Original Article

CRISPR Technology- A New Horizon in Clinical Dentistry- A Review

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Abstract:

Clustered Regularly InterSpaced Palindromic Repeats is known as CRISPR. It is a protein that can be programmed to change, eliminate, or activate the genome. This cutting-edge technology offers a broad range of possible implementation and is poised to revolutionise oral healthcare in the years to come. The most widely utilised genome editing techniques include homing endonucleases, Transcription Activator-Like Effector Nucleases, Zinc-Finger Nucleases, and CRISPR- CRISPR-associated protein 9 (Cas9). These adaptable genome-editing tools can alter the genomes in ways that are sequence-specific. Because it is highly effective and accurate, the genome editing method CRISPR-Cas9 has drawn attention as a potent weapon in the fight against cancer. This method and its uses, particularly in dental field, are covered in this review.

Keywords: CRISPR-Cas 9 system, Oral cancer, Genome editing

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Introduction:

All cancers are brought on by alterations in the DNA sequence of cancer cell genomes.[1] Oral squamous cell carcinoma (OSCC) of the oral cavity is the most prevalent type of oral cancer.[2] Survival of individuals with OSCC has improved as a consequence of current improvements in surgical procedures and diagnostic precision. However, treatment failure for OSCC has the potential to be fatal because of its aggressive nature [3] hence, developing technology is necessary to treat cancer patients from underlying causes. The molecular biology technique of gene editing recognized as CRISPR (clustered regularly interspaced short palindromic repeats) enables the altering of the genomes of living organisms. By introducing the Cas9 nuclease complexes with a synthetic guide RNA (gRNA) into the cell, a specific area of the cell's genome can be cut. This makes it possible to change genes in vivo, either by removing others or by adding new ones (in living organisms). The method is regarded as being tremendously important in biotechnology and medicine since it makes it possible to easily, cheaply,

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and with exceptionally high precision modify genomes in vivo. In addition to managing illnesses and pests, it can be used to generate novel medications, food items, and genetically modified organisms. It has the potential to be employed to treat somatic mutation-related disorders including cancer as well as hereditary genetic diseases. CRISPR technology offers a number of potential dental applications besides the management of oral cancer, while its medical uses may be far off; this technology has the potential to permanently alter the dentistry field.

History:

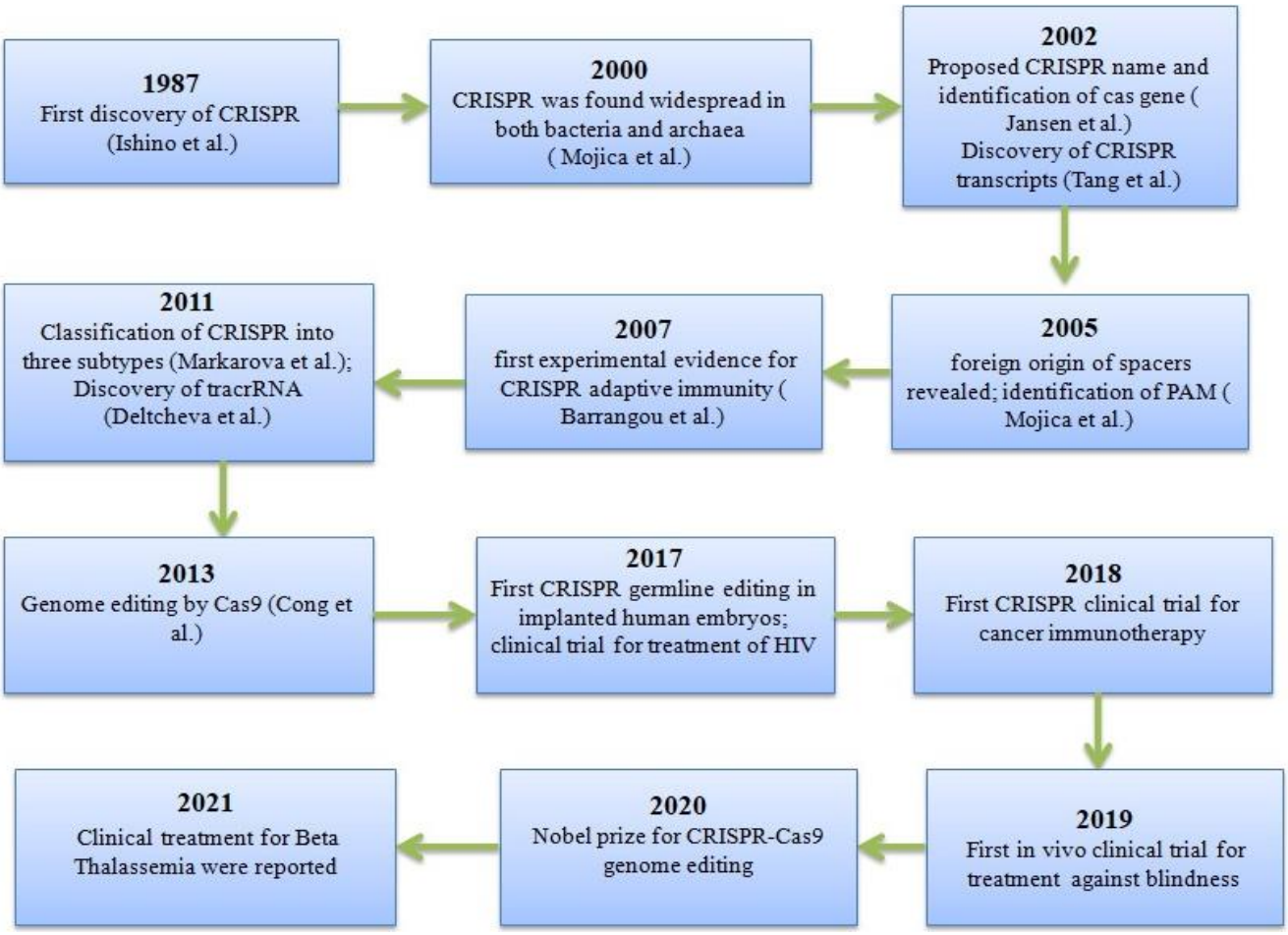

Figure 1: History of CRISPR in brief

Mechanism of CRISPR Genome Editing:

Three distinct CRISPR/Cas systems are available, according to the most widely used classification. [4] The most well-known type II CRISPR/Cas system has 3 parts:

1. Endonuclease (Cas9),
2. CRISPR RNA (crRNA),
3. Transactivating crRNA (tracrRNA).

The guide RNA is a duplex configuration made up of molecules crRNA and tracrRNA (gRNA). The option

to replace guide RNA with a synthetic fused chimeric single gRNA (sgRNA) makes it easier to use CRISPR/Cas9 for genome engineering. [5]

Brief DNA sequence is recognized as the "Protospacer-adjacent motif" (PAM), follows the sgRNA's distinctive twenty base-pair sequence is intended to be corresponding to the target DNA position. In the absence of PAM, Cas9 will be unable to unwind the target by separating the target DNA from its own genome. [6] When produced in the cell, the Cas9 nuclease and sgRNA combine to create a ribonucleoprotein (RNP) complex that is directed by sgRNA to a target DNA region. To create a double stand break (DSB), Cas9 neatly cuts the DNA, leaving blunt ends then sgRNA attaches to the target sequence using Watson - Crick Model. Homology-directed repair (HDR) or non-homologous ends joining (NHEJ) are two methods that eukaryotes can use to repair DSBs, based on the state of cell and the existence of a repair template. To accomplish precise repair, the HDR process combines a donor DNA template at the site of the DSB. NHEJ frequently leads in insertions/deletions of random base pairs interrupting the target sequence in the absence of a homologous repair template. [7] Based on the disorders we intend to treat, more gene editing is performed after DSBs are produced.

CRISPR-Cas9 Genome Editing's Use in Dentistry:

This system has drawn a lot of attention because to its many benefits in genome editing and researchers are progressively coming to see it as a viable therapeutic apparatus for treating disorders caused by genetic abnormalities.

Cancer:

Cancer poses serious threat to both human life and public health since it is a complicated disease that results from numerous genetic and epigenetic changes, [8] because it is so simple to utilise, CRISPR/Cas9 may cure malignancy by rising making of therapeutic immune cells, like by creating CAR-T cells, knocking down programmed cell death protein 1.[9] Most impressive application of CRISPR-Cas9 technology in cancer treatment is unquestionably the development of CAR-T cells. This normally has an outside single-chain variable segment that may accurately discriminate tumour antigens and an interior chimeric signaling domain that might activate T lymphocytes.[10] These genetically altered T lymphocytes expressing tumour-targeting receptors have successfully treated patients with a variety of haematological malignancies, including leukaemia and lymphomas.[11]

Negative immune system modulation requires PD-1 and programmed cell death ligands, particularly on T-cells. They dramatically raise the overall survival rate of cancer patients by attenuating the immunological response, which aids tumour cells in surviving by escaping the immune system.[12]

Oral Malignancy:

OSCC and oral leukoplakia (OL) were studied by Kiyosue et al. (2013) for immunohistochemistry expression of the p75 neurotrophin receptor (p75NTR). The study's conclusions indicated that the expression of p75NTR in populations of undifferentiated cells in OL and OSCC. Additionally, this research came to the conclusion that p75NTR may have a role in OSCC invasion and poor prognosis. [13] In OSCC cell lines, Six out of fourteen (43%) of the cell lines responded to the genome-scale CRISPR-Cas9 Knock-Out (GeCKO) libraries presented by Ludwig M et al. in 2017.[14] Using CRISPR/Cas9 technology, In 2017, Huang et al. investigated the significance of the p75NTR in human tongue SCCs. This study shows that deletion of p75NTR suppress various tumor-promoting features of SCC-9 cells, indicating that p75NTR is a viable target for the improvement of innovative treatment methods for tongue malignancy. [3]

This technology is very effective at identifying genes linked to the pathobiology of oral cancer and treating it by a procedure called gene knockout.

Dental Caries:

The main causative factor for dental caries in humans is streptococcus mutans. The oral cavity already has a normal bacterial flora, but as that flora multiplies, disease might start to develop. Therefore, we should be aware of how to control bacterial composition so that the oral ecology can maintain a healthy, dynamic balance. Dental caries eventually develops as a result

of the biofilm's dysbiosis, which is followed by alternate in the bacterial composition and, in particular, an increase of *S. mutans*. The tooth surface became demineralized as a result of this.[15] Researchers have created antimicrobials with a predetermined range of activity using RNA-guided nucleases (RGNs) CRISPR/Cas technology. RGNs also allow for the selective knockdown of specific strains based on genetic markers, which modifies complicated bacterial communities.[16]

Dental Plaque:

Role of Streptococccus Mutans:

Glucosyltransferases (Gtfs), a key component of Streptococcus mutans' pathogenicity, use sucrose to create extracellular polysaccharides (EPS), that result in the development of biofilmSelf-targeting CRISPR arrays with spacer sequences identifying with gtfB were created and cloned onto plasmids in research by Gong T et al. This plasmid was changed into UA159 to get the required mutations (self-targeting). EPS production was significantly reduced as a result, which in turn caused the disintegration of biofilm production.[17]

P. Gingivalis:

The main pathogen causing microbial dysbiosis has been identified as *Porphyromonas gingivalis*, a Gram-negative anaerobic rod. Nearly 95% of *P. gingivalis* clinical strains have been discovered to contain CRISPR arrays. This bacterial genetic immune system most likely plays a role in the control of the microbiome in chronic periodontitis. In order to avoid the development of dental plaque and ultimately to prevent periodontitis, dental clinics can benefit greatly from and be encouraged by the application of CRISPR technology. [18]

Salivary Dysfunction:

Previous research has shown that aquaporin 1 (AQP1) gene expression is crucial for the therapy of salivary dysfunction in cancer patients undergoing ionising radiation. By creating a gRNA sequence and a homology directed repair (HDR) template for the endogenous promoter of the cytomegalovirus (CMV), Wang Z et al. improved the expression of the AQP1 gene using the CRISPR/Cas9 system. He proposed that substituting endogenous promoter could potentially alleviate salivary gland dysfunction.[19]

Herpes Virus:

The prevalence of herpes in adult humans is almost universal (large DNA viruses). Herpes viruses encompass several significant human diseases that result in oral lesions. In current times, this system has been utilized to target and modify particular areas of the virus-infected cells' genomes. It has been possible to eradicate the viral genome from infected cells, restrict viral replication, or in some situations, inactivate the virus.[20]

In Chronic Pain:

Many dentists are concerned about chronic discomfort in a variety of orofacial illnesses. Numerous drugs, ranging from NSAIDs to opioids, temporarily relieve symptoms, but once the effect of the prescription wears off, the pain returns. It was discovered that some variations in this gene render the affected person incapable of feeling pain. The cause of this is a defective gene that inhibits the neural pathways ability to transmit pain signals by controlling certain chemicals implicated in this procedure that are present on the surface of neurons. CRISPR allows for the editing of epigenetic markers that activated this pathway.

CRISPR in COVID-19:

It is being developed by the Tata Group and the CSIR Institute of Genomics and IGIB. They discovered FELUDA Paper strip test for detection of COVID-19 infection (Figure 2).

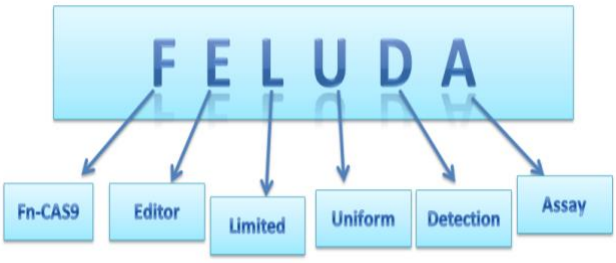

Figure 2: Detection of COVID-19 by FELUDA

This test is done by utilization of CRISPR-Cas9 genome editing technology.

In this technique Fn Cas9 protein will bind with guide RNA (gRNA) to identify the viral genes.

Conclusions:

Oral cancer is one of several diseases brought on by abnormalities in the genes that can be treated with precision using the CRISPR/Cas9. 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). It helps dentists identify the organisms or defective genes that cause the numerous oral disorders discussed in this article. By manipulating (causative) complicated bacterial populations or defective genes, CRISPR can treat these oral disorders. Even while research into treating oral disorders has begun, clinical applications of those uses are still in their infancy and have the potential to fundamentally alter dentistry. A CRISPR-based test for diagnosing the current pandemic COVID 19, which has spread around the globe, has recently received FDA approval. By this manner, CRISPR can achieve remarkable human goals from diagnosis to treatment.

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