ORIGINAL RESEARCH PAPER

Oral Pathology

CRISPR (CLUSTERED REGULARLY INTERSPACED PALINDROMIC REPEATS)- ITS ROLE IN DENTISTRY: A NARRATIVE REVIEW

KEY WORDS: CRISPR, Genome editing, Palindrome, Gene mutations, Dentistry

Neeralya Nandhini. S — Intern, Adhiparasakthi dental college and hospital, Melmaruvathur

Kokila Sivakumar* — Assistant Professor, Department of Oral Pathology, Adhiparasakthi dental college and hospital, Melmaruvathur *Corresponding Author

Janani. I — Associate Professor, Department of Oral Pathology, Adhiparasakthi dental college and hospital

ABSTRACT

Background: There have been numerous genome editing technologies created. CRISPR/Cas9 is creating an outstanding upheaval in scientific research. Because CRISPR is more effective at editing than the current technologies, the field has undergone a revolution. Most significantly, using it is considerably easier and more adaptable. **Aim:** The aim of the article is to understand what is CRISPR, how it works, different systems of CRISPR and it's application in medical and dental field. **Conclusion:** A major development in human genetic engineering is CRISPR. CRISPR can locate and connect to any genomic sequence in a sample swiftly, producing results in under an hour. The avoidance and treatment of human diseases is the significant area of focus for CRISPR. By manipulating (causative) complicated bacterial populations or defective genes, CRISPR can treat the oral disorders. In this manner, CRISPR can achieve astounding human goals from diagnosis to treatment.

INTRODUCTION–

The entirety of an organism's genetic makeup is included in its genome. It is made up of the DNA's nucleotide sequence. It offers all the data necessary for the organism to grow and work. Scientists can alter an organism's DNA using a technique called genome editing or gene editing. They facilitates addition, deletion, or modification of genetic material in certain sites of the genome. The non-reproductive cells which are also known as the somatic cells, other than germ cells or the reproductive cells, are the only ones that undergoes the majority of the changes brought about by gene editingand they are not inherited from one generation to the following generation.[1]

There have been numerous genome editing technologies created. To target different sites in the genome re-designing or re-engineering of a new set of proteins is needed, even though the invention of artificially created mega nucleases, Zinc finger nucleases (ZFNs), and Transcription activator-like effector (TALENs) constantly enhanced the effectiveness of genome editing. Due to the challenges allied with replicating and protein engineering, ZFNs and TALENs were not broadly practiced by the scientific community.[3(fig 1)] CRISPR/Cas9 is creating an outstanding upheaval in scientific research.[2] Because CRISPR is more effective at editing than the current technologies, the field has undergone a revolution. Most significantly, using it is considerably easier and more adaptable.[4]

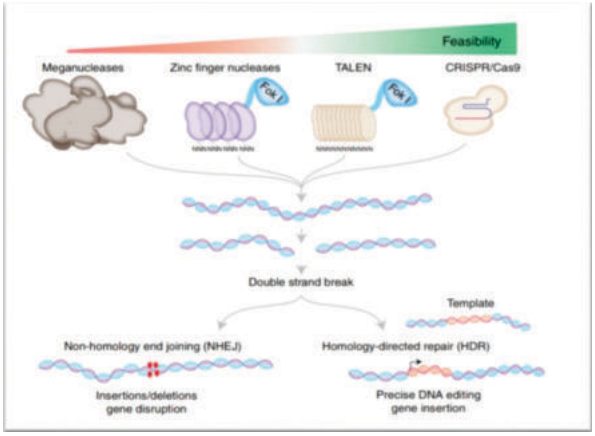

Fig 1. Gene-editing technologies

What is CRISPR?

Clustered Regularly Interspaced Short Palindromic Repeats is known as CRISPR. Like other species, bacteria have genetic material called DNA. Nucleotides are the four main building units that make up DNA. Nucleotide consists of a phosphate group, deoxyribose sugar and nitrogenous bases. The order of the four bases namely, Adenine (A), guanine (G), cytosine (C), and thymine (T) is crucial for the instructions needed to create the creature. The key sometimes creates a palindromic pattern that can be read both forward and backward. The word "madam," for instance, is a palindrome. Like the word "madam," a DNA palindrome can be TAGCGAT, which is readable both forward and backward. The TAGCGAT sequence, which is repeated numerous times in DNA, is known as a short palindromic "repeat" because of this. And it is referred to as "clustered" if these palindromic repeats are found in clusters throughout the DNA code. These palindromic repeats are referred to as "interspaced" because other DNA sequences isolate them.[5]

A type of significant genetic adaptive immune system is the CRISPR system, which can be discovered in the genomes of bacteria and archaea. The CRISPR sequence is a member of the DNA-specific sequence family. CRISPR loci are typically composed of short, highly conserved repeated sequences of 21–48 base pairs in length, parted by non-repeated gap sequences of 26–72 base pairs. With these intervals, CRISPR recognises the foreign DNA sequence.[8]

Because Cas-9 is a crucial protein utilised by the CRISPR system, it is included in the system.[6] The endonuclease protein that constitutes the CRISPR gene-editing system has a DNA-targeting specificity and slicing activity that can be regulated by a short guide RNA.[1]

How does CRISPR work?

There are three processes that make up the CRISPR system. Step 1: Acquisition of foreign DNA, use of the host's Cas protein to recognise foreign DNA, and insertion of protospacers, or small segments of bacterial DNA, into the host's CRISPR system. Step 2: CRISPR RNA (crRNA) biosynthesis, in which CRISPR DNA is translated into pre-crRNA, which is then converted by an endonuclease enzyme into a library of short crRNAs (crRNAs) that contain a complementary sequence to the foreign DNA. Stage 3: Target Interference: Mature crRNA instructs a specific Cas to bind to

a complementary target, interfering with the target sequence.[7(fig2)]

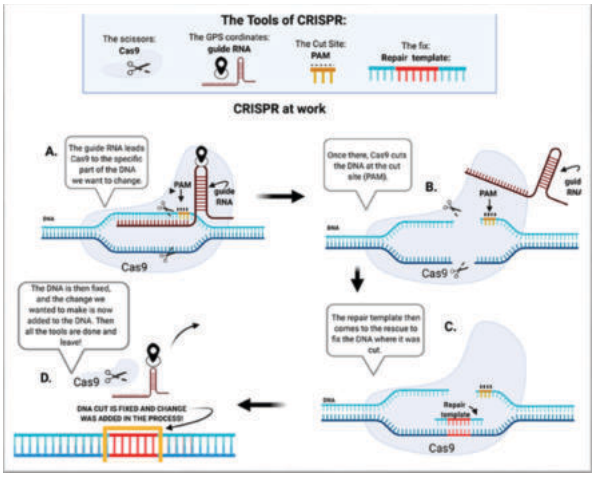

Fig 2. Mechanism of CRISPR

Different CRISPR systems –

The structure of CRISPR-associated (Cas) genes, which are usually observed next to the CRISPR arrays, is used to group these CRISPR systems.[9] The categorization work is still ongoing as scientists discover new systems and to upgrade the categorization scheme with subclasses, groups, and types depending on relative genomic analysis, structures, and biochemical activities of CRISPR components.

There are six distinct CRISPR/Cas systems; type I, type III, and type IV make up class one. The other three types II, V, and VI are comprised in the class two system. Since the most common Type II CRISPR/Cas needs only one Cas9 (Csn1/Cas5) and has a very simpler uncomplicated structure, CRISPR/Cas9 has anextensive potential applications.[7]

Potential Clinical Uses of CRISPR –

The avoidance and treatment of human diseases is the significant area of focus for CRISPR. Many laboratory uses it for the promptinvention of animal and cellular models, functional genomic assays, and instant cellular genome visualising.[10] Fewer studies have shown that CRISPR is used to restore impaired DNA in mice, treating them of genetic abnormalities, and it has been proposed that human embryos can bealtered as well.Gene therapy has further possible clinical applications like the treatment of infectious diseases like HIV, and the engineering of autologous patient material to treat cancer and other disorders.[2]

The potential use of CRISPR/Cas9 to treat genetic ailments caused by single gene mutations is one of the most intriguing applications. It includes haemoglobinopathies, Duchenne's muscular dystrophy, and cystic fibrosis.[2]

Hu et al, showed that the HIV-1 genomic activity may be targeted using the CRISPR/Cas9 system. This hindered the expression and duplication of the HIV gene in a range of cells that could cultivate a latent HIV infection. Moreover, cells might be given immunization against HIV-1 infection.[2]

The potential to alter patient-derived T-cells and stem/progenitor cells using CRISPR/Cas9 and subsequently reintroduce them into patients to treat disease has attracted growing interest. In addition to having the potential to cure solid tumours, primary immunological deficits, and autoimmune disorders, T-cell genome editing has already demonstrated efficacy in treating haematological malignancies.[11]

CRISPR in Dentistry –

Nowadays, genomic dentistry achieves two important goals: predictive and preventative.[12] It may take decades to develop and apply CRISPR therapies in the dental industry due to the controversial nature of the technology and the fact that it is a new one with little known about its possibilities.[13]

Through the use of CRISPR technology, the two most common dental disorders, tooth decay and periodontal disease, might be significantly reduced and even completely eliminated. The reduction in tooth caries and the emergence of periodontal disease that would follow from the development of a CRISPR therapy that prevents biofilm formation would be unprecedented.[14]

Dental Caries:-

Serbanescu et al. reported in 2005 that the Streptococcus mutans CRISPR system played a role in preventing the uptake and spread of the antibiotic resistance genes. This is related to dental caries. This discovery suggested a potential method for using S. Mutans' antibiotic resistance by concentrating on its CRISPR System.

Research have demonstrated that the CRISPR Cas RNA guided nucleases (RGN) technology can be utilised to create antimicrobials whose range of activity is specifically chosen. RGNs also allow for the selective suppression of specific strains based on genetic markers, which can modulate complicated bacterial populations.[15]

Periodontitis:-

As a screening method to find cellular pathways implicated in the aetiology of periodontitis, CRISPR Cas9 is beneficial for creating knockouts in vitro and in vivo. Genes implicated in the progression of periodontitis can also have their transcriptome and gene expression altered using alternative CRISPR systems as CRISPRa, CRISPRi, and Cas 13. While creating novel methods to lessen or get rid of periodontal infections, Cas3 might be employed to target the periodontal biofilm.[15]

Oral Cancer:-

To explore the mechanisms of tumour genesis and development, CRISPR Cas9 can be employed in the field of cancer research to modify genomes. Amazing achievements have been attained in recent years as the CRISPR Cas9 technology has been used more and more in cancer research and treatment.[16] Using CRISPR/Cas9 technology, Huang et al. 2017 examined the significance of the p75NTR in human tongue squamous carcinoma cells. This study shows that deletion of p75NTR suppresses various tumor-promoting features of SCC-9 cells, indicating that p75NTR is a viable target for the development of innovative treatment methods for tongue cancer. The CRISPR/Cas9 technology is very effective at identifying genes linked to the pathobiology of oral cancer and treating it by a procedure called gene knockout.[17]

Temporomandibular Disorders:-

Daya et al. discussed how the CRISPR Cas 9 method can target gene alteration to treat temporomandibular disorders. The new CRISPR Cas9 technology creates RNA-guided transcriptional activators and repressors that are specifically targeted to human genes known to be involved in pain pathways.[18]

Salivary Dysfunction:-

Water-specific protein aquaporin 1 (AQP1) gene expression was improved by Wang Z et al using the CRISPR Cas9 technology. AQPs are essential because they maintain water flow. At least three of the primary characteristics of cancer—angiogenicity, invasion, and metastasis—are fundamentally impacted by AQPs. Angiogenesis is induced by AQP1, while cellular invasion, proliferation, or migration are stimulated by AQP3. Cancer migration and proliferation are correlated with AQP5 expression, and AQP5 and AQP9

control chemotherapy resistance. Utilizing the CRISPR-Cas system we can enhance AQP1 transcription and protein levels using CMV promoter.[19]

Tooth Regeneration:-

CRISPR technology with new iPSC(induced pluripotent stem cells) means patient and disease-specific pluripotent stem cells that may be used in regenerative treatment comes for teeth or its components. iPSCs, which can be programmed to a pluripotent state and are produced from somatic cells, can develop into several cell lines similarly to embryonic stem cells. By producing cells with the relevant mutation, CRISPR-edited iPSCs can be used to study particular disease-causing mutations or to develop particular cell lines implicated in tooth regeneration.[20]

Craniofacial Abnormalities:-

CRISPR also aid in the treatment of craniofacial malformations by giving importance to the mutations that cause anomalies or by understanding the mechanisms of the diseases and regulating the genes connected to the abnormalities. The periodontal ligament, tooth pulp, and alveolar bone all include mesenchymal stem cells with the capacity for multi-lineage differentiation and immunosuppressive characteristics. Hence, CRISPR modified MSCs might be useful for treating craniofacial, periodontal, and oral abnormalities.[20(fig3)]

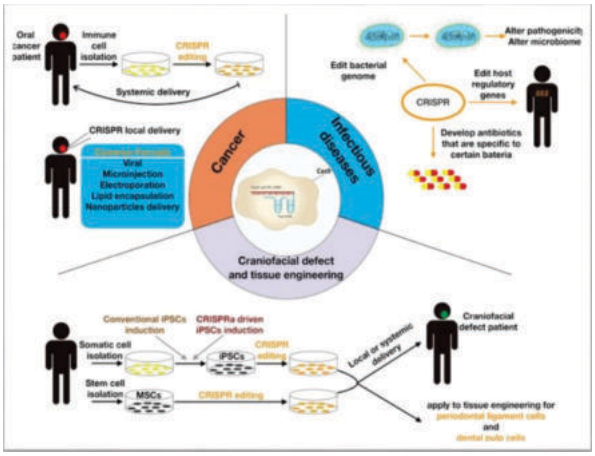

Fig 3. Applications in dentistry

Limitations –

The ethical issues raised by the potential for producing off-target mutations—which is the result of the Cas nuclease's non-specific activity in non-target areas of the genome—limit the technology's applicability in clinical settings at the moment. Gene editing in embryos is one of the most divisive CRISPR/Cas9-related topics.CRISPR/Cas9 technology can modify human embryos' genomes and this capacity of the technology may one day be employed to the preimplantation diagnosis and therapy of genetic illnesses. However the extended effects of any genetic change to the germ cells cannot be reversed.

Variability from patient to patient – Cas9 antibodies are found spontaneously in the serum of some donors, which might cause low editing efficiency and could trigger a catastrophic immunological storm in patients undergoing CRISPR-Cas9 treatment.

A CRISPR-Cas9 protocol's development can be difficult and time-consuming. ''Genetic drive'' largest worry of CRISPR – following editing or inclusion of the genes, these genome lies within cells which theoretically can be moved on to other species and passed on generation to generation.[21]

CONCLUSION –

A major development in human genetic engineering is CRISPR. China's CRISPR gene-editing research has advanced quickly in recent years, but there is still room for quality improvement.It helps dentists identify the organisms or defective genes that cause the numerous oral disorders. By manipulating (causative) complicated bacterial populations or defective genes, CRISPR can treat the oral disorders. CRISPR can locate and connect to any genomic sequence in a sample swiftly, producing results in under an hour. In this manner, CRISPR can achieve astounding human goals from diagnosis to treatment. Oral cancer is one of several diseases brought on by abnormalities in the genes that can be treated with precision using the CRISPR/Cas9 system. It is a DNA-free method suitable for both in vivo and in vitro experiments. This approach cures diseases from their underlying source, which is addressing genes in various ways, despite the fact that it requires optimization (effectiveness, safety, and specificity).

Financial Support-Nil

Conflicts Of Interest - Nil

REFERENCES:

1. Genomic Research- Reprinted from MedlinePlus Genetics U.S. National Library of Medicine National Institutes of Health, Department of Health & Human Services.
2. Redman M, King A, Watson C, King D. What is CRISPR/Cas9?. Archives of Disease in Childhood-Education and Practice. 2016 Aug 1;101(4):213-5.
3. Adli M. The CRISPR tool kit for genome editing and beyond. Nature communications. 2018 May 15;9(1):1911.
4. Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. Molecular microbiology. 2002 Mar;43(6):1565-75.
5. Marnik EA, Bautista C, Drangowska-Way A, Simopoulos CM, Merritt TJ. CRISPR: A New Way for Scientists to Edit DNA. Frontiers for Young Minds. 2021 Oct 28;9.
6. Zhang S, Guo F, Yan W, Dai Z, Dong W, Zhou J, Zhang W, Xin F, Jiang M. Recent advances of CRISPR/Cas9-based genetic engineering and transcriptional regulation in industrial biology. Frontiers in Bioengineering and Biotechnology. 2020 Jan 28;7:459.
7. Gao C, Wang R, Zhang L, Yue C. Visualization analysis of CRISPR gene-editing knowledge map based on CiteSpace. Biology Bulletin. 2021 Nov;48:705-20.
8. Heler R, Marraffini LA, Bikard D. Adapting to new threats: the generation of memory by CRISPR Cas immune systems. Molecular microbiology. 2014 Jul;93(1):1-9.
9. Haft DH, Selengut J, Mongodin EF, Nelson KE. A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS computational biology. 2005 Nov;1(6):e60.
10. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014 Jun 5;157(6):1262-78.
11. Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, Haliburton GE, Ye CJ, Bluestone JA, Doudna JA, Marson A. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proceedings of the National Academy of Sciences. 2015 Aug 18;112(33):10437-42.
12. Chavez-Granados PA, Manisekaran R, Acosta-Torres LS, Garcia-Contreras R. CRISPR/Cas gene-editing technology and its advances in dentistry. Biochimie. 2022 Mar 1;194:96-107.
13. Whitaker B. CRISPR: The Gene Editing Tool Revolutionizing Biomedical Research. 60 Minutes. 2018.
14. Gong T, Tang B, Zhou X, Zeng J, Lu M, Guo X, Peng X, Lei L, Gong B, Li Y. Genome editing in Streptococcus mutans through self targeting CRISPR arrays. Molecular oral microbiology. 2018 Dec;33(6):440-9.
15. Barbour A, Glogauer J, Grinfeld L, OstadsharifMemar R, Fine N, Tenenbaum H, Glogauer M. The role of CRISPR Cas in advancing precision periodontics. Journal of Periodontal Research. 2021 Jun;56(3):454-61.
16. Zhang H, Qin C, An C, Zheng X, Wen S, Chen W, Liu X, Lv Z, Yang P, Xu W, Gao W. Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Molecular Cancer. 2021 Dec;20:1-22.
17. Huang P, Tong D, Sun J, Li Q, Zhang F. Generation and characterization of a human oral squamous carcinoma cell line SCC-9 with CRISPR/Cas9-mediated deletion of the p75 neurotrophin receptor. Archives of Oral Biology. 2017 Oct 1;82:223-32.
18. Munzenmaier DH, Wilentz J, Cowley Jr AW. Genetic, epigenetic, and mechanistic studies of temporomandibular disorders and overlapping pain conditions.
19. D'Agostino C, Elkashty OA, Chivasso C, Perret J, Tran SD, Delporte C. Insight into salivary gland aquaporins. Cells. 2020 Jun 25;9(6):1547.
20. Bales LE. CRISPR-Cas in the Field of Dentistry: A Comprehensive Collection of the Potential Uses of CRISPR-Cas9 in Dental Health Care.
21. Jangam D, Talreja KM. Applications of Crispr technology in dentistry: a review. Eur J Biomed Pharmaceut Sci. 2020;7:314-20.