelta/Delta;Kras(G12D/+);Trp53Delta/Delta (AKP) mouse colon organoids and human CRC organoids engraft in the distal colon and metastasize to the liver. Finally, we apply the orthotopic transplantation model to characterize the clonal dynamics of Lgr5(+) stem cells and demonstrate sequential activation of an oncogene in established colon adenomas. These experimental systems enable rapid in vivo characterization of cancer-associated genes and reproduce the entire spectrum of tumor progression and metastasis.

Sakuma, T. (2017). "[Current advances and future prospects of genome editing technology in the field of biomedicine.]." Clin Calcium **27**(12): 1788-1793.

Genome editing technology can alter the genomic sequence at will, contributing the creation of cellular and animal models of human diseases including hereditary disorders and cancers, and the generation of the mutation-corrected human induced pluripotent stem cells for ex vivo regenerative medicine. In addition, novel approaches such as drug development using genome-wide CRISPR screening and cancer suppression using epigenome editing technology, which can change the epigenetic modifications in a site-specific manner, have also been conducted. In this article, I summarize the current advances and future prospects of genome editing technology in the field of biomedicine.

Salsman, J. and G. Dellaire (2017). "Precision genome editing in the CRISPR era." Biochem Cell Biol **95**(2): 187-201.

With the introduction of precision genome editing using CRISPR-Cas9 technology, we have entered a new era of genetic engineering and gene therapy. With RNA-guided endonucleases, such as Cas9, it is possible to engineer DNA double strand breaks (DSB) at specific genomic loci. DSB repair by the error-prone non-homologous end-joining (NHEJ) pathway can disrupt a target gene by generating insertions and deletions. Alternatively, Cas9-mediated DSBs can be repaired by homology-directed repair (HDR) using an homologous DNA repair template, thus allowing precise gene editing by incorporating genetic changes into the repair template. HDR can introduce gene sequences for protein epitope tags, delete genes, make point mutations, or alter enhancer and promoter activities. In anticipation of adapting this technology for gene therapy in human somatic cells, much focus has been placed on increasing the fidelity of CRISPR-Cas9 and increasing HDR efficiency to improve precision genome editing. In this review, we will discuss applications of CRISPR technology for gene inactivation and genome editing with a focus on approaches to enhancing CRISPR-Cas9-mediated HDR for the generation of cell and animal models, and conclude with a discussion of recent advances and challenges towards the application of this technology for gene therapy in humans.

Sanchez-Rivera, F. J., et al. (2014). "Rapid modelling of cooperating genetic events in cancer through somatic genome editing." Nature **516**(7531): 428-431.

Cancer is a multistep process that involves mutations and other alterations in oncogenes and tumour suppressor genes. Genome sequencing studies have identified a large collection of genetic alterations that occur in human cancers. However, the determination of which mutations are causally related to tumorigenesis remains a major challenge. Here we describe a novel CRISPR/Cas9-based approach for rapid functional investigation of candidate genes in well-established autochthonous mouse models of cancer. Using a Kras(G12D)-driven lung cancer model, we performed functional characterization of a panel of tumour suppressor genes with known loss-of-function alterations in human lung cancer. Cre-dependent somatic activation of oncogenic Kras(G12D) combined with CRISPR/Cas9-mediated genome editing of tumour suppressor genes resulted in lung adenocarcinomas with distinct histopathological and molecular features. This rapid somatic genome engineering approach enables functional characterization of putative cancer genes in the lung and other tissues using autochthonous mouse models. We anticipate that this approach can be used to systematically dissect the complex catalogue of mutations identified in cancer genome sequencing studies.

Shao, Y., et al. (2014). "CRISPR/Cas-mediated genome editing in the rat via direct injection of one-cell embryos." Nat Protoc **9**(10): 2493-2512.

Conventional embryonic stem cell (ESC)-based gene targeting, zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) technologies are powerful strategies for the generation of genetically modified animals. Recently, the CRISPR/Cas system has emerged as an efficient and convenient alternative to these approaches. We have used the CRISPR/Cas system to generate rat strains that carry mutations in multiple genes through direct injection of RNAs into one-cell embryos, demonstrating the high efficiency of Cas9-mediated gene editing in rats for simultaneous generation of compound gene mutant models. Here we describe a stepwise procedure for the generation of knockout and knock-in rats. This protocol provides guidelines for the selection of genomic targets, synthesis of guide RNAs, design and construction of homologous recombination (HR) template vectors, embryo microinjection, and detection of mutations and insertions in founders or their progeny. The procedure from target design to identification of founders can take as little as 6 weeks, of which <10 d is actual hands-on working time.

Shi, Z. D., et al. (2017). "Genome Editing in hPSCs Reveals GATA6 Haploinsufficiency and a Genetic Interaction with GATA4 in Human Pancreatic Development." Cell Stem Cell **20**(5): 675-688.e676.

Human disease phenotypes associated with haploinsufficient gene requirements are often not recapitulated well in animal models. Here, we have investigated the association between human GATA6 haploinsufficiency and a wide range of clinical phenotypes that include neonatal and adult-onset diabetes using CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9-mediated genome editing coupled with human pluripotent stem cell (hPSC) directed differentiation. We found that loss of one GATA6 allele specifically affects the differentiation of human pancreatic progenitors from the early PDX1+ stage to the more mature PDX1+NKX6.1+ stage, leading to impaired formation of glucose-responsive beta-like cells. In addition to this GATA6 haploinsufficiency, we also identified dosage-sensitive requirements for GATA6 and GATA4 in the formation of both definitive endoderm and pancreatic progenitor cells. Our work expands the application of hPSCs from studying the impact of individual gene loci to investigation of multigenic human traits, and it establishes an approach for identifying genetic modifiers of human disease.

Shinkuma, S., et al. (2016). "Site-specific genome editing for correction of induced pluripotent stem cells derived from dominant dystrophic epidermolysis bullosa." Proc Natl Acad Sci U S A **113**(20): 5676-5681.

Genome editing with engineered site-specific endonucleases involves nonhomologous end-joining, leading to reading frame disruption. The approach is applicable to dominant negative disorders, which can be treated simply by knocking out the mutant allele, while leaving the normal allele intact. We applied this strategy to dominant dystrophic epidermolysis bullosa (DDEB), which is caused by a dominant negative mutation in the COL7A1 gene encoding type VII collagen (COL7). We performed genome editing with TALENs and CRISPR/Cas9 targeting the mutation, c.8068_8084delinsGA. We then cotransfected Cas9 and guide RNA expression vectors expressed with GFP and DsRed, respectively, into induced pluripotent stem cells (iPSCs) generated from DDEB fibroblasts. After sorting, 90% of the iPSCs were edited, and we selected four gene-edited iPSC lines for further study. These iPSCs were differentiated into keratinocytes and fibroblasts secreting COL7. RT-PCR and Western blot analyses revealed gene-edited COL7 with frameshift mutations degraded at the protein level. In addition, we confirmed that the gene-edited truncated COL7 could neither associate with normal COL7 nor undergo triple helix formation. Our data establish the feasibility of mutation site-specific genome editing in dominant negative disorders.

Shuvalov, O., et al. (2015). "Current genome editing tools in gene therapy: new approaches to treat cancer." Curr Gene Ther **15**(5): 511-529.

Gene therapy suggests a promising approach to treat genetic diseases by applying genes as pharmaceuticals. Cancer is a complex disease, which strongly depends on a particular genetic make-up and hence can be treated with gene therapy. From about 2,000 clinical trials carried out so far, more than 60% were cancer targeted. Development of precise and effective gene therapy approaches is intimately connected with achievements in the molecular biology techniques. The field of gene therapy was recently revolutionized by the introduction of "programmable" nucleases, including ZFNs, TALENs, and CRISPR, which target specific genomic loci with high efficacy and precision. Furthermore, when combined with DNA transposons for the delivery purposes into cells, these programmable nucleases represent a promising alternative to the conventional viral-mediated gene delivery. In addition to "programmable" nucleases, a new class of TALE- and CRISPR-based "artificial transcription effectors" has been developed to mediate precise regulation of specific genes. In sum, these new molecular tools may be used in a wide plethora of gene therapy strategies. This review highlights the current

status of novel genome editing tools and discusses their suitability and perspectives in respect to cancer gene therapy studies.

Singh, N., et al. (2017). "Genome-Editing Technologies in Adoptive T Cell Immunotherapy for Cancer." Curr Hematol Malig Rep **12**(6): 522-529.

PURPOSE OF REVIEW: In this review, we discuss the most recent developments in gene-editing technology and discuss their application to adoptive T cell immunotherapy. RECENT FINDINGS: Engineered T cell therapies targeting cancer antigens have demonstrated significant efficacy in specific patient populations. Most impressively, CD19-directed chimeric antigen receptor T cells (CART19) have led to impressive responses in patients with B-cell leukemia and lymphoma. CTL019, or KYMRIAH (tisagenlecleucel), a CD19 CAR T cell product developed by Novartis and the University of Pennsylvania, was recently approved for clinical use by the Food and Drug Administration, representing a landmark in the application of adoptive T cell therapies. As CART19 enters routine clinical use, improving the efficacy of this exciting platform is the next step in broader application. Novel gene-editing technologies like CRISPR-Cas9 allow facile editing of specific genes within the genome, generating a powerful platform to further optimize the activity of engineered T cells.

Smith, C., et al. (2015). "Efficient and allele-specific genome editing of disease loci in human iPSCs." Mol Ther **23**(3): 570-577.

Efficient and precise genome editing is crucial for realizing the full research and therapeutic potential of human induced pluripotent stem cells (iPSCs). Engineered nucleases including CRISPR/Cas9 and transcription activator like effector nucleases (TALENs) provide powerful tools for enhancing gene-targeting efficiency. In this study, we investigated the relative efficiencies of CRISPR/Cas9 and TALENs in human iPSC lines for inducing both homologous donor-based precise genome editing and nonhomologous end joining (NHEJ)-mediated gene disruption. Significantly higher frequencies of NHEJ-mediated insertions/deletions were detected at several endogenous loci using CRISPR/Cas9 than using TALENs, especially at nonexpressed targets in iPSCs. In contrast, comparable efficiencies of inducing homologous donor-based genome editing were observed at disease-associated loci in iPSCs. In addition, we investigated the specificity of guide RNAs used in the CRISPR/Cas9 system in targeting disease-associated point mutations in patient-specific iPSCs. Using myeloproliferative neoplasm patient-derived iPSCs that carry an acquired JAK2-V617F point mutation and alpha1-antitrypsin (AAT) deficiency patient-derived iPSCs that carry an inherited Z-AAT point mutation, we demonstrate that Cas9 can specifically target either the mutant or the wild-type allele with little disruption at the other allele differing by a single nucleotide. Overall, our results demonstrate the advantages of the CRISPR/Cas9 system in allele-specific genome targeting and in NHEJ-mediated gene disruption.

Spisak, S., et al. (2015). "CAUSEL: an epigenome- and genome-editing pipeline for establishing function of noncoding GWAS variants." Nat Med **21**(11): 1357-1363.

The vast majority of disease-associated single-nucleotide polymorphisms (SNPs) mapped by genome-wide association studies (GWASs) are located in the non-protein-coding genome, but establishing the functional and mechanistic roles of these sequence variants has proven challenging. Here we describe a general pipeline in which candidate functional SNPs are first evaluated by fine mapping, epigenomic profiling, and epigenome editing, and then interrogated for causal function by using genome editing to create isogenic cell lines followed by phenotypic characterization. To validate this approach, we analyzed the 6q22.1 prostate cancer risk locus and identified rs339331 as the top-scoring SNP. Epigenome editing confirmed that the rs339331 region possessed regulatory potential. By using transcription activator-like effector nuclease (TALEN)-mediated genome editing, we created a panel of isogenic 22Rv1 prostate cancer cell lines representing all three genotypes (TT, TC, CC) at rs339331. Introduction of the 'T' risk allele increased transcription of the regulatory factor 6 (RFX6) gene, increased homeobox B13 (HOXB13) binding at the rs339331 region, and increased deposition of the enhancer-associated H3K4me2 histone mark at the rs339331 region compared to lines homozygous for the 'C' protective allele. The cell lines also differed in cellular morphology and adhesion, and pathway analysis of differentially expressed genes suggested an influence of androgens. In summary, we have developed and validated a widely accessible approach that can be used to establish functional causality for noncoding sequence variants identified by GWASs.

Stone, D., et al. (2016). "Genome editing and the next generation of antiviral therapy." Hum Genet **135**(9): 1071-1082.

Engineered endonucleases such as homing endonucleases (HEs), zinc finger nucleases (ZFNs), Tal-effector nucleases (TALENS) and the RNA-guided engineered nucleases (RGENs or CRISPR/Cas9) can target specific DNA sequences for cleavage, and are

proving to be valuable tools for gene editing. Recently engineered endonucleases have shown great promise as therapeutics for the treatment of genetic disease and infectious pathogens. In this review, we discuss recent efforts to use the HE, ZFN, TALEN and CRISPR/Cas9 gene-editing platforms as antiviral therapeutics. We also discuss the obstacles facing gene-editing antiviral therapeutics as they are tested in animal models of disease and transition towards human application.

Sun, W., et al. (2015). "Self-assembled DNA nanoclews for the efficient delivery of CRISPR-Cas9 for genome editing." Angew Chem Int Ed Engl **54**(41): 12029-12033.

CRISPR-Cas9 represents a promising platform for genome editing, yet means for its safe and efficient delivery remain to be fully realized. A novel vehicle that simultaneously delivers the Cas9 protein and single guide RNA (sgRNA) is based on DNA nanoclews, yarn-like DNA nanoparticles that are synthesized by rolling circle amplification. The biologically inspired vehicles were efficiently loaded with Cas9/sgRNA complexes and delivered the complexes to the nuclei of human cells, thus enabling targeted gene disruption while maintaining cell viability. Editing was most efficient when the DNA nanoclew sequence and the sgRNA guide sequence were partially complementary, offering a design rule for enhancing delivery. Overall, this strategy provides a versatile method that could be adapted for delivering other DNA-binding proteins or functional nucleic acids.

Tothova, Z., et al. (2017). "Multiplex CRISPR/Cas9-Based Genome Editing in Human Hematopoietic Stem Cells Models Clonal Hematopoiesis and Myeloid Neoplasia." Cell Stem Cell **21**(4): 547-555.e548.

Hematologic malignancies are driven by combinations of genetic lesions that have been difficult to model in human cells. We used CRISPR/Cas9 genome engineering of primary adult and umbilical cord blood CD34(+) human hematopoietic stem and progenitor cells (HSPCs), the cells of origin for myeloid pre-malignant and malignant diseases, followed by transplantation into immunodeficient mice to generate genetic models of clonal hematopoiesis and neoplasia. Human hematopoietic cells bearing mutations in combinations of genes, including cohesin complex genes, observed in myeloid malignancies generated immunophenotypically defined neoplastic clones capable of long-term, multi-lineage reconstitution and serial transplantation. Employing these models to investigate therapeutic efficacy, we found that TET2 and cohesin-mutated hematopoietic cells were sensitive to azacitidine treatment. These findings demonstrate the potential for generating genetically defined models of human myeloid diseases, and they are suitable for examining the biological consequences of somatic mutations and the testing of therapeutic agents.

Tsai, S. Q., et al. (2014). "Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing." Nat Biotechnol **32**(6): 569-576.

Monomeric CRISPR-Cas9 nucleases are widely used for targeted genome editing but can induce unwanted off-target mutations with high frequencies. Here we describe dimeric RNA-guided FokI nucleases (RFNs) that can recognize extended sequences and edit endogenous genes with high efficiencies in human cells. RFN cleavage activity depends strictly on the binding of two guide RNAs (gRNAs) to DNA with a defined spacing and orientation substantially reducing the likelihood that a suitable target site will occur more than once in the genome and therefore improving specificities relative to wild-type Cas9 monomers. RFNs guided by a single gRNA generally induce lower levels of unwanted mutations than matched monomeric Cas9 nickases. In addition, we describe a simple method for expressing multiple gRNAs bearing any 5' end nucleotide, which gives dimeric RFNs a broad targeting range. RFNs combine the ease of RNA-based targeting with the specificity enhancement inherent to dimerization and are likely to be useful in applications that require highly precise genome editing.

Uddin, B., et al. (2015). "Genome editing through large insertion leads to the skipping of targeted exon." BMC Genomics **16**: 1082.

BACKGROUND: Highly efficient genome editing can be achieved through targeting an endonuclease to specific locus of interest. Engineered zinc-finger nuclease (ZFN) and CRISPR-associated protein-9 nuclease (Cas9) offer such an elegant approach for genome editing in vertebrate cells. In this study, we have utilized ZFN and Cas9-catalyzed double strand break followed by homologous recombination-mediated incorporation of premature stop codon and selection marker to target human cell division cycle 14A (hCDC14A) and cell division cycle 14B (hCDC14B) genes. RESULTS: Targeting of the hCDC14A and hCDC14B loci in telomerase immortalized human retinal pigment epithelium (hTERT-RPE1) and human colon cancer (HCT116) cells were confirmed by Southern blot hybridization. Nevertheless, DNA sequence analysis of reverse transcription polymerase chain reaction (RT-PCR) products confirmed that in all the single/double allele ablations, the targeted exon was spliced out. The phenomenon of exon skipping was independent of the genome editing approaches exploited, Cas9 or ZFN. Because the exons had a nucleotide number that could be divided by 3, the reading frame of the exon deletion

was maintained. This indicates an exon-skipping event possibly due to the insertion of large DNA fragment (1.7 to 2.5 Kb) within the targeted exons. As a proof-of-principle, we have used gene disruption followed by non-homologous end joining (NHEJ) approach. Small alterations in the exon (one to fifteen bases) were transcribed to mRNA without exon skipping. Furthermore, loxP site-mediated removal of selection markers left a 45 bp scar within the targeted exon that can be traced in mRNA without exon skipping. CONCLUSION: From this study, we conclude that insertion of a large DNA fragment into an exon by genome editing can lead to its skipping from the final transcript. Hence, more cautious approach needs to be taken while designing target sites in such that the possible skipping of targeted exon causes a frame-shift mediated incorporation of pre-mature stop codon. On the other hand, exon skipping may be a useful strategy for the introduction of protein deletions.

Vartak, S. V. and S. C. Raghavan (2015). "Inhibition of nonhomologous end joining to increase the specificity of CRISPR/Cas9 genome editing." FEBS J **282**(22): 4289-4294.

DNA repair, one of the fundamental processes occurring in a cell, safeguards the genome and maintains its integrity. Among various DNA lesions, double-strand breaks are considered to be the most deleterious, as they can lead to potential loss of genetic information, if not repaired. Nonhomologous end joining (NHEJ) and homologous recombination are two major double-strand break repair pathways. SCR7, a DNA ligase IV inhibitor, was recently identified and characterized as a potential anticancer compound. Interestingly, SCR7 was shown to have several applications, owing to its unique property as an NHEJ inhibitor. Here, we focus on three main areas of research in which SCR7 is actively being used, and discuss one of the applications, i.e. genome editing via CRISPR/Cas, in detail. In the past year, different studies have shown that SCR7 significantly increases the efficiency of precise genome editing by inhibiting NHEJ, and favouring the error-free homologous recombination pathway, both in vitro and in vivo. Overall, we discuss the current applications of SCR7 to shed light on the unique property of the small molecule of having distinct applications in normal and cancer cells, when used at different cellular concentrations.

Wang, D., et al. (2015). "Adenovirus-Mediated Somatic Genome Editing of Pten by CRISPR/Cas9 in Mouse Liver in Spite of Cas9-Specific Immune Responses." Hum Gene Ther **26**(7): 432-442.

CRISPR/Cas9 derived from the bacterial adaptive immunity pathway is a powerful tool for genome editing, but the safety profiles of in vivo delivered Cas9 (including host immune responses to the bacterial Cas9 protein) have not been comprehensively investigated in model organisms. Nonalcoholic steatohepatitis (NASH) is a prevalent human liver disease characterized by excessive fat accumulation in the liver. In this study, we used adenovirus (Ad) vector to deliver a Streptococcus pyogenes-derived Cas9 system (SpCas9) targeting Pten, a gene involved in NASH and a negative regulator of the PI3K-AKT pathway, in mouse liver. We found that the Ad vector mediated efficient Pten gene editing even in the presence of typical Ad vector-associated immunotoxicity in the liver. Four months after vector infusion, mice receiving the Pten gene-editing Ad vector showed massive hepatomegaly and features of NASH, consistent with the phenotypes following Cre-loxP-induced Pten deficiency in mouse liver. We also detected induction of humoral immunity against SpCas9 and the potential presence of an SpCas9-specific cellular immune response. Our findings provide a strategy to model human liver diseases in mice and highlight the importance considering Cas9-specific immune responses in future translational studies involving in vivo delivery of CRISPR/Cas9.

Wang, D. Y., et al. (2016). "[The application of CRISPR/Cas9 genome editing technology in cancer research]." Yi Chuan **38**(1): 1-8.

The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 nuclease) genome editing technology has become more and more popular in gene editing because of its simple design and easy operation. Using the CRISPR/Cas9 system, researchers can perform site-directed genome modification at the base level. Moreover, it has been widely used in genome editing in multiple species and related cancer research. In this review, we summarize the application of the CRISPR/Cas9 system in cancer research based on the latest research progresses as well as our understanding of cancer research and genome editing techniques.

Wang, J., et al. (2016). "Highly efficient homology-driven genome editing in human T cells by combining zinc-finger nuclease mRNA and AAV6 donor delivery." Nucleic Acids Res **44**(3): e30.

The adoptive transfer of engineered T cells for the treatment of cancer, autoimmunity, and infectious disease is a rapidly growing field that has shown great promise in recent clinical trials. Nuclease-driven genome editing provides a method in which to precisely target genetic changes to further enhance T cell function in vivo. We describe the development of a highly efficient method to genome edit both primary human CD8 and CD4 T cells by homology-directed repair at a pre-defined site of the genome. Two

different homology donor templates were evaluated, representing both minor gene editing events (restriction site insertion) to mimic gene correction, or the more significant insertion of a larger gene cassette. By combining zinc finger nuclease mRNA delivery with AAV6 delivery of a homologous donor we could gene correct 41% of CCR5 or 55% of PPP1R12C (AAVS1) alleles in CD8(+) T cells and gene targeting of a GFP transgene cassette in >40% of CD8(+) and CD4(+) T cells at both the CCR5 and AAVS1 safe harbor locus, potentially providing a robust genome editing tool for T cell-based immunotherapy.

Wang, J., et al. (2015). "TALENs-mediated gene disruption of FLT3 in leukemia cells: Using genome-editing approach for exploring the molecular basis of gene abnormality." Sci Rep **5**: 18454.

Novel analytic tools are needed to elucidate the molecular basis of leukemia-relevant gene mutations in the post-genome era. We generated isogenic leukemia cell clones in which the FLT3 gene was disrupted in a single allele using TALENs. Isogenic clones with mono-allelic disrupted FLT3 were compared to an isogenic wild-type control clone and parental leukemia cells for transcriptional expression, downstream FLT3 signaling and proliferation capacity. The global gene expression profiles of mutant K562 clones and corresponding wild-type controls were compared using RNA-seq. The transcriptional levels and the ligand-dependent autophosphorylation of FLT3 were decreased in the mutant clones. TALENs-mediated FLT3 haplo-insufficiency impaired cell proliferation and colony formation in vitro. These inhibitory effects were maintained in vivo, improving the survival of NOD/SCID mice transplanted with mutant K562 clones. Cluster analysis revealed that the gene expression pattern of isogenic clones was determined by the FLT3 mutant status rather than the deviation among individual isogenic clones. Differentially expressed genes between the mutant and wild-type clones revealed an activation of nonsense-mediated decay pathway in mutant K562 clones as well as an inhibited FLT3 signaling. Our data support that this genome-editing approach is a robust and generally applicable platform to explore the molecular bases of gene mutations.

Wang, P., et al. (2017). "Genome Editing for Cancer Therapy: Delivery of Cas9 Protein/sgRNA Plasmid via a Gold Nanocluster/Lipid Core-Shell Nanocarrier." Adv Sci (Weinh) **4**(11): 1700175.

The type II bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 (CRISPR-associated protein) system (CRISPR-Cas9) is a powerful toolbox for gene-editing, however, the nonviral delivery of CRISPR-Cas9 to cells or tissues remains a key challenge. This paper reports a strategy to deliver Cas9 protein and single guide RNA (sgRNA) plasmid by a nanocarrier with a core of gold nanoclusters (GNs) and a shell of lipids. By modifying the GNs with HIV-1-transactivator of transcription peptide, the cargo (Cas9/sgRNA) can be delivered into cell nuclei. This strategy is utilized to treat melanoma by designing sgRNA targeting Polo-like kinase-1 (Plk1) of the tumor. The nanoparticle (polyethylene glycol-lipid/GNs/Cas9 protein/sgPlk1 plasmid, LGCP) leads to >70% down-regulation of Plk1 protein expression of A375 cells in vitro. Moreover, the LGCP suppresses melanoma progress by 75% on mice. Thus, this strategy can deliver protein-nucleic acid hybrid agents for gene therapy.

Webster, D. E., et al. (2014). "Enhancer-targeted genome editing selectively blocks innate resistance to oncokinase inhibition." Genome Res **24**(5): 751-760.

Thousands of putative enhancers are characterized in the human genome, yet few have been shown to have a functional role in cancer progression. Inhibiting oncokinases, such as EGFR, ALK, ERBB2, and BRAF, is a mainstay of current cancer therapy but is hindered by innate drug resistance mediated by up-regulation of the HGF receptor, MET. The mechanisms mediating such genomic responses to targeted therapy are unknown. Here, we identify lineage-specific enhancers at the MET locus for multiple common tumor types, including a melanoma lineage-specific enhancer 63 kb downstream from the MET TSS. This enhancer displays inducible chromatin looping with the MET promoter to up-regulate MET expression upon BRAF inhibition. Epigenomic analysis demonstrated that the melanocyte-specific transcription factor, MITF, mediates this enhancer function. Targeted genomic deletion (<7 bp) of the MITF motif within the MET enhancer suppressed inducible chromatin looping and innate drug resistance, while maintaining MITF-dependent, inhibitor-induced melanoma cell differentiation. Epigenomic analysis can thus guide functional disruption of regulatory DNA to decouple pro- and anti-oncogenic functions of a dominant transcription factor and block innate resistance to oncokinase therapy.

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.

Wen, J., et al. (2017). "Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing." J Hematol Oncol **10**(1): 119.

BACKGROUND: Sickle cell disease (SCD) is a disorder of red blood cells (RBCs) expressing abnormal hemoglobin-S (HbS) due to genetic inheritance of homologous HbS gene. However, people with the sickle cell trait (SCT) carry a single allele of HbS and do not usually suffer from SCD symptoms, thus providing a rationale to treat SCD. METHODS: To validate gene therapy potential, hematopoietic stem cells were isolated from the SCD patient blood and treated with CRISPR/Cas9 approach. To precisely dissect genome-editing effects, erythroid progenitor cells were cloned from single colonies of CRISPR-treated cells and then expanded for simultaneous gene, protein, and cellular function studies. RESULTS: Genotyping and sequencing analysis revealed that the genome-edited erythroid progenitor colonies were converted to SCT genotype from SCD genotype. HPLC protein assays confirmed reinstallation of normal hemoglobin at a similar level with HbS in the cloned genome-edited erythroid progenitor cells. For cell function evaluation, in vitro RBC differentiation of the cloned erythroid progenitor cells was induced. As expected, cell sickling assays indicated function reinstitution of the genome-edited offspring SCD RBCs, which became more resistant to sickling under hypoxia condition. CONCLUSIONS: This study is an exploration of genome editing of SCD HSPCs.

Wu, H. Y. and C. Y. Cao (2018). "The application of CRISPR-Cas9 genome editing tool in cancer immunotherapy." Brief Funct Genomics.

Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) system was originally discovered in prokaryotes functioned as a part of the adaptive immune system. Because of its high efficiency and easy operability, CRISPR-Cas9 system has been developed to be a powerful and versatile gene editing tool shortly after its discovery. Given that multiple genetic alterations are the main factors that drive genesis and development of tumor, CRISPR-Cas9 system has been applied to correct cancer-causing gene mutations and deletions and to engineer immune cells, such as chimeric antigen receptor T (CAR T) cells, for cancer immunotherapeutic applications. Recently, CRISPR-Cas9-based CAR T-cell preparation has been an important breakthrough in antitumor therapy. Here, we summarize the mechanism, delivery and the application of CRISPR-Cas9 in gene editing, and discuss the challenges and future directions of CRISPR-Cas9 in cancer immunotherapy.

Wyvekens, N., et al. (2015). "Dimeric CRISPR RNA-Guided FokI-dCas9 Nucleases Directed by Truncated gRNAs for Highly Specific Genome Editing." Hum Gene Ther **26**(7): 425-431.

Monomeric clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated 9 (Cas9) nucleases have been widely adopted for simple and robust targeted genome editing but also have the potential to induce high-frequency off-target mutations. In principle, two orthogonal strategies for reducing off-target cleavage, truncated guide RNAs (tru-gRNAs) and dimerization-dependent RNA-guided FokI-dCas9 nucleases (RFNs), could be combined as tru-RFNs to further improve genome editing specificity. Here we identify a robust tru-RFN architecture that shows high activity in human cancer cell lines and embryonic stem cells. Additionally, we demonstrate that tru-gRNAs reduce the undesirable mutagenic effects of monomeric FokI-dCas9. Tru-RFNs combine the advantages of two orthogonal strategies for improving the specificity of CRISPR-Cas nucleases and therefore provide a highly specific platform for performing genome editing.

Wyvekens, N., et al. (2015). "Genome Editing in Human Cells Using CRISPR/Cas Nucleases." Curr Protoc Mol Biol **112**: 31.33.31-18.

The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) system has been broadly adopted for highly efficient genome editing in a variety of model organisms and human cell types. Unlike previous genome editing technologies such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas technology does not require complex protein engineering and can be utilized by any researcher proficient in basic molecular biology and cell culture techniques. This unit describes protocols for design and cloning of vectors expressing single or multiplex gRNAs, for transient transfection of human cell lines, and for quantitation of mutation frequencies by T7 endonuclease I assay. These protocols also

include guidance for using two improvements that increase the specificity of CRISPR/Cas nucleases: truncated gRNAs and dimeric RNA-guided FokI nucleases.

Xi, L., et al. (2015). "A novel two-step genome editing strategy with CRISPR-Cas9 provides new insights into telomerase action and TERT gene expression." Genome Biol **16**: 231.

BACKGROUND: To facilitate indefinite proliferation, stem cells and most cancer cells require the activity of telomerase, which counteracts the successive shortening of telomeres caused by incomplete DNA replication at the very end of each chromosome. Human telomerase activity is often determined by the expression level of telomerase reverse transcriptase (TERT), the catalytic subunit of the ribonucleoprotein complex. The low expression level of TERT and the lack of adequate antibodies have made it difficult to study telomerase-related processes in human cells. RESULTS: To overcome the low CRISPR-Cas9 editing efficiency at the TERT locus, we develop a two-step "pop-in/pop-out" strategy to enrich cells that underwent homologous recombination (HR). Using this technique, we fuse an N-terminal FLAG-SNAP-tag to TERT, which allows us to reliably detect TERT in western blots, immunopurify it for biochemical analysis, and determine its subcellular localization by fluorescence microscopy. TERT co-localizes detectably with only 5-7 % of the telomeres at a time in S-phase HeLa cells; no nucleolar localization is detected. Furthermore, we extend this approach to perform single base-pair modifications in the TERT promoter; reverting a recurrent cancer-associated TERT promoter mutation in a urothelial cancer cell line results in decreased telomerase activity, indicating the mutation is causal for telomerase reactivation. CONCLUSIONS: We develop a two-step CRISPR-Cas9 genome editing strategy to introduce precise modifications at the endogenous TERT locus in human cell lines. This method provides a useful tool for studying telomerase biology, and suggests a general approach to edit loci with low targeting efficiency and to purify and visualize low abundance proteins.

Yamanaka, Y. (2016). "CRISPR/Cas9 Genome Editing as a Strategy to Study the Tumor Microenvironment in Transgenic Mice." Methods Mol Biol **1458**: 261-271.

Development of engineered site-specific endonucleases like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 has been revolutionizing genetic approaches in biomedical research fields. These new tools have opened opportunities to carry out targeted genome editing in mouse zygotes without the need for manipulating embryonic stem cells, which have a higher technical burden and many constraints in strain availability. Specific genetic modifications can be directly generated in working genetic backgrounds. This new approach saves time and costs associated with generation and backcrossing of genetically modified animals and will facilitate their use in various cancer research fields.

Yang, H., et al. (2018). "Break Breast Cancer Addiction by CRISPR/Cas9 Genome Editing." J Cancer **9**(2): 219-231.

Breast cancer is the leading diagnosed cancer for women globally. Evolution of breast cancer in tumorigenesis, metastasis and treatment resistance appears to be driven by the aberrant gene expression and protein degradation encoded by the cancer genomes. The uncontrolled cancer growth relies on these cellular events, thus constituting the cancerous programs and rendering the addiction towards them. These programs are likely the potential anticancer biomarkers for Personalized Medicine of breast cancer. This review intends to delineate the impact of the CRSPR/Cas-mediated genome editing in identification and validation of these anticancer biomarkers. It reviews the progress in three aspects of CRISPR/Cas9-mediated editing of the breast cancer genomes: Somatic genome editing, transcription and protein degradation addictions.

Yang, Y., et al. (2016). "Targeting ABCB1-mediated tumor multidrug resistance by CRISPR/Cas9-based genome editing." Am J Transl Res **8**(9): 3986-3994.

The RNA-guided clustered regularly interspaced short palindromic (CRISPR) in combination with a CRISPR-associated nuclease 9 (Cas9) nuclease system is a new rapid and precise technology for genome editing. In the present study, we applied the CRISPR/Cas9 system to target ABCB1 (also named MDR1) gene which encodes a 170 kDa transmembrane glycoprotein (P-glycoprotein/P-gp) transporting multiple types of chemotherapeutic drugs including taxanes, epipodophyllotoxins, vinca alkaloids and anthracyclines out of cells to contribute multidrug resistance (MDR) in cancer cells. Our data showed that knockout of ABCB1 by CRISPR/Cas9 system was succesfully archieved with two target sgRNAs in two MDR cancer cells due to the alteration of genome sequences. Knockout of ABCB1 by CRISPR/Cas9 system significantly enhances the sensitivity of ABCB1 substrate chemotherapeutic agents and the intracellular accumulation of rhodamine 123 and doxorubicin in MDR cancer cells. Although now there are lots of limitations to the application of CRISPR/Cas9 for editing cancer genes in human patients, our study provides valuable clues for the use of the

CRISPR/Cas9 technology in the investigation and conquest of cancer MDR.

Yang, Y., et al. (2018). "Recent Advances in Therapeutic Genome Editing in China." Hum Gene Ther **29**(2): 136-145.

Editing of the genome to correct disease-causing mutations is a promising approach for the treatment of human diseases. Recent advances in the development of programmable nuclease-based genome editing tools have substantially improved the ability to make precise changes in the human genome. Genome editing technologies are already being used to correct genetic mutations in affected tissues and cells to treat diseases that are refractory to traditional gene therapies. Chinese scientists have made remarkable breakthroughs in the field of therapeutic genome editing, particularly with the first clinical trial involving the clustered regularly interspaced short palindromic repeats-caspase 9 system that began in China. Herein, current progress toward developing programmable nuclease-based gene therapies is introduced, as well as future prospects and challenges in China.

Yao, S., et al. (2015). "CRISPR/Cas9-Mediated Genome Editing of Epigenetic Factors for Cancer Therapy." Hum Gene Ther **26**(7): 463-471.

Advances in engineered recombinant nuclease have provided facile and reliable methods for genome editing. Especially with the development of the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein-9 nuclease) system, the discovery of various versions of Cas9 proteins and delivery carriers, it is now practicable to introduce desired mutations into the genome, to correct disease-related mutations, and to activate or suppress genes of interest. Epigenetic regulators are often disturbed in cancer cells and are essential for the transformation of normal to cancerous cells. Tumor-related epigenetic alterations or epigenetic factor mutations play a major part during the various steps of carcinogenesis and affect a variety of cancer-related genes and a wide range of cancerous phenotypes. Therefore, epigenetic regulatory enzymes might be candidate targets for cancer therapy. In this review, we discuss prospects of CRISPR/Cas9-based genome editing in targeting epigenetics for cancer gene therapy.

Yin, H., et al. (2017). "Delivery technologies for genome editing." Nat Rev Drug Discov **16**(6): 387-399.

With the recent development of CRISPR technology, it is becoming increasingly easy to engineer the genome. Genome-editing systems based on CRISPR, as well as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs), are becoming valuable tools for biomedical research, drug discovery and development, and even gene therapy. However, for each of these systems to effectively enter cells of interest and perform their function, efficient and safe delivery technologies are needed. This Review discusses the principles of biomacromolecule delivery and gene editing, examines recent advances and challenges in non-viral and viral delivery methods, and highlights the status of related clinical trials.

Yin, H., et al. (2016). "Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo." Nat Biotechnol **34**(3): 328-333.

The combination of Cas9, guide RNA and repair template DNA can induce precise gene editing and the correction of genetic diseases in adult mammals. However, clinical implementation of this technology requires safe and effective delivery of all of these components into the nuclei of the target tissue. Here, we combine lipid nanoparticle-mediated delivery of Cas9 mRNA with adeno-associated viruses encoding a sgRNA and a repair template to induce repair of a disease gene in adult animals. We applied our delivery strategy to a mouse model of human hereditary tyrosinemia and show that the treatment generated fumarylacetoacetate hydrolase (Fah)-positive hepatocytes by correcting the causative Fah-splicing mutation. Treatment rescued disease symptoms such as weight loss and liver damage. The efficiency of correction was >6% of hepatocytes after a single application, suggesting potential utility of Cas9-based therapeutic genome editing for a range of diseases.

Yin, H., et al. (2018). "Partial DNA-guided Cas9 enables genome editing with reduced off-target activity." Nat Chem Biol **14**(3): 311-316.

CRISPR-Cas9 is a versatile RNA-guided genome editing tool. Here we demonstrate that partial replacement of RNA nucleotides with DNA nucleotides in CRISPR RNA (crRNA) enables efficient gene editing in human cells. This strategy of partial DNA replacement retains on-target activity when used with both crRNA and sgRNA, as well as with multiple guide sequences. Partial DNA replacement also works for crRNA of Cpf1, another CRISPR system. We find that partial DNA replacement in the guide sequence significantly reduces off-target genome editing through focused analysis of off-target cleavage, measurement of mismatch tolerance and genome-wide profiling of off-target sites. Using the structure of the Cas9-sgRNA complex as a guide, the majority of the 3' end of crRNA can be replaced with DNA nucleotide, and the 5 - and 3'-DNA-replaced crRNA enables efficient

genome editing. Cas9 guided by a DNA-RNA chimera may provide a generalized strategy to reduce both the cost and the off-target genome editing in human cells.

Yin, H., et al. (2017). "Structure-guided chemical modification of guide RNA enables potent non-viral in vivo genome editing." Nat Biotechnol **35**(12): 1179-1187.

Efficient genome editing with Cas9-sgRNA in vivo has required the use of viral delivery systems, which have limitations for clinical applications. Translational efforts to develop other RNA therapeutics have shown that judicious chemical modification of RNAs can improve therapeutic efficacy by reducing susceptibility to nuclease degradation. Guided by the structure of the Cas9-sgRNA complex, we identify regions of sgRNA that can be modified while maintaining or enhancing genome-editing activity, and we develop an optimal set of chemical modifications for in vivo applications. Using lipid nanoparticle formulations of these enhanced sgRNAs (e-sgRNA) and mRNA encoding Cas9, we show that a single intravenous injection into mice induces >80% editing of Pcsk9 in the liver. Serum Pcsk9 is reduced to undetectable levels, and cholesterol levels are significantly lowered about 35% to 40% in animals. This strategy may enable non-viral, Cas9-based genome editing in the liver in clinical settings.

Yin, H., et al. (2014). "Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype." Nat Biotechnol **32**(6): 551-553.

We demonstrate CRISPR-Cas9-mediated correction of a Fah mutation in hepatocytes in a mouse model of the human disease hereditary tyrosinemia. Delivery of components of the CRISPR-Cas9 system by hydrodynamic injection resulted in initial expression of the wild-type Fah protein in approximately 1/250 liver cells. Expansion of Fah-positive hepatocytes rescued the body weight loss phenotype. Our study indicates that CRISPR-Cas9-mediated genome editing is possible in adult animals and has potential for correction of human genetic diseases.

Zafra, M. P. and L. E. Dow (2016). "Somatic Genome Editing Goes Viral." Trends Mol Med **22**(10): 831-833.

Generating new mouse models of cancer is a challenging and laborious task that can require years of investment to interrogate a single gene. Now, Jonkers and colleagues describe the first application of CRISPR-based genome editing in the mammary gland, providing a new approach to dissect complex genetic interactions in vivo.

Zhu, P., et al. (2017). "CRISPR/Cas9-Mediated Genome Editing Corrects Dystrophin Mutation in Skeletal Muscle Stem Cells in a Mouse Model of Muscle Dystrophy." Mol Ther Nucleic Acids **7**: 31-41.

Muscle stem cells (MuSCs) hold great therapeutic potential for muscle genetic disorders, such as Duchenne muscular dystrophy (DMD). The CRISP/Cas9-based genome editing is a promising technology for correcting genetic alterations in mutant genes. In this study, we used fibrin-gel culture system to selectively expand MuSCs from crude skeletal muscle cells of mdx mice, a mouse model of DMD. By CRISP/Cas9-based genome editing, we corrected the dystrophin mutation in expanded MuSCs and restored the skeletal muscle dystrophin expression upon transplantation in mdx mice. Our studies established a reliable and feasible platform for gene correction in MuSCs by genome editing, thus greatly advancing tissue stem cell-based therapies for DMD and other muscle disorders.

Zhu, Z., et al. (2016). "Genome Editing of Lineage Determinants in Human Pluripotent Stem Cells Reveals Mechanisms of Pancreatic Development and Diabetes." Cell Stem Cell **18**(6): 755-768.

Directed differentiation of human pluripotent stem cells (hPSCs) into somatic counterparts is a valuable tool for studying disease. However, examination of developmental mechanisms in hPSCs remains challenging given complex multi-factorial actions at different stages. Here, we used TALEN and CRISPR/Cas-mediated gene editing and hPSC-directed differentiation for a systematic analysis of the roles of eight pancreatic transcription factors (PDX1, RFX6, PTF1A, GLIS3, MNX1, NGN3, HES1, and ARX). Our analysis not only verified conserved gene requirements between mice and humans but also revealed a number of previously unsuspected developmental mechanisms with implications for type 2 diabetes. These include a role of RFX6 in regulating the number of pancreatic progenitors, a haploinsufficient requirement for PDX1 in pancreatic beta cell differentiation, and a potentially divergent role of NGN3 in humans and mice. Our findings support use of systematic genome editing in hPSCs as a strategy for understanding mechanisms underlying congenital disorders.

Zuris, J. A., et al. (2015). "Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo." Nat Biotechnol **33**(1): 73-80.

Efficient intracellular delivery of proteins is needed to fully realize the potential of protein therapeutics. Current methods of protein delivery commonly suffer from low tolerance for serum, poor endosomal escape and limited in vivo efficacy. Here we report that common cationic lipid nucleic acid transfection reagents can potently deliver proteins that

are fused to negatively supercharged proteins, that contain natural anionic domains or that natively bind to anionic nucleic acids. This approach mediates the potent delivery of nM concentrations of Cre recombinase, TALE- and Cas9-based transcription activators, and Cas9:sgRNA nuclease complexes into cultured human cells in media containing 10% serum. Delivery of unmodified Cas9:sgRNA complexes resulted in up to 80% genome modification with substantially higher specificity compared to DNA transfection. This approach also mediated efficient delivery of Cre recombinase and Cas9:sgRNA complexes into the mouse inner ear in vivo, achieving 90% Cre-mediated recombination and 20% Cas9-mediated genome modification in hair cells.

Zych, A. O., et al. (2018). "Application of Genome Editing Techniques in Immunology." Arch Immunol Ther Exp (Warsz).

The idea of using the effector immune cells to specifically fight cancer has recently evolved into an exciting concept of adoptive cell therapies. Indeed, genetically engineered T cells expressing on their surface recombinant, cancer-targeted receptors have been shown to induce promising response in oncological patients. However, in addition to exogenous expression of such receptors, there is also a need for disruption of certain genes in the immune cells to achieve more potent disease-targeted actions, to produce universal chimeric antigen receptor-based therapies or to study the signaling pathways in detail. In this review, we present novel genetic engineering methods, mainly TALEN and CRISPR/Cas9 systems, that can be used for such purposes. These unique techniques may contribute to creating more successful immune therapies against cancer or prospectively other diseases as well.

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