MedCrave
Step into the World of Research

Journal of Microbiology & Experimentation

Review Article

Open Access

CrossMark

Crispr-a novel approach towards a fortified immune system

Abstract

Human genome is extremely enigmatic and is known for its intricacy. The high level of its complex nature has indeed provoked several investigators to disclose the hidden insights which till date have been accomplished on a partial basis. Several facts in relation to the human genome has triggered curiosity among scientific demonstrators to further dig in to the roots of these fascinating molecular entities and one such fact that serves as the main basis for this article is the relation between human immunity and its genome through microbial perspective. Prokaryotes are considered as one of the most primitive biological facets that have led to the origin of complex life forms and evolution has made these unicellular contenders as an integral part of several biological forms including human beings. One of the latest and most researched aspects in prokaryotes is certain regions of the genome consisting of interspersed short palindromic sequence that confers resistance against bacteriophages (bacterial viruses) which is a consequence of viral infection. The prime objective of this review is to explore the same from the context of immunity in human beings. The current article attempts to emphasize on the use of CRISPR technology towards the benefit of human beings and its role as apromising tool to counteract clinical manifestation and safeguard the immune system.

Volume 11 Issue 3 - 2023

Vasudevan, Padma, Prerana Shankpal, Charitha Sheri
Aurora's Degree & PG College, Department of Microbiology, India

Correspondence: Vasudevan Ranganathan, Department of Microbiology, Aurora's Degree & PG College, Accredited by NAAC with B++ Grade, Chikadpally, Hyderabad, India, Tel 9100001639/8121119692, Email vasudeva123@gmail.com

Received: March 08, 2023 | **Published:** June 20, 2023

Introduction

Genome editing over the last few years have indeed revolutionized the attempts towards the betterment of human genome with the intent of exploring hidden molecular insights. This enhanced the understanding of the human genome from the context of their products which fulfills a cascade of molecular interactions.[1] The onset of genome editing technology has gained its importance over the last decade and has dominated the field of biotechnology through a cohesive approach that enabled genetic manipulation.[2,3] Altercations in the double stranded DNA has provided a comprehensive understanding on genetic recombination in living cells.[4]

Cas 9

CRISPR/CAS 9 technology is consisting of Cas 9 nucleases and single guide RNA.[5,6] Cas 9 is RNA dependent DNA endonucleases capable of degrading foreign sequences.Demonstrative studies have authenticated the significance of Cas 9 in counteracting the foreign sequences.[7] *Streptococcus pyogenes* makes use of CRISPR to recognize the foreign sequences which in turn triggers the activity of Cas 9 gene responsible for the activation of a cascade of reactions conferring the defense mechanism which is a part of adaptive system. The Cas 9 then starts cleaving the foreign DNA which could be a bacteriophage DNA or a plasmid DNA by unwinding the foreign DNA with the assistance of guide RNA.[8,9]

Variants of Cas enzyme

Since Cas 9 is an RNA dependent DNA endonucleases, it's recruitment at the specific site is assisted by the guide RNA which supports the employment of Cas 9 at the site of action. Cas 9 action also depends on the extent of complementarity between the guide RNA and the foreign sequence. Several studies have claimed the importance of guide RNA in binding with the foreign DNA followed by the recruitment of Cas 9 which starts degrading the foreign. Cas 9 has the ability of cleaving any sequence that is complementary to guide RNA and scientific investigators have claimed the binding of guide RNA to foreign DNA causing the onset of degradation.[10] The Cas 9 protein in *Streptococcuspyogenes* has been thoroughly studied to comprehend the CRISPR system through the different components that is responsible for the integrity and configuration of the protein. Initial studies have revealed the existence of four components system comprising of two small molecules of trans activating CRISPR RNA and crRNA which was later re-constructed in to a more ccompact two component system by the fusion of the two smaller molecules giving rise to a single guide RNA which in collaboration with the Cas 9 protein degrades the foreign DNA. This engineering work was performed by Jennifer Doudna and Emmanuelle Charpentier for which they were awarded the prestigious Nobel Prize in 2020.[5] After the discovery of CRISPR-CAS 9 technology in *Streptococcuspyogenes*, several studies have reported a similar kind of system in other organisms including *Saccharomyces cerevisiae*, *candida*, nematodes including monkeys and human embryos.[11,12,13] In addition to Cas 9, several other variants of the Cas family have been studied which includes Cas 12 a and Cas 13.

Different Cas enzymes have the abilities of generating different types of cuts and the role of Cas 9 is making blunt end cuts have been validated through several demonstrative studies.[14,15] The cas12a slightly differs from that of cas9 as the former rely on T rich sequences providing alternative targeting sites with the assistance of CRISPR RNA (crRNA) in contrast cas 9 activity depends on CRISPR RNA and trans-activating crRNA.[16,17] The CRISPR/CAS9 is guided by the sg RNA which in turn binds to tracer RNA and the entire complex is referred as ribo nucleoprotein complex.[18,5]

Studies have authenticated the prominence of these sequences in causing affirmative outcomes in host organism.[19,20] These two types of classes are further divided in to six systems which are further divided into different subtypes. Each of these subtypes is characterized by their uniqueness from the view point of their mode of action. The classification is based on the Cas protein as several studies have authenticated the involvement of different kinds of Cas proteins in triggering the cleavage mechanism. Involvement of several subunits of Cas protein or a single large protein has been validated through demonstrative studies.[21,22]

Gene editing through CRISPR technology

CRISPR-Cas9 technology is one of the technologies that has emerged in the recent past and has revolutionized the scientific community. This technology has been widely employed for gene editing and genome manifestation. The technology involves the association of the cluster of short palindromic sequences with specific endonuclease causing the degradation of the foreign nucleic acid.[23,24]

Scientific investigators have explored this technology to comprehend the immune defense in the organism and have used it as a means of editing DNA for optimistic outcomes. This technology has indeed led to the discovery of a short piece of RNA known as the guide sequence which attaches itself to the specific target and assists in the cleavage mechanism. The guide sequence also favors the attachment of Cas9 enzyme which degrades the foreign nucleic acid. The guide RNA initially recognizes the target nucleic acid sequence and further provides the room for the attachment of Cas9 enzyme which degrades the intended foreign nucleic acid at specific location. Genome editing through CRISPR technology has been of great research interest and was investigated from the view point of probing possible diagnostic measures for several human clinical manifestations. Ongoing research in various cells lines including animal models has served as a platform for comprehending the significance of CRISPR technology to counteract several gene related disorders. The current clinical aspects involve lab level attempts and clinical trials with the intent of disclosing the affirmative side of CRISPR technology. Scientists are working to figure out the possible measures for single gene associated disorders like hemophilia, cystic fibrosis and sickle cell anemia through genome editing by employing CRISPR technology.[6]

Prominence of CRISPR technology in disease models

Cystic fibrosis is a genetic consequence that was corrected through CRISPR technology. Cystic fibrosis is due to the homozygous mutation in the CFTR gene that causes severe pulmonary manifestations. CRISPR technology was employed to counteract the genetic consequence where the intestinal stem cells of the patient were corrected through invitro procedures. CRISPR/Cas9 technology has allowed the genome editing of the stem cells and has enhanced the differentiation of these cells in to vital organs with functional form of the CFTR gene. CRISPR technology has been widely employed in case of liver transplant and metabolic diseases associated with the liver.[25] CRISPR technology has also been employed for comprehending metabolic pathways as several studies have validated the role of this technology is reprogramming various pathways associated with metabolism by amending genes. This technology has been used for down regulating genes that have been associated with clinical manifestation by suppressing gene expression. The promising impact of CRISPR technology has been demonstrated in mice certain genes for the regulation of metabolic pathways. Mutation of 4 hydroxy phenylpyruvate dioxygenase genes has resulted in the increase in tyrosine catabolism which in turn avoids the accumulation of tyrosine and toxic metabolites.[26]

Hence it is very obvious that the loss of this receptor prevents the viral infection. CRISPR technology is widely employed to edit the chemokine receptor through gene editing mechanism leading to an altered receptor that doesn't allow the binding of the virus on to the receptor and averts the infection.[27,28] Studies have also authenticated the prominence of CRISPR technology as an appropriate remedy for hepatitis B virus. The covalently closed circular DNA poses a problem for treating hepatitis B. Eradication of hepatitis B was possible through gene editing by CRISPR technology which alters the covalently closed circular DNA. This has in turn reduced the intensity of the clinical condition by the removal of hepatocytic viral load.[29]

Discussion

Determination of a gene function is very vital as it plays a crucial role on the characterization of the gene of interest. Homologous recombination or blocking of the RNA through RNA interference has been commonly employed in order to disclose the significance of the gene. This approach has been demonstrated at the *invitro* level involving cultured cells and *invivo* employing living models for characterizing the gene. Recent advances have allowed the manipulation of a gene at a specific locus in a cell that can be used for affirmative outcomes. Gene editing in a broad range of species has enhanced the understanding of molecules at the genetic and molecular echelon and served as a means of exploiting the specific loci on the genes with the intent of deriving diagnostic assistance.[30] Genome editing has been made possible with the help of specific nucleases capable of inducing cuts in single or double stranded nucleic acids. The process is very complicated which follows a series of cellular mechanisms after the activity of nucleases. Several repair systems have been affiliated with the cascade of cellular reactions that ensures the authenticity of the host genome. CRISPR technology is one of the recently emerging techniques that are employed for editing genes for deriving positive results. As a matter of fact, this technology is often employed to counteract consequences causing clinical manifestation due to gene alterations. CRISPR technology makes use of several other biological entities including specific proteins and RNA sequences that will assist the direction of the complex towards the target nucleic acid sequence for degradation.[31] CRISPR technology makes use of Cas 9 enzyme which is assisted by guide sequence towards the target sequence for initiating double strand breakage. Several studies have validated the prominence of this technology in eubacteria and archaeabacteria as a means of defense mechanism against the external nucleic acid sequences as a consequence of biological threat from the outside. Various variants of the endonucleases associated with CRISPR sequences have been studied with the ability of selective action against external RNA or DNA sequences contributing towards acquired immunity in the host.[32] This system accounts to about 50% and 87% respectively in eubacteria and archaea and is responsible for fortifying the host from the context of adapting to high temperatures, rearrangement of chromosomes, replication and DNA repair mechanism.[33,34] A series of repeated fragment of with interspaced variable segments in *Escherichiacoli* has led to the discovery of CRISPR technology in 1987 which served as one of the recent achievements in biological sciences as the Nobel Prize for this technology was awarded in 2020. Repeats of 29 nucleotides in length were found in *Escherichiacoli* that were separated by interspaced sequences of 32 nucleotides in length.[35] Mojica have validated the association of CRISPR system with Cas genes and discovered the repeats of short palindromic sequences of 24 to 40 nucleotides in prokaryotes. The Cas genes were located adjacent to the CRISPR site which further authenticates the affiliation of these genes with CRISPR segments.[36, 37]

Conclusion

Genetic engineering and molecular gene technology have redefined the chemistry of bio-molecules which allowed manipulations of bio molecules. Though genetic recombination is usually regarded as a process taking place at its own pace, the significance of gene editing technologies as prominent tool in genes and genomes cannot be denied. The prevalence of CRISPR technology widens the understanding of

Citation: Vasudevan, Padma, Shankpal P, et al. Crispr-a novel approach towards a fortified immune system. *J Microbiol Exp*. 2023;11(3):73–75.
DOI: 10.15406/jmen.2023.11.00391

how genes play a vital role in offering resistance against a foreign sequence by employing a cascade of bio molecules and the intricacies involved in this technology further questions the authenticity of this mechanism in multi cellular organisms. However, the employment of this technology at acceptable echelon still remains tentative and demands further research for the complete dissection of the hidden insights in accordance with this technology.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

References

1. Rothstein RJ. One-step gene disruption in yeast. *Meth Enzymol*. 1983;101:202–211.
2. Ghosh D, Venkataramani P, Nandi S, et al. CRISPR-Cas9 a boon or bane: the bumpy road ahead to cancer therapeutics. *Cancer Cell Int.* 2019;19:12.
3. Gaj T, Gersbach CA, Barbas CF. 3rd ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol*. 2013;31(7):397–405.
4. Rouet P, Smih F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. *Proc Natl Acad Sci USA*. 1994;91:6064–6068.
5. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–21.
6. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157(6):1262–1278.
7. Deltcheva E, Chylinski K, Sharma CM, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*. 2011;471(7340):602–607.
8. Heler R, Samai P, Modell JW, et al. Cas9 specifies functional viral targets during CRISPR-Cas adaptation. *Nature*. 2015;519 (7542):199–202.
9. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;315(5819):1709–1712.
10. Mali P, Esvelt KM, Church GM. Cas9 as a versatile tool for engineering biology. *Nat Methods*. 2013;10(10): 957–63.
11. Baltimore D, Berg P, Botchan M, et al. Biotechnology. A prudent path forward for genomic engineering and germline gene modification. *Science*. 2015;348(6230):36–38.
12. Liu JJ, Kong II, Zhang GC, et al. Metabolic Engineering of Probiotic Saccharomyces boulardii. *Appl Environ Microbiol*. 2016;82(8):2280–2287.
13. Friedland AE, Tzur YB, Esvelt KM, et al. Heritable genome editing in C. elegans via a CRISPR-Cas9 system. *Nat Methods*. 2013;10(8):741–743.
14. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*. 2015;163(3):759–771.
15. Fonfara I, Richter H, Bratovič M, et al. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. *Nature*. 2016;532(7600):517–521.
16. Broughton JP, Deng X, Yu G, et al. CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol*. 2020;38(7):870–874.
17. Nguyen LT, Smith BM, Jain PK. Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection. *Nat Commun*. 2020;11(1):4906.
18. Garneau JE, Dupuis M, Villion M, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature*. 2010;468:67–71.
19. Tyson GW, Banfield JF. Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses. *Environ Microbiol*. 2008;10(1):200–207.
20. Wright AV, Nuñez JK, Doudna JA. Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering. *Cell*. 2016;164(1–2):29–44.
21. Wiedenheft B, Sternberg SH, Doudna JA. RNA-guided genetic silencing systems in bacteria and archaea. *Nature*. 2012;482(7385):331–338.
22. Deng L, Garrett RA, Shah SA, et al. A novel interference mechanism by a type IIIB CRISPR-Cmr module in Sulfolobus. *Mol Microbiol*. 2013;87(5):1088–1099.
23. Ormond KE, Mortlock DP, Scholes DT, et al. Human Germline Genome Editing. *Am J Hum Genet*. 2017;101(2):167–176.
24. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *J Clin Invest*. 2014; 124(10):4154-4161.
25. Yin H, Xue W, Chen S, et al. Genomeediting with Cas9 in adult mice corrects a disease mutation and phenotype. *Nat Biotechnol*. 2014;32:551–553.
26. Lin SR, Yang HC, Kuo YT, et al. The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. *Mol Ther Nucleic Acids* 2014;3:e186.
27. Puschnik AS, Majzoub K, Ooi YS, et al. A CRISPR toolbox to study virus-host interactions. *Nat Rev Microbiol*. 2017;15:351–364.
28. Kang H, Minder P, Park MA, et al. CC R5 disruption in induced pluripotent stem cells using CRISPR/Cas9 provides selective resistance of immune cells to CC R5-tropic HIV-1 virus. *Mol Ther Nucleic Acids*. 2015;4:e268.
29. Zhen S, Hua L, Liu YH, et al. Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. *Gene Ther*. 2015;22(5):404–412.
30. Im W, Moon J. Applications of CRISPR/Cas9 for gene editing in hereditary movement disorders. *J Mov Disord*. 2016;9:136–143.
31. Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: Prospects and challenges. *Nat Med*. 2015;21:121–131.
32. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. *Science*. 2010;327:167–170.
33. Mojica FJ, Ferrer C, Juez G, et al. Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferaxmediterranei and Haloferaxvolcanii and could be involved in replicon partitioning. *Mol Microbiol*. 1995;17:85–93.
34. Riehle MM, Bennett AF, Long AD. Genetic architecture of thermal adaptation in Escherichia coli. *Proc Natl Acad Sci USA*. 2001;98:525–530.
35. Ishino Y, Shinagawa H, Makino K, et al. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol*. 1987;169:5429–5433.
36. Mojica FJ, Diez-Villasenor C, Soria E, et al. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. *Mol Microbiol*. 2000;36:244–246.
37. Jansen R, Embden JD, Gaastra W, et al. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol.* 2002;43:1565–1575.