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Dentistry

APPLICATIONS OF CRISPR TECHNOLOGY IN PEDIATRIC DENTISTRY: A REVIEW

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ABSTRACT The technology driven biologic advances can be called as Bio-technology This field of science has profoundly impacted human life in myriad ways. Recombinant DNA technologies encompass the chemical splicing (recombination) of different strands of DNA generally using either bacteria (such as Escherichia coli) or bacteriophages (viruses that infect bacteria, such as λ phage), or by direct microinjection. when virus(bacteriophage) invade bacterial cells in response to that a defence mechanism is developed. acronym "CRISPR," which stands for clustered regularly interspaced short palindromic repeats. Many scientists are in quest to utilize this natural prokaryotic adaptive immunity to be manipulated in eukaryotic cells. Currently many clinical trials are going on and promising applications of this gene therapy for cancer, hematologic diseases are known. The focus of this review is to gain insights into CRISPR technology and its diverse applications in Pediatric Dentistry.

KEYWORDS : CRISPR, Recombinant DNA technology, Prokaryotic Immunity, Pediatric Dentistry

INTRODUCTION

The technology driven biologic advances can be called as Bio-technology. The term was coined in last century by Hungarian engineer Karl Ereky.(Ledford & Callaway, 2020) This field of science has profoundly impacted human life in myriad ways. Genetic engineering consists of various techniques for the manipulation of genetic material (primarily DNA) to alter, repair, or enhance form or function. Recombinant DNA technologies encompass the chemical splicing (recombination) of different strands of DNA generally using either bacteria (such as Escherichia coli) or bacteriophages (viruses that infect bacteria, such as λ phage), or by direct microinjection.(Robert & Baylis, 2008). Such R-DNA technologies have been used in various fields such as agriculture, Medicine, formulations of various vaccines, gene therapies as well as molecular diagnostics, etc. Genetic modification carried out in a prokaryotic bacterial cell through bacteriophages is well described in literature; however, it is essential to understand that when virus(bacteriophage) invade bacterial cells in response to that a defense mechanism is developed. For bacteria, this mechanism is the host restriction/modification system (Aksan Kurnaz, n.d.). This is noteworthy because this observation has opened new dynamics in field of biotechnology.

The defense systems of prokaryotes can be classified into two broad groups that differ in their modes of action. The first group includes those defense systems that function on the self, non-self-discrimination principle, DNA is the target of the recognition; this mechanism is prokaryotic immunity. They use methylation to label the 'self' genomic DNA and identify and cleave any unmodified 'non-self' DNA. Another protective mechanism within this category is the DNA phosphorothioation system, referred to as the DND system, which marks DNA through phosphothiolation and eliminates DNA that lacks this modification. The R-M and DND systems represent the prokaryotic version of innate immunity. The second defense mechanism is by programmed cell death or dormancy induced by infection. Unlike R-M and DND systems, prokaryotic adaptive immunity is able to memorize the encounters with infectious agent and attack it specifically afterwards(Makarova et al., 2013). This was traced in 1987 by Japanese scientist Yoshizumi Ishino and his team in *E.*(Ishino et al., 1987) In the genome of bacteria, researchers observed some viral sequences. Characteristically, these sequences were positioned at regular intervals and at short distances to each other. Bacterial DNA in between these sequences exhibited palindromic repeating patterns. It was found that when needed, these DNA sequences can be transformed into guide RNA (gRNA) to cut invading viral DNA with the assistance of Cas enzyme, particularly if the same type of virus attempts to infect the bacterium again (DNA Technologies, n.d.) Initially perceived as components of the DNA repair system, the genes located in close proximity to CRISPR genes were later identified to be predominantly associated with CRISPR and were consequently termed CRISPR-associated (Cas) genes(Makarova, 2002; Nidhi et al., 2021) In 2005, Mojica proposed the hypothesis that these sequences constituted a microbial immune system. Collaborating with Ruud Jansen at Utrecht University in the Netherlands, Mojica coined the now-Nobel-prize-winning acronym "CRISPR," which stands for clustered regularly interspaced short palindromic repeats (Ledford & Callaway, 2020) Kira S. Makarova and colleagues conducted an updated analysis of the evolutionary relationships between CRISPR and Cas proteins (Makarova et al., 2011) fascinatingly such immune system is not found in eukaryotic cell. Many scientists are in quest to utilize this natural prokaryotic adaptive immunity to be manipulated in eukaryotic cells. Feng Zhang (Broad Institute, USA) was first to patent and attempt this gene editing. (Gostimskaya, 2022) Currently many clinical trials are going on and promising applications of this gene therapy for cancer, hematologic diseases are known. The focus of this review is to gain insights into CRISPR technology and its diverse applications in Pediatric Dentistry.

CRISPR Technology and Pediatric Dentistry

Tooth Development

Tooth development relies on epithelial cells migrating into mesenchymal tissue. The initial placement of these cells determines the location of primary and later secondary teeth. However, controlling genes for the cells' migration and location isn't clear. The CRISPR gene editing method offers a precise way to edit DNA during embryogenesis, potentially preventing abnormal tooth development. This could empower dentists to manage gene expression, preventing issues like ectopic odontogenesis and malocclusion. (Rossomando, 2021) Disorders like primary failure of eruption (PFE) affect tooth eruption and are challenging to diagnose and treat. PFE is linked to disruptions in dental follicle, periodontal ligament, and signaling pathways. Research in this area is crucial for understanding genetic control of tooth eruption and developing effective therapies for better dental health.

Cleft Lip And Cleft Palate

Cleft lip and cleft palate have a multifactorial etiology, comprising both genetic and environmental factors The etiology of CLP seems complex, with genetics playing a major role. Numerous genes associated with syndromic cleft lip and palate (CLP) have been identified. Three of them TBX22, PVRL1, and IRF6 are responsible for causing X-linked cleft palate, As the causes for the mistake Remain obscure, it is known that there is a strong genetic Components to CLP

Feng Zhang (Broad Institute, USA).

Dental Dysplasia

Dental dysplasia is a congenital anomaly observed in various conditions and birth defects. this dysplasia affects primary and permanent dentition, enamel, and dentin mineralization and include hypoplasia and hypomineralization and the imperfectas of both enamel and dentin. As is the case with all birth defects, diagnosis and treatment occur postnatally. There are a multitude of genes involved. For instance, these genes govern cellular differentiation, the synthesis and processing of extracellular matrix proteins, modulation of cellular functions during enamel formation, regulation of ion movement, and acidity regulation. Given the multitude of steps involved, there are numerous opportunities for errors to occur, but concurrently, there are also opportunities for CRISPR technology to intervene. Top of Form Mutations responsible for dentin dysplasia type II and Mutations in the DSPP gene and for those with dentinogenesis imperfecta types II and III have been Identified. Moreover, amelogenesis imperfecta can arise due to mutations in the AMELX, ENAM, MMP20, and FAM83H genes. This is because the AMELX, ENAM, and MMP20 genes play a crucial role in coding for proteins essential to tooth development. (Rossomando, 2021)

Caries And Periodontal Diseases

Caries and periodontal diseases, both linked to bacterial plaque, are prevalent infectious diseases in humans. Natural CRISPR loci are found in the majority of human oral microbiota(Rho et al., 2012). (Serbanescu et al., 2015) revealed that Streptococcus mutans' CRISPR system prevents the uptake and spread of antibiotic resistance genes, suggesting the potential to exploit S. mutans' antibiotic resistance by targeting its CRISPR system. Comparative analysis of CRISPR loci in dental plaque biofilm showed greater similarity in healthy individuals, indicating a robust bacterial community resistant to bacteriophages (Zhou et al., 2015). The CRISPR system may play a role in maintaining oral microbial community equilibrium, making it a potential target for disease control. Additionally, CRISPR could be employed to modify host regulatory genes to combat infectious diseases.

Oral Lesions

Herpes Virus: Nearly 100% of the adult human population carries herpes viruses, which are large DNA viruses. Herpesviruses encompass several significant human pathogens responsible for oral lesions, such as herpes simplex viruses (HSV) type 1 and 2 (causing gingivostomatitis, herpes labialis, and mucocutaneous ulcers), human cytomegalovirus (HCMV) (associated with infectious mononucleosis), and Epstein-Barr virus (EBV) (linked to conditions like hairy leukoplakia and mucocutaneous ulcers). Despite the use of antiviral drugs to treat these infections, complete clearance of viruses has not been achieved. Recently, the CRISPR/Cas9 system has been employed to target and modify specific regions within the genome of virus-infected cells. This represents a novel approach to addressing oral lesions caused by herpes viruses. Inactivation, inhibition of viral replication or in some cases eradication of viral genome from Infected cells has been achieved.

Haematological Diseases

CRISPR–Cas9 genome-editing tool can alter the DNA of bone-marrow stem cells, which has a potential treatment for certain blood diseases. CRISPR/Cas9 gene editing has been used to modify a subset of blood stem cells to reverse the clinical symptoms of sickle cell disease and beta thalassemia. Treatment of leukemia, lymphomas, multiple myeloma is in current clinical trials in hematology using the CRISPR/Cas9 system.

Limitation Of CRISPR Technology

A major concern for application CRISPR/Cas9 is the high frequency of off-target effects (OTEs), which have been observed at a frequency of ≥50% (Zhang et al., 2015). Efforts to address the concern of off-target effects (OTEs) in CRISPR/Cas9 gene editing involve the development of engineered Cas9 variants with reduced OTE and the optimization of guide designs. One strategy for minimizing OTEs involves the use of Cas9 nickase (Cas9n), a variant that induces single-stranded breaks (SSBs), in conjunction with a pair of single-guide RNAs (sgRNAs) targeting both strands of the DNA. the intended location to produce the DSB(Ran et al., 2013; Uddin et al., 2020).

Ethical Issues

Currently, there are no internationally agreed-upon laws or regulations on gene editing, leaving scientific research and application of CRISPR technology to the discretion of individual countries(Uddin et al., 2020). Indian protocols explicitly forbid human germline editing and reproductive cloning, as outlined in the National Guidelines for Stem Cell Research by the Indian Council of Medical Research. However, the concern lies in the fact that these guidelines have not yet been converted into specific laws (Uddin et al., 2020; Udwadia & Singh, 2019)

CONCLUSION

CRISPR cas9 system is a DNA free approach suited in both in vivo and vitro. Though it requires optimization (efficacy, safety and specialty) this technology treats disease from its root cause that is treating genes in various ways in pediatric dentistry. It has a role in identification of causative organisms or totally genre in various oral pathologies mentioned in this article.

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