CRISPR-Cas9

CRISPR-Cas9 Genome Editing: CRISPR-Cas9 is a revolutionary genome editing technology that allows precise modification of specific DNA sequences. It has numerous applications in functional genomics, gene therapy, and biotechnology.

CRISPR, is a family of DNA sequences in bacteria that contains snippets of DNA from viruses that have attacked the bacteria. These snippets are used by the bacterium to detect and destroy DNA from further attacks by similar viruses. These sequences play a key role in a bacterial defense system and form the basis of a genome editing technology known as CRISPR/Cas9 that allows permanent modification of genes within organisms.

Li et al. hypothesized that chromosomal translocation, such as t(1;19)(q10;p10) resulting in the 1p/19q co-deletion, may be induced by simultaneously introducing DNA double-strand breaks (DSBs) into chromosome 1p and 19q using CRISPR/Cas9. We developed a CRISPR/Cas9-based strategy to induce t(1;19)(q10;p10) and droplet digital PCR (ddPCR) assays to detect the hybrid 1q/19p and 1p/19q chromosomes.

After translocation induction, we detected both 1p/19q and 1q/19p hybrid chromosomes by PCR amplification of the junction regions in HEK 293T, and U-251 and LN-229 glioblastoma cells. Sequencing analyses of the PCR products confirmed DNA sequences matching both chromosomes 1 and 19. Furthermore, the 1p/19q hybrid chromosome was rapidly lost in all tested cell lines. The 1q/19p hybrid chromosome also become undetectable over time likely due to cell survival disadvantage.

They demonstrated that t(1;19)(q10;p10) may be induced by CRISPR/Cas9-mediated genomic editing. This method represents an important step toward engineering the 1p/19q co-deletion to model oligodendrogliomas. This method may also be generalizable to engineering other cancer-relevant translocations, which may facilitate the understanding of translocation roles in cancer progression [1)].

The CRISPR-Cas9 system is a powerful gene-editing tool with wide-ranging applications, but the safe and efficient intracellular delivery of CRISPR components remains a challenge. In this study, we utilized biodegradable poly(beta-amino ester) nanoparticles to codeliver plasmid DNA encoding Cas9 and short guide RNA (sgRNA), respectively, to enable gene knockout following a CRISPR-mediated cleavage at one genomic site (1-cut edit), as well as gene deletion following DNA cleavage at two sites flanking a region of interest (2-cut edits). We designed a reporter system that allows for easy evaluation of both types of edits: gene knockout can be assessed by a decrease in near-infrared fluorescent protein (iRFP) fluorescence, whereas deletion of an expression stop cassette turns on a red-enhanced nanolantern fluorescence/luminescence dual reporter. Nanoparticles enabled up to 70% gene knockout due to small indels, as well as 45% gain-of-function expression after a 600-bp deletion edit. The efficiency of 2-cut edits is more sensitive than 1-cut edits to Cas9 and the sgRNA expression level. We demonstrate promising biodegradable nanoparticle formulations for gene editing. Our findings also provide new insights into the screening and transfection requirements for different types of gene edits, which are applicable for designing nonviral delivery systems for the CRISPR-Cas9 platform [2)].

Cas9 (CRISPR associated protein 9) is an RNA-guided DNA endonuclease enzyme associated with the CRISPR (Clustered Regularly Interspersed Palindromic Repeats) adaptive immunity system in Streptococcus pyogenes, among other bacteria. S. pyogenes utilizes Cas9 to memorize and later interrogate and cleave foreign DNA, such as invading bacteriophage DNA or plasmid DNA. Cas9 performs this interrogation by unwinding foreign DNA and checking for sites complementary to the 20 basepair spacer region of the guide RNA. If the DNA substrate is complementary to the guide RNA, Cas9 cleaves the invading DNA. In this sense, the CRISPR-Cas9 mechanism has a number of parallels with the RNA interference (RNAi) mechanism in eukaryotes.

Genetic modification is an indispensable tool to study gene function in normal development and disease. The recent breakthrough of creating human induced pluripotent stem cells (iPSCs) by defined factors (Takahashi et al., Cell 131:861-872, 2007) provides a renewable source of patient autologous cells that not only retain identical genetic information but also give rise to many cell types of the body including neurons and glia. Meanwhile, the rapid advancement of genome modification tools such as gene targeting by homologous recombination (Capecchi, Nat Rev Genet 6:507-512, 2005) and genome editing tools such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) system, TALENs (Transcription activator-like effector nucleases), and ZFNs (Zinc finger nucleases) (Wang et al., Cell 153:910-918, 2013; Mali et al., Science 339:823-826, 2013; Hwang et al., Nat Biotechnol 31:227-229, 2013; Friedland et al., Nat Methods 10(8):741-743, 2013; DiCarlo et al., Nucleic Acids Res 41:4336-4343, 2013; Cong et al., Science 339:819-823, 2013) has greatly accelerated the development of human genome manipulation at the molecular level. This chapter describes the protocols for making neural lineage reporter lines using homologous recombination and the CRISPR/Cas system-mediated genome editing, including construction of targeting vectors, guide RNAs, transfection into hPSCs, and selection and verification of successfully targeted clones. This method can be applied to various needs of hPSC genetic engineering at high efficiency and high reliability [3].

Chow et al.e developed an adeno associated virus-mediated, autochthonous genetic CRISPR screen in glioblastoma. Stereotaxic delivery of a virus library targeting genes commonly mutated in human cancers into the brains of conditional-Cas9 mice resulted in tumors that recapitulate human glioblastoma. Capture sequencing revealed diverse mutational profiles across tumors. The mutation frequencies in mice correlated with those in two independent patient cohorts. Co-mutation analysis identified co-occurring driver combinations such as B2m-Nf1, Mll3-Nf1 and Zc3h13-Rb1, which were subsequently validated using AAV minipools. Distinct from Nf1-mutant tumors, Rb1-mutant tumors are undifferentiated and aberrantly express homeobox gene clusters. The addition of Zc3h13 or Pten mutations altered the gene expression profiles of Rb1 mutants, rendering them more resistant to temozolomide. The study provides a functional landscape of gliomagenesis suppressors in vivo [4].

1)

Li C, Liu Z, Zhang X, Wang H, Friedman GK, Ding Q, Zhao X, Li H, Kim K, Yu X, Burt Nabors L, Han X, Zhao R. Generation of chromosome 1p/19q co-deletion by CRISPR/Cas9-guided genomic editing. Neurooncol Adv. 2022 Aug 18;4(1):vdac131. doi: 10.1093/noajnl/vdac131. PMID: 36225650; PMCID: PMC9547542.

2)

Rui Y, Varanasi M, Mendes S, Yamagata HM, Wilson DR, Green JJ. Poly(Beta-Amino Ester)

Nanoparticles Enable Nonviral Delivery of CRISPR-Cas9 Plasmids for Gene Knockout and Gene Deletion. Mol Ther Nucleic Acids. 2020 Apr 21;20:661-672. doi: 10.1016/j.omtn.2020.04.005. [Epub ahead of print] PubMed PMID: 32380416.

3)

Xue H, Wu J, Li S, Rao MS, Liu Y. Genetic Modification in Human Pluripotent Stem Cells by Homologous Recombination and CRISPR/Cas9 System. Methods Mol Biol. 2016;1307:173-90. doi: 10.1007/7651_2014_73. PubMed PMID: 24615461.

4)

Chow RD, Guzman CD, Wang G, Schmidt F, Youngblood MW, Ye L, Errami Y, Dong MB, Martinez MA, Zhang S, Renauer P, Bilguvar K, Gunel M, Sharp PA, Zhang F, Platt RJ, Chen S. AAV-mediated direct in vivo CRISPR screen identifies functional suppressors in glioblastoma. Nat Neurosci. 2017 Aug 14. doi: 10.1038/nn.4620. [Epub ahead of print] PubMed PMID: 28805815.