International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

A Review of Genome Editing Strategies by Crisper/Cas 9 System

Shafeeque Kalody

Uwin life sciences, Malappuram, Kerala, India, 676505

Abstract: ***Clustered regularly interspaced short palindromic repeats (CRISPR) and associated Proteins Cas9 are part of bacterial defense mechanism against foreign genetics elements such as those present in the plasmids and phages .CRISPR-cas9 allows researchers for knock out or knock in of specific DNA sequences in animals models and human cells lines. Hence CRISPR-cas9 system could assist in treatment of cancer, Genome editing in genetic disorder like DMD, β thalasemia, Hemophilia and cystic fibrosis, Genetic engineering in primary human B cells and treatment of hematological disorder etc. Here the latest applications of CRISPR-cas9 editing system in various fields of biomedical researchers are reviewed and discussed***

Keywords: CRISPR/Cas9, guide RNA, Single guide RNA, Genetic disorder

1. Introduction

CRISPR stand for clustered regularly interspaced short palindromic repeats. These are the DNA sequences found within the genome of microorganism like bacteria and archaea. After emergence of the Central dogma of molecular biology, Researchers have attempted to manipulate genome by developing new technologies. CRISPR /Cas-9 biology has been turning point in the field of molecular biology of gene editing system. CRIPR / Cas -9 system consist of two major components: the CRISPR gene and the Cas 9 protein. CRISPR first found in E.coli, the spacer's fragments of DNA gathered from phage that previously tried to attack the cell. The complex also holds the DNA for the Cas-9 protein, this Cas -9 guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. It is necessary to understand the function of given gene through precise editing and regulation of genomic information .CRISPR / Cas (CRISPR-associated Protein) is prokaryotic immune system.RNA guided Cas protein is highly efficient and specific genome editing in various organism [1].CRSIPR / Cas genome editing system is based on recognition of specific in the genome and introduce break on nucleic acid with the use of nuclease. Since earlier time several of these nucleases reported, such as Zing finger nuclease and Transcription Activator like effectors nucleases (TALEN). In which Cas 9 protein makes cut in DNA [2].In 2002 *Drosophila Melanogaster* (fruit fly) genome edited by using Zinc finger nuclease, it also used to repair a mutation human cells [3, 4]. Models organism like *Arabidopsis Thaliana* and *Caenorhabditis elegans* genome altered with use of TALEN in 2011[5,6]

CRISPR/Cas 9Biology

CRISPR (Clustered regularly Interspaced short palindromic repeats)/Cas 9 protein system is an adaptive immune system , that evolved in bacteria .It is RNA –guided DNA nuclease, giving them safe guard against attacking phages and plasmids .In prokaryote three type of CRISPR mechanism are founded, in which type 2 extensively studied [7].CRISPR /Cas 9 editing tool work like restriction endonuclease ,that is guided by a hybrid strand of RNA and abbreviated to Cr RNA which is approximately 20 bp in length ,form complex with Cas 9 endonuclease. CRISPR RNA bind with complementary DNA sequences in the host cells through normal Watson and crick base pairing, this specificity has changed CRISPR/Cas 9 gene editing system as new tool in molecular biology in the category of restriction endonuclease. CRISPR/Cas 9 is similar to eukaryote immune system. In type two CRISPR mechanism trRNA and crRNA combined to form single guide RNA (sgRNA), followed by sgRNA and Cas 9 forms into a CRISPR ribonucleo protein complex [8].

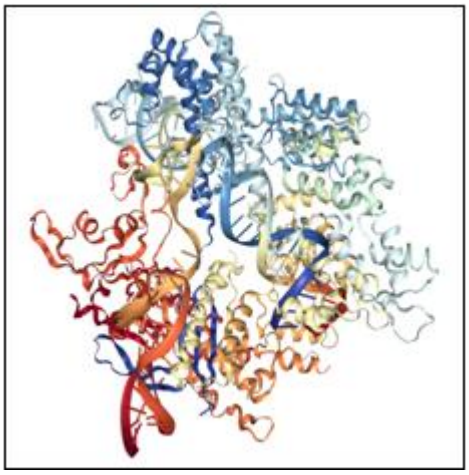

Figure: Crystal structure of Cas-9 in complex with guide RNA target DNA (*Streptococcus pyogens*) [9]

Genetic engineering in primary human B cells

Involvement of B cells in various autoimmune and infectious diseases turns to an attractive platform for genome editing. One report described targeting gene knock-out to study V (D) J recombination of pro-B cell lines in mouse. B cells has Critical role in humoral immunity ,therapies via genetic engineered B cells would have broad potential applications .Other studies have demonstrated ,genetic manipulation of primary human B cells isolated from human peripheral blood ,by deliver of CRISPR/Cas 9 ribonucleoproteins through electroporation ,this study confirmed that efficient editing at both genomic and protein expression levels [10]

Potential uses of CRISPR/Cas 9 in Genetic Disorder

Genetic disorder associated with physical or mental problems .Since advent of Recombinant DNA technology, Genome editing technologies rapidly emerged for manipulate genome at specific locations. CRISPR /Cas 9 system has been using last few years to treat genetic disease like Cystic fibrosis, β-thalassemia, Hemophilia and Duchenne muscular dystrophy etc

DMD is genetic disorder caused by in-frame deletions of dystrophin gene .It is inherited X-linked fatal genetic muscle disease. Dystrophin has critical role in muscle development .Absence of dystrophin cause progressive muscle weakening and affected person leads to premature death .Till now there is no effective treatment for DMD discovered. Genome correction approaches for muscular dystrophy involve exon removal and c DNA knocking using different techniques has been tried. Bengston et al reported that adenovirus associated viral (AAV) –mediated muscle specific gene correction has potential for treatment of neuro muscular disorder. Recently CRISPR –Cpf1 isolated from (*Prevotella* and *Francisella* -1)used to correction of muscular dystrophy mutation animal disease model and human cardiomyocytes .Therapeutic potential of CRISPR/Cas 9 tool encouraging to cure human genetic disorder including DMD[11]

Cystic fibrosis is another genetic disease, caused by mutation in CFTR gene on the long arm of chromosome 7 .CFTR stand for Cystic fibrosis transmembrane conductance regulator. It causes fluid retention and disturbance in ion transport. Recently reported that correction of mutation in CFTR gene by CRISPR/Cas 9 in intestinal stem cell. [12, 13, 14]

Deficiency in coagulation factor cause heredity disorder hemophilia .It is common inherited blood disease. There are two types of hemophilia reported .Deficiency in coagulation factor VIII(F VIII) Cause hemophilia A (HA),second type hemophilia B(HB) is caused by mutation in coagulation factor XI. Several research trials have been demonstrated to cure hemophilia using gene therapy recently. Nathwani et al, 2011 and McIntosh et al, 2013 reported that adenovirus associated virus vectors expression of clotting factor. One research group from china discovered a novel mutation Y371D In human coagulation factor F9(FIX).They inserted this mutated gene into mice via CRISPR /Cas 9 system leads to Hemophilia B phenotype . Guan et al, 2016 demonstrated that somatic gene correction via CRISPR /Cas 9 in coagulation factor IX gene mutation in muse model.

CRISPR/Cas 9 in Cancer Research

CRISPR /Cas 9 system can assist in tracking Oncogenic progression and analyzing the expression patterns of the Cancer genes .In 2016 two attempts for malignancy treatment have been demonstrated in China and United states. Both which are conjectures to use CRISPR /Cas 9 to design and understanding T cells in vitro to suppress oncogenic cells .Current progress in the field of gene editing system via CRISPR/Cas 9 have made an advance towards to produce lung cancer treatments .Cas nuclease most commonly isolates from the bacterium *Streptococcus pyogens* is use to communicate in side of cells of interest. To and colleagues findings showed that significance as it possible to create specific mutation in Ras genes in cancer patients. Now days use of mouse model become effective with CRISPR/Cas 9 therapeutics .Recently several nuclear oncology research laboratories reported that both NANOG and NANOG8 express in cancer cell lines and cancer tissues, which can be corrected with CRISPR /Cas 9 or in combination with ZFNs or either TALENs [15-20]

CRISPR/Cas 9 leads to large deletions

The efficacy of the Crisper/Cas9 therapeutics for genome editing in humans and mouse models have been understated and extensively researched .Consequently single guide RNA were used to instigate deletion of up to 600bp in mouse zygotes .Whereas researchers guess that a substantial proportion of potential genotypes may have missed by on target Cas9 repair and cutting, some of which may produce pathogenic consequence following genome editing of mitotically active cells .One of the research investigated that allelic diversity produced by Cas9 at the X linked Pig A locus .Which is hemizygous in male embryonic stem cells .In this study Single guide RNA resulted out Pig A loss (59-97%)by targeting exons 2 to 4 yielded very high rate of frequencies .Both Pacbio cluster reads and FLAER staining supports this results.[21]

2. Conclusion

With the rapid development of Crisper/Cas9 based gene editing strategy in current molecular research, we prospect this gene editing system will change general picture of cancer research, hematological disorder treatment and genetic disorder surgery .Recent works show Crisper/Cas9 permanently correct gene mutation in various genetic disorder. I believe its natural gift to upcoming young researchers and it will be turning point in medical research

References

[1] Haifeng Wang,1 Marie La Russa,1,2 and Lei S. Qi1,3,4 ; CRISPR/Cas9 in Genome Editing and Beyond ; doi: 10.1146/annurev-biochem-060815-014607
[2] Gaj T et al. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 31: 397-405
[3] Bibikova M et al. (2002). Targeted chromosomal cleavage and mutagenesis in Drosophila using zincfinger nucleases. Genetics 161: 1169-1175
[4] Urnov RD et al. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435: 646-651
[5] Cermak T et al. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 39: e82
[6] Wood AJ et al. (2011). Targeted genome editing across species using ZFNs and TALENs. Science 333: 30
[7] Rudrarup Bhattacharjee1, Karishma Das Purkayastha1, Dattatreya Adapa2 and Amarendranath Choudhury3,4*CRISPR/Cas9 genome editing system in the diagnosis and treatment of cancer J RNAi Gene Silencing, 2017, Vol 13, 585-591ISSN

[8] Andrew B Bonner* CRISPR/Cas9 Gene Editing and its Potential to Treat Common Diseases ; DOI: 10.26717/BJSTR.2018.05.001142
[9] https://www.rcsb.org/3d-view/4OO8/1
[10] Chung-An M. Wua,1, Theodore L. Rothb,1, Yuriy Baglaenkoh,i,1,2, Dario M. Ferrih,i, Patrick Braueri,j[, Juan Carlos Zuniga-Pfluckeri,j, Kristina W. Rosbef, Joan E. Witherh,i,k,∗∗∗, Alexander Marsonb,c,d,e,∗∗, Christopher D.C. Allena,g,∗ Genetic engineering in primary human B cells with CRISPR-Cas9 ribonucleoproteins https://doi.org/10.1016/j.jim.2018.03.009
[11] Vivek K Pandey1, Anima Tripathi2, Ravi Bhushan1, Akhtar Ali1, Pawan K Dubey1; Application of CRISPR/Cas9 Genome Editing in Genetic Disorders: A Systematic Review Up to Date; DOI: 10.4172/2157-7412.1000321
[12] Schwank G, Koo BK, Sasselli V (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13: 653-658
[13] Crane AM, Kramer P, Bui JH (2015) Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Rep 4: 569-577.
[14] Firth AL, Menon T, Parker GS (2015) Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs. Cell Rep 12: 1385-1390.
[15] Reardon S. 2016. First CRISPR clinical trial gets green light from US panel. Nature.
[16] CRISPR/Cas9 therapeutics: a cure for cancer and other genetic diseases.Faheem Ahmed Khan1, Nuruliarizki Shinta Pandupuspitasari1, Huang Chun-Jie1, Zhou Ao1, Muhammad Jamal2, Ali Zohaib3, Farhan Ahmed Khan4, Muthia Raihana Hakim5 and Zhang ShuJun1.Oncotarget, Advance Publications 2016
[17] Feldser DM, Kostova KK, Winslow MM, Taylor SE, Cashman C WhittakerCA, et al. Stage-specific sensitivity to p53 restoration during lung cancer Progression. Nature. 2010; 468: 572-5.
[18] Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, Jaenisch R. One-step Generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. Cell. 2013; 154: 1370-9.
[19] Xue W, Chen S, Yin H, Tammela T, Papagiannakopoulos T, Joshi NS, et al. CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature.2014; 514: 380-4.
[20] Maddalo D, Manchado E, Concepcion CP, Bonetti C, Vidigal JA, Han YC, et al.In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014; 516: 423-7
[21] Repair of double strand break induced by CRIPR /Cas9 leads to large deletion and complex rearrangements. Michael Kosicki, Kart Tomberg ,Allan Bradley; Nature biotechnology 36/765-771(2018)

.