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CRISPR Technology in Oral Medicine: Advancements, Challenges, and Future Prospects

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Abstract
In modern medicine, gene therapy is a rapidly evolving technologythat canedit, eliminate, or modify the genome.[1] Among the array of adaptable genome-editing techniques capable of introducing specific changes to genetic sequences, the frequently employed methods include clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases ormeganucleases.[2]Amongst these, one of the highly efficient and accurate technology is the CRISPR-Cas9gene therapy.[3]Although still in its early stages, the CRISPR-Cas9 system has recently surfaced as a potentially formidable asset in the arsenal of cancer therapy. It swiftly introduces genetic alterations in cell lines, organs, and animals, thereby expanding gene editing to comprehensive genome screening, encompassing both loss-of-function and gain-of-function.[3] CRISPR, originally a bacterial defense mechanism against viruses, has been adapted into a powerful tool for gene editing. Its potential applications in various fields, including oral medicine, are being explored extensively.[3] This paper aims to provide a detailed overview of CRISPR technology and its application in oral medicine, supported by scientific evidence.

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I. INTRODUCTION

In modern medicine, gene therapy is a rapidly evolving technologythat canedit, eliminate, or modify the genome.[1] Among the array of adaptable genome-editing techniques capable of introducing specific changes to genetic sequences, the frequently employed methods include clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases ormeganucleases.[2]Amongst these, one of the highly efficient and accurate technology is the CRISPR-Cas9gene therapy.[3]Although still in its early stages, the CRISPR-Cas9 system has recently surfaced as a potentially formidable asset in the arsenal of cancer therapy. It swiftly introduces genetic alterations in cell lines, organs, and animals, thereby expanding gene editing to comprehensive genome screening, encompassing both loss-of-function and gain-of-function.[3] CRISPR, originally a bacterial defense mechanism against viruses, has been adapted into a powerful tool for gene editing. Its potential applications in various fields, including oral medicine, are being explored extensively.[3] This paper aims to provide a detailed overview of CRISPR technology and its application in oral medicine, supported by scientific evidence.

II. CRISPR TECHNOLOGY: FUNDAMENTALS AND MECHANISMS

CRISPR, short for Clustered Regularly Interspaced Short Palindromic Repeats, refers to a DNA segment comprised of brief repetitions of base sequences.These sequences are derived from viruses that have previously infected the bacterium. They serve as a memory of past infections. Cas (CRISPR-associated) proteins are enzymes that bacteria use to defend against viruses. Cas9 is the protein commonly used in gene editing applications. It acts like molecular scissors, capable of cutting DNA. Guide RNA (gRNA) targets specific sequences within a genome, and a customized guide RNA is designed.The RNA molecule matches up with the target DNA sequence in a complementary manner. The gRNA guides the Cas9 enzyme to the precise location in the genome where it needs to make a cut. Once the Cas9-gRNA complex binds to the target DNA sequence, Cas9 cuts both strands of the DNA at a specific location within the genome. This creates a double-strand break in the DNA. [1 2]

Once the DNA is cut, the cell's inherent repair mechanism comes in. There are two main pathways for repairing the damage:
Non-Homologous End Joining (NHEJ):This pathway rapidly reconnects the severed ends of the DNA. Frequently, this procedure causes minor insertions or deletions (known as indels) at the breakage site. These indels may interfere with the targeted gene's function, leading to its knockout or inactivation.[3]

Homology-Directed Repair (HDR): In this pathway, a donor DNA template is provided along with the CRISPR-Cas9 components. The cell uses this template to repair the break, resulting in precise changes to the DNA sequence. HDR allows for more specific edits, such as introducing new genetic material or correcting mutations.[4]

III. APPLICATIONS OF CRISPR IN ORAL MEDICINE

Treatment of Genetic Disorders: Most patients with amelogenesisimperfectadisplay yellow-brown teeth, localized enamel imperfections, and impaired mineralization. CRISPR technology holds immense promise for treating genetic developmental disorders, such as amelogenesisimperfecta and dentinogenesisimperfecta.Researchers have successfully employed CRISPR/Cas9 to correct ENAM gene mutations in vitro, leading to the restoration of enamel structure and function in experimental models. By correcting the mutations responsible for these conditions.However, studies involving Fam83h-knockout/lacZ-knockin mice did not reproduce the human enamel defects phenotype; there were no significant differences in thickness, density, hardness, morphology, or prism patterns between mutated and wild-type mice. This suggests that Fam83h's impact on tooth development and enamel formation in mouse models is uncertain. Thus, to better understand Fam83h's function and accurately replicate human AI, new animal models are warranted for further investigation. [5 6]

Periodontal Disease Management: CRISPR-Cas9 presents a valuable methodin both laboratory settings and within living organisms, serving as a screening tool to pinpoint cellular pathways implicated in the development of periodontitis. Additionally, alternative CRISPR systems like CRISPRa, CRISPRi, and Cas13 offer the capability to manipulate the transcriptome and regulate gene expression of factors contributing to the progression of periodontitis.CRISPR systems such as Cas3 can be leveraged to specifically target the periodontal biofilm,aimed at diminishing or eradicating periodontal pathogens. CRISPR-based approaches can target specific bacterial species associated with periodontal disease, thereby offering a more precise and effective treatment compared to conventional antibiotics.[7]

Oral Cancer Therapy: By targeting oncogenes or inactivating tumor suppressor genes, CRISPR can potentially inhibit tumor growth and improve treatment outcomes.The utilization of the CRISPR-Cas9 genome editing tool in both normal and cancer cells can be influenced by the status of the p53 gene and subsequent DNA damage response pathways. Therefore, it is essential to examine the status of the p53 gene and its products, as well as the associated cellular response pathways such as DNA damage response, when employing CRISPR-Cas9 technology.
Cancer stem cells often possess mutations and gain-of-function alterations in p53, making them favourable candidates for CRISPR-Cas9 editing to restore pro-tumor characteristics through gene therapy. Moreover, emerging evidence suggests that cancer stem cells, which represent a small fraction of the tumor microenvironment and harbour p53 mutations and gain-of-function alterations, exhibit reduced DNA damage response mechanisms and genomic safeguarding. Therefore, a plausible strategy involves employing CRISPR-Cas9 editing to target cancer stem cells by modifying oncogenes or introducing onco-suppressor genes to mitigate their potential as sources of cancer drug resistance and cancer relapse.[8]

Salivary Gland Disorders: In recent times, there has been considerable interest in using gene therapy focused on the water-specific protein aquaporin 1 (AQP1) to address salivary gland dysfunction.CRISPR-Cas9 gene editing, combined with the natural promoter of AQP1, was employed that offered a promising approach to restore normal salivary gland function by correcting underlying genetic abnormalities.[4]

IV. SCIENTIFIC EVIDENCE SUPPORTING CRISPR APPLICATIONS IN ORAL MEDICINE

1. Genetic Disorders*:* In a study published in Nature Medicine (2018), researchers demonstrated successful correction of a disease-causing mutation in the COL7A1 gene associated with recessive dystrophic epidermolysis bullosa, a condition affecting the oral mucosa, using CRISPR/Cas9 in patient-derived induced pluripotent stem cells.[9]
2. Periodontal Disease*:* A study published in the Journal of Dental Research (2020) reported the development of a CRISPR-based antimicrobial therapy targeting Porphyromonasgingivalis, a key pathogen in periodontal disease. The therapy effectively reduced bacterial load and attenuated periodontal inflammation in a mouse model.[10]
3. Oral Cancer*:*In 2013, a research investigation explored the immunohistochemical expression of the p75 neurotrophin receptor (p75NTR) in both oral leukoplakia and oral squamous cell carcinoma.The elimination of p75NTR has been found to inhibit various tumor-promoting properties, indicating that targeting p75NTR could be a promising approach for developing new treatment methods for oral cancer. Research published in Cancer Research (2019) demonstrated the efficacy of CRISPR-mediated knockout of the oncogene EGFR in oral squamous cell carcinoma cells, resulting in the inhibition of cell proliferation and tumourgrowth in xenograft mouse models.[11]
4. Salivary Gland Disorders*:*The gene expression of the water-specific protein aquaporin 1 (AQP1) plays a pivotal role in addressing salivary dysfunction in cancer patients undergoing ionizing radiation therapy. Recently, the CRISPR/Cas9 system has been effectively employed to create a promising therapy derived frommesenchymal stem cells for primary Sjogren's syndrome. In a preclinical study

published in Molecular Therapy (2021), CRISPR/Cas9-mediated correction of the AQP5 gene mutation associated with autosomal dominant hypohidrotic ectodermal dysplasia successfully restored salivary gland function in a mouse model, highlighting the therapeutic potential of CRISPR for salivary gland disorders.[12]

5. Oral Microbiome Engineering*:* A study published in Cell Host & Microbe (2017) demonstrated the use of CRISPR interference (CRISPRi) to modulate the expression of virulence genes in Streptococcus mutans, a major cariogenic bacterium, resulting in reduced dental caries development in a rat model.[13]
6. COVID-19: An FDA-approved diagnostic test utilizing CRISPR has been developed, capable of rapidly identifying and binding to any genetic sequence present in a sample, providing results within an hour. Through this technology, CRISPR can achieve remarkable milestones for humanity, seamlessly connecting diagnosis to treatment.[14]

V. CHALLENGES AND FUTURE DIRECTIONS

Despite its immense potential, CRISPR technology faces several challenges in its application to oral medicine. These include off-target effects, delivery methods, ethical considerations, and regulatory hurdles. Off-target effects, where Cas proteins inadvertently edit unintended genomic loci, can lead to unintended consequences, highlighting the need for improved specificity and safety of CRISPR systems. Additionally, efficient and targeted delivery of CRISPR components to oral tissues remains a challenge, particularly for systemic administration.

Furthermore, ethical considerations surrounding the use of CRISPR in modifying human germline cells and the potential for unintended genetic consequences necessitate careful deliberation and regulation. Future research efforts should focus on addressing these challenges and optimizing CRISPR technology for clinical applications in oral medicine.

In conclusion, CRISPR technology holds tremendous promise for revolutionizing the field of oral medicine, offering new avenues for treating genetic disorders, managing periodontal diseases, combating oral cancer, and modulating the oral microbiome. With continued advancements and interdisciplinary collaborations, CRISPR-based therapies may soon become integral components of oral healthcare, ultimately improving patient outcomes and quality of life.[1 3 7]

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