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The Role Of Crispr-Cas 9 Gene Editing Technology In Dentistry: A Review

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I. Introduction

Since the creation of the central dogma of molecular biology, researchers have made great efforts to develop new technologies to edit, modify, or manipulate genomes.

Precise editing of genomic information is essential to understand the functions of a given gene and more importantly to introduce useful genes into genomes or replace deficient genes in genomes, termed gene therapy.

Genomes of eukaryotic organisms are composed of billions of DNA bases. The abilityto change these DNA bases at precisely predetermined locations holds tremendous value not only for molecular biology but also for medicine and biotechnology to be able to modify these DNA bases at specific predefined sites. Genome editing, also known as genetic engineering, has seen significant changes in the last ten years due tothe scientific discovery of a novel genetic modification technique called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its related nucleases.[1,2] This approach was developed to overcome and address various challenges in the earlier generation gene manipulation technologies. These earlier generations relied on synthesized transcription activators like effector nuclease (TALEN) and endonucleases like zinc finger endonuclease (ZFN) to function[3,4].

The third generation of CRISPR-Cas9 gene-editing technology targets the genomic sequence more precisely and accurately with little off-target effects compared to earlier gene-editing techniques. There are many possible uses for this technique in thetreatment of different genetic disorders[5–7] In recent times, genomic dentistry has served two primary purposes like preventive and predictive.[8] Due to the severity and morbidity associated with various disorders and developmental anomalies, researchers are now more focused on investigating gene activities on a genome-scale that will in turn help in the management of these conditions. Moreover, genome editing has substantial translational and therapeutic promise.[2] Various studies have been conducted in this regard about human genome variation for numerous oral and craniofacial conditions.[8] Hence this review focused on providing a comprehensive summary of major genome-editing nucleases, along with various aspects of the CRISPR/Cas9 system in dentistry.

II. Mechanism Of CRISPR–Cas9 Technology

The CRISPR-Cas9 pathway is Activated by a bacterium as a natural immune responseto a virus. When a virus infects a bacteria, a little section of its DNA gets incorporated as spacers in the bacterial genome's CRISPR locus. As a result, the bacteria develop an adaptive immunity against the virus. Subsequent infection with a similar virus activates the CRISPR locus to form pre-rRNA and tracrRNA. They facilitate the formation of guide RNAs (gRNAs). The guide RNAs (gRNAs) form a cleavage in the complementary segments of the attacking virus genome, using Cas9 [9, 10.]

Wound healing and tissue regeneration

Tissue and wound healing may be accelerated by gene therapy. CRISPR may open the door to new approaches to the repair and regeneration of skin wounds, muscles, cartilage, nerves, and cleft palates, among other birth defects.

Cell therapy and tissue engineering

CRISPR techniques can be used to generate or modify a patient's autologous cells totransplant or swap damaged tissue, encourage cell growth, or modulate immune function, for generating skin grafts with therapeutic potential.

Flap biology and grafts

To improve tolerance and avoid immune system rejection, vascularized complexallografts of the hand or face can be reprogrammed through the use of CRISPR gene editing techniques in addition to modifying tissue flaps.

Gingivitis and periodontitis

Porphyromonas gingivalis is a gram-negative anaerobic rod that causes chronic periodontitis. Ninety-five percent of these bacteria were found to carry CRISPR arrays. As a result, CRISPR technology appears to be a viable technique in dentalclinics for preventing plaque buildup, which leads to periodontitis [11.]

Salivary dysfunction

Cancer patients treated with ionizing radiation often suffer from xerostomia. Here, it is possible to increase the expression of the AQP1 gene by using the CRISPR Cas9 system. [11] Aquaporin 1 (AQP1), a water-specific protein, may promote salivation. The CRISPR/Cas9 system has recently been utilized to successfully target key genes in thetreatment of primary Sjogren's syndrome [12,13]

Genome Editing Techniques in the Oral and Craniofacial Field

There is limited literature available on the application of genome editing techniques in oral and craniofacial biology since it has emerged over a period of only a few years

1)Head And Neck Cancer

Cancer is a genetically complex disease that arises from a sequence of genetic and epigenetic alterations. therefore, cancer research benefits tremendously from the CRIPSR/Cas9 techniques. CRISPR/Cas9 has been used to change several genes in several head and neck cancer cell lines. Those attempts confirm the involvements of fibronectin and LDB1 for cancer cells and invasiveness[14] and identify novel therapeutic targets such as p75NTR and MUL1- HSPA5 axis (Huang et al. 2017; Kimet al. 2018). CRISPR may provide greater breakthroughs in treating head and neck cancer.

The ongoing clinical trials use CRISPR/Cas9-edited immune cells to fight against cancer cells, which could be applied to treat head and neck cancer. Lessons learned from treating monogenic diseases such as Duchenne muscular dystrophy by direct local injection also highlight the possibility of utilizing the same strategy to treat headand neck cancer.

Craniofacial Defects and Tissue Engineering

In vertebrates, the development of the craniofacial region entails the precise and tightly controlled patterning of tissues from the neural crest and all three germ layers.A failure in any of the precise spatiotemporal sequences of events leads to oral and craniofacial anomalies, such as cleft lip and palate[15]

The CRISPR technology has made significant improvements that can impact cells, particularly stem cells, within the tissue engineering triad.

Currently, two types of stem cells serve as research tools: one is pluripotent stem cellsfeaturing ESCs and induced pluripotent stem cells (iPSCs); the other is multipotent tissue-specific stem cells that reside in native tissues such as hematopoietic stem cells and mesenchymal stem cells (MSCs). Since they may quickly repopulate after editing,ESCs and iPSCs are perfect targets for CRISPR.[16]

Generations of mutant animals have been substantially aided by CRISPR-edited embryonic stem cells. Genetic modification of the embryonic tissues is particularly desirable since many craniofacial defects appear early in the embryonic phase.

However, there is controversy surrounding this issue. As a result, before clinicalapplications, extensive preclinical research is required.

Through the ectopic activation of the master transcription factors (e.g., OCT4, SOX2,KFL4, MYC) in somatic cells to activate the pluripotency network, CRISPR also plays a role in the generation of iPSCs.

The traditional transfection method used in iPSC induction showed a high level of cytotoxicity along with poor effectiveness. The CRISPR activation system has been proven to boost the endogenous OCT4 or SOX2 expression that is sufficient to triggerpluripotency reprogramming.[17]

When it comes to treatments for diseases of the oral cavity and cranium, MSCs have drawn more interest lately. There is proof that certain MSC subsets are present in the tooth pulp, periodontal ligament, and alveolar bone. MSCs with CRISPR/Cas9 editingmay prove to be a useful tool in the treatment of craniofacial,

periodontal, and oral abnormalities.

Applications of Genome Editing Techniques in the Oral InfectionsInfectious diseases

Dental caries are usually caused by Streptococcus mutans [18] They produce biofilm dysbiosis and imbalances in bacterial populace, which leads to tooth surface demineralization and the possibility of cavities. [19] Scientists used RNA-gated nucleases (RGN) CRISPR / Cas to produce antimicrobial drugs with pre-selected activity ranges. RGN also manipulates bacterial populations by knocking out specific strains depending on genetic fingerprints [20]

Caries and periodontal diseases are the most prevalent infectious diseases affecting humans. Both diseases are etiologically attributed to bacterial plaque. Naturally occurring CRISPR loci can be found in most human oral microbiota (Rho et al.2012)[21]

Gong Tao et al. in his in vitro study, highlighted the significance of CRISPR-Cas9 system elements in the defence against mobile genetic elements and created a novel technology that precisely and successfully edits the genome of S. mutans UA159 using self-targeting CRISPR arrays, reducing the synthesis of EPS and the formation of biofilms.[22]According to Serbanescu et al. (2015), the CRISPR system of Streptococcus mutans has an effect on stopping the absorption and spread of antibioticresistance genes. This discovery suggested that S. mutans's CRISPR system may be used to target its antibiotic resistance. When the CRISPR loci in the dental plaque biofilm of healthy and periodontitis patients were compared, it was found that the CRISPR components in the healthy patients were more similar to one another, forming a strong and functional bacterial community that could prevent bacteriophageinvasion(Zhou et al.2015).

Since the elucidation of CRISPR-Cas system bacterial adaptive immunity in *S. thermophilus* and programmable editing demonstrated using the *S. pyogenes*-derived SpyCas9, there has been a boom and boon of CRISPR-Cas research and applications thereof. The classification scheme of CRISPR-Cas systems has expanded substantially in the past decade, including the recent notable increase of class 2 systems, and new subtypes and variants are sure to be uncovered and characterized inthe near future[23].

CRISPR/Cas9 genome editing technology in herpes simplex virus

Since the discovery of the 29 nt tandem interval repeat sequences in E. coli. in 1987, the next-generation sequencing technology facilitated the discovery of the CRI host defence mechanism of bacteria and archaea against virus infection in 2005

Zhang et al. invented the CRISPR/Cas9 technology in 2013 based on the host defence mechanism, which made genome editing of mammalian cells efficient and convenient[24,25,26.] The CRISPR/Cas9 technology has reached high efficiency and specificity although there is still room to improve.

Soon after the invention, the CRISPR/Cas9 technology was successfully used in genome editing of HSVs in 2016. The efficiency and specificity of HSV genomeediting using the CRISPR/Cas9 technology were even higher than those of mammalian genome editing because HSV genomes are relatively smaller than mammalian genomes [27]

CRISPR/Cas9 cleavage rate was 80% or higher, and HDR was increased about 10,000–1,000,000 times than naturally occurring HDR, and the increased HDR efficiency was about 1–10% [27] Future studies would increase the HDR efficiency.

First, Lin et al. developed the automated screening of recombinant HSVs produced by HDR following CRISPR/Cas9 cleavage. The development of this ground-breaking idea would greatly speed up HSV research. By streamlining the experimental processes, future research could increase the effectiveness of producing recombinant HSVs.

Current studies have set good examples of how the CRISPR/Cas9 technology accelerated HSV research in basic biology, pathogen treatment, and gene therapy. As the CRISPR/Cas9 and flow cytometric technologies made gene editing efficient, accurate, and automated, we could give the perspective that their application would trigger great advances in the fields of HSV and other virus research.

Future studies on unknown functions of specific viral genes would be possible because almost every gene of HSVs can be targeted by gRNA-guided Cas9 [28-32]. Considering that the functions of >79 genes of HSVs and some non-coding sequenceshave not been fully understood, future studies would focus on these functional researches using the CRISPR/Cas9 technology.

We believe that the CRISPR/Cas9 technology would potentially be worthwhile for clinical trials of HSV treatment in the future since it completely suppressed HSV replication by using two sets of gRNAs [39]. The novel technology invented by Lin etal. will become a revolutionary way to create novel oncolytic HSVs to kill tumors more effectively [38]

The role of CRISPR-Cas in advancing precision periodontics

Currently, the utility of CRISPR–Cas application in clinical settings is limited. CRISPR–Cas9 can be a

useful tool to create knockouts (CRISPR KO) in vitro and in animal models as a screening tool to identify cellular pathways involved in the pathogenesis of periodontitis, alternative CRISPR systems such as CRISPRa, CRISPRi, and Cas13 can be used to modify the transcriptome and gene expression of certain genes involved in periodontitis progression without altering the genome sequence, and CRISPR systems such as Cas3 can be used to target the periodontal biofilm and to develop new strategies to reduce or eliminate periodontal pathogens.[33]

Application of CRISPR-Cas in HPV-driven oral squamous carcinoma tumorgrowth

The current standard treatments for oropharyngeal cancer (OSC) consist of radiotherapy or chemoradiotherapy [34]. It is well known that human papillomavirus positive (HPVþ) cancers present better prognosis than HPV-negative (HPV-) cancers

[35]. However, the lines of treatment for both HPVþ and HPV- OSCs have not changed much over time and have remained limited [36]. Finding a novel therapeutic approach that improves the quality of life of patients undergoing current standard treatment regimens is crucial.

Danyelle Assis Ferreira, Nigel A.J. McMillan, and Adi Idris in their in vivo study using an inducible CRISPR platform have successfully regressed HPVþ OSC tumoursby deleting E7.[37]

Application of genome editing in oral potentially malignant disorders (OPMD)

Tumors develop through a succession of changes in cellular genomes and epigenomes that confer growth advantages to certain cell populations.

This ultimately causes either an increase in cell division or a suppression of cell death,which results in the growth of those cells that display these genotypes and phenotypes. Each of the clonal expansions generates cell populations with increased neoplastic phenotypes until finally, a primary tumor develops[38]. Some argue that these changes occur in the stem cell population of a tissue [39] whilst others support the notion that the cells of origin are non-stem cell transit amplifying cells [40].

The molecular profile of head and neck cancer has been extensively documented by researchers and the information available about OSCC is frequently derived from the HNSCC data.[41,42-45]Notably there is no early intervention to prevent OSCC because OPMD is relatively little known about condition[46].Furthermore, surgical biopsy is the conventional method of monitoring OPMD, although it only provides a moment in time. An understanding of the temporal events leading up to malignant transformation may be more important for clinical management.

The clinical management of OPMD is extremely difficult. S.S. Prime et al In their review, examined what is already known about some of the genetic changes associated with OPMD and made recommendations for change based on these findings. S.S.Prime proposed that OPMD can be monitored more dynamically and at a molecular level using liquid biopsies and suggested an appropriate biomarker of transformation in their context. The molecular profile of OPMD, however, can also beused to identify tractable gene targets and, in the short term, re-purposed existing drugs may be useful in the development of new treatment options. they highlighted recent work that identifies the interactions between genes associated with common driver mutations and, also, new advances in the identification of survival genes using CRISPR-Cas9 technology, both of which have translational implications. Further, we propose that digital technology has the potential to create novel datasets that will facilitate the use of machine learning and artificial intelligence to improve clinical outcomes.[47] Oral cancer is a global health problem that warrants a preventative strategy. It has been suggested that the management of the early premalignant stage should be central to the prevention of this debilitating malignancy. It has been suggested that the use of re-purposed drugs and/or new drug development should be based on what is already known about the molecular landscape of OPMD. The prevention of malignant transformation in this way is likely to have therapeutic benefit, this approach could also be a paradigm for the management of other aerodigestive tract cancers. It is an exciting challenge but eminently achievable.

Limitations of CRISPR technology

The safety and effectiveness of CRISPR-Cas9 technology still require the validation of extensive research therefore there is a scope for more research in this field. Further,the CRISPRCas9 methodology can be complicated and time-consuming to develop.

Selecting a target location and creating a sgRNA are challenging procedures. The universality of CRISPR-Cas9 technology is not high.[48] Unexpected results can be obtained in the experimental treatment of critically ill patients. The greatest fear of CRISPR is that the modified gene may be passed down from generation to generation 49

III. Conclusions

By modulating or completely removing defective genes CRISPR technology treats oral diseases. While this technology appears to be life-saving, related research is not sufficient to truly consider CRISPR Cas9

technology for oral diseases as a treatment. Changes in the human genetic code can have eternal and random consequences for future generations. Consequently, for their application in dentistry, evidence-based research is required.

References

[1] Naldini L. Gene Therapy Returns To Centre Stage. Nature. 2015;526:351–60

[2] Yu N, Yang J, Mishina Y, Giannobile Wv. Genome Editing: A New Horizon For Oral And Craniofacial Research. J Dent Res 2019;98(1):36-45.

[3] Doudna Ja, Charpentier E. Genome Editing. The New Frontier Of GenomeEngineering With Crisprcas9. Sci 2014;346:1258096.

[4] Hryhorowicz M, Lipianki D, Zeyland J, Slomski R. Crispr/Cas9 Immune System As A Tool For Genome Engineering. Arch Immunol Ther Ex 2017;65(3):233-240.

[5] Beaudet Al, Meng L. Gene-Targeting Pharmaceuticals For Single-Gene Disorders.Hum Mol Genet 2016;25(R1):R18-R26.

[6] Morishige S, Mizuno S, Ozawa H, Nakamura T, Mazahery A, Nomura K, Et Al. Crispr/Cas9- Mediated Gene Correction In Hemophilia B Patientderived Ipscs. Int JHematol 2020;111(2):225-233.

[7] Schwank G, Koo Bk, Sasselli V, Dekkers Jf, Heo I, Demircan T, Et Al. Functional Repair Of Cftr By Crispr/Cas9 In Intestinal Stem Cell Organoids Of Cystic Fibrosis Patients. Cell Stem Cell 2013;13(6):653-658

[8] Chavez-Granados Pa, Manisekaran R, Acostatorres Ls, Garcia-Contreras R.Crispr/Cas Geneediting Technology And Its Advances In Dentistry. Biochimie 2022;194:96-107.

[9] Xia Al, He Q, Wang Jc, Et Al. Applications And Advances Of Crisprcas9 In CancerImmunotherapy. Journal Of Medical Genetics 2019;56:4-9.

[10] Wang D, Wang Xw, Peng Xc, Xiang Y, Song Sb, Wang Yy, Et Al. Crispr/Cas9 Genome Editing Technology Significantly Accelerated Herpes Simplex Virus Research. Cancer Gene Therapy 2018;25:93-105.

[11] Chen T, Olsen I. Porphyromonas Gingivalis And Its Crispr-Cas System. Journal OfOral Microbiology 2019; 11(1): 1638196

[12] Wang Z, Wang Y, Wang S, Zhang Lr, Zhang N, Cheng Z, Et Al. Crispr-Cas9 Hdr System Enhances Aqp1 Gene Expression. Oncotarget 2017 4;8(67):111683- 111696.

[13] Chen W, Yu Y, Ma J, Olsen N, Lin J. Mesenchymal Stem Cells In Primary Sjogren"S Syndrome: Prospective And Challenges. Stem Cells International 2018; 2018: 4357865.

[14] Ryu S, Yoon Sh, Song J, Choi Y, Lee S, Baek M, Lee Hb, Jeon Sy, Jon S, Lee D,Kim Hs. Impact Of Media Compositions And Culture Systems On The Immunophenotypes Of Patient-Derived Breast Cancer Cells. Bmc Cancer. 2023 Sep 6;23(1):831.

[15] Dixon Mj, Marazita Ml, Beaty Th, Murray Jc. Cleft Lip And Palate: Understanding Genetic And Environmental Influences. Nature Reviews Genetics. 2011Mar;12(3):167-78.

[16] Zhao Mt, Chen H, Liu Q, Shao Ny, Sayed N, Wo Ht, Zhang Jz, Ong Sg, Liu C, Kim Y, Yang H. Molecular And Functional Resemblance Of Differentiated Cells DerivedFrom Isogenic Human Ipscs And Scnt-Derived Escs. Proceedings Of The National Academy Of Sciences. 2017 Dec 26;114(52):E11111-20.

[17] Knijnenburg Ta, Wang L, Zimmermann Mt, Chambwe N, Gao Gf, Cherniack Ad, Fan H, Shen H, Way Gp, Greene Cs, Liu Y. Genomic And Molecular Landscape Of Dna Damage Repair Deficiency Across The Cancer Genome Atlas. Cell Reports. 2018 Apr3;23(1):239-54.

[18] Gao L, Xu Ts, Huang G, Jiang S, Gu Y, Chen F. Oral Microbiomes: More And More Importance In Oral Cavity And Whole Body. Protein & Cell 2018; 9(5): 488-500.

[19] He X, Lux R, Kuramitsu Hk, Anderson Mh, Shi W. Achieving Probiotic Effects Via Modulating Oral Microbial Ecology. Advances In Dental Research 2009; 21(1): 53-56.

[20] Citorik Rj, Mimee M, Lu Tk. Sequence-Specific Antimicrobials Using Efficiently Delivered Rna-Guided Nucleases. Nature Biotechnology 2014; 32(11):1141-1145.

[21] Rho M, Wu Yw, Tang H, Doak Tg, Ye Y. Diverse Crisprs Evolving In Human Microbiomes. Plos Genetics. 2012 Jun 13;8(6):E1002441.

[22] 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.

[23] Roberts A, Barrangou R. Applications Of Crispr-Cas Systems In Lactic Acid Bacteria. Fems Microbiology Reviews. 2020 Sep 1;44(5):523-37.

[24] C.R. Pickering, J. Zhang, S.Y. Yoo, L. Bengtsson, S. Moorthy, D.M. Neskey, M. Zhao, M.V. Ortega Alves, Changk, J. Drummond, E. Cortez, T.-X. Xie, D. Zhang, W.Chung, J.-P.J. Issa, P.A. Zweidler-Mckay, X. Wu, A.K. El-Naggar, J. N. Weinstein, J. Wang, D.M. Muzney, R.A. Gibbs, D.A. Wheeler, J.N. Myers, M. J. Frederick, Integrative Genomic Characterization Of Oral Squamous Cell Carcinoma Identifies Frequent Somatic Drivers, Canc. Discov. 3 (2013) 770–781.

[25] Cancer Genome Atlas, Comprehensive Genomic Characterization Of Head And Neck Squamous Cell Carcinomas, Nature 517 (2015) 576–582.

[26] C.R. Leemans, P.J.F. Snijders, R.H. Brakenhoff, The Molecular Landscaoe Of Head And Neck Cancer, Nat. Rev. Canc. 18 (2018) 269–282.

[27] A.W.Y. Chai, K.P. Lim, S.C. Cheong, Translational Genomics And Recent Advances In Oral Squamous Cell Carcinoma, Semin. Canc. Biol. 61 (2020) 71–83.

[28] Lin C, Li H, Hao M, Xiong D, Luo Y, Huang C, Et Al. Increasing The Efficiency Of Crispr/Cas9-Mediated Precise Genome Editing Of Hsv-1 Virus In Human Cells. Sci Rep. 2016;6:34531

[29] Nelles Da, Fang My, O'connell Mr, Xu Jl, Markmiller Sj, Doudna Ja, Et Al. Programmable Rna Tracking In Live Cells With Crispr/Cas9. Cell. 2016;165:488–96.

[30] Bi Y, Sun L, Gao D, Ding C, Li Z, Li Y, Et Al. High-Efficiency Targeted Editing Of Large Viral Genomes By Rna-Guided Nucleases. Plos Pathog. 2014;10:E1004090.

[31] Suenaga T, Kohyama M, Hirayasu K, Arase H. Engineering Large Viral DnaGenomes Using The Crispr-Cas9 System. Microbiol Immunol. 2014;58:513–22.

[32] Komor Ac, Kim Yb, Packer Ms, Zuris Ja, Liu Dr. Programmable Editing Of A Target Base In Genomic Dna Without Doublestranded Dna Cleavage. Nature. 2016;533:420–4.

[33] Barbour A, Glogauer J, Grinfeld L, Ostadsharif Memar R, Fine N, Tenenbaum H, Glogauer M. The Role Of Crispr-Cas In Advancing Precision Periodontics. Journal OfPeriodontal Research. 2021 Jun;56(3):454-61.

[34] A. Hay, I. Ganly, Targeted Therapy In Oropharyngeal Squamous Cell Carcinoma: The Implications Of Hpv For Therapy, Rare Cancers Ther. 3 (1) (2015) 89–117.

[35] F.K. Keane, Y.H. Chen, B.A. Neville, R.B. Tishler, J.D. Schoenfeld, P.J. Catalano, Et Al., Changing Prognostic Significance Of Tumor Stage And Nodal Stage In Patients With Squamous Cell Carcinoma Of The Oropharynx In The Human Papillomavirus Era, Cancer 121 (15) (2015) 2594–2602.
[36] M.R. Posner, J.H. Lorch, O. Goloubeva, M. Tan, L.M. Schumaker, N.J. Sarlis, Et Al., Survival And Human Papillomavirus In Oropharynx Cancer In Tax 324: A Subset Analysis From An International Phase Iii Trial, Ann. Oncol. 22 (5) (2011) 1071–1077.
[37] Ferreira Da, Mcmillan Na, Idris A. Genetic Deletion Of Hpv E7 Oncogene Effectively Regresses Hpv Driven Oral Squamous Carcinoma Tumour Growth. Biomedicine & Pharmacotherapy. 2022 Nov 1;155:113782.
[38] P.C. Nowell, The Clonal Evolution Of Tumor Cell Populations, Science 194 (1976)23–28.
[39] J.E. Visvader, Cells Of Origin In Cancer, Nature 469 (2011) 314–322
[40] C.L. Chaffer, R.A. Weinberg, How Does Multistep Tumorigenesis Really Proceed?Canc. Discov. 5 (2015) 22–24.
[41] C.R. Pickering, J. Zhang, S.Y. Yoo, L. Bengtsson, S. Moorthy, D.M. Neskey, M. Zhao, M.V. Ortega Alves, Changk, J. Drummond, E. Cortez, T.-X. Xie, D. Zhang, W.Chung, J.-P.J. Issa, P.A. Zweidler-Mckay, X. Wu, A.K. El-Naggar, J. N. Weinstein, J. Wang, D.M. Muzney, R.A. Gibbs, D.A. Wheeler, J.N. Myers, M. J. Frederick, Integrative Genomic Characterization Of Oral Squamous Cell Carcinoma Identifies Frequent Somatic Drivers, Canc. Discov. 3 (2013) 770–781.
[42] Cancer Genome Atlas, Comprehensive Genomic Characterization Of Head And Neck Squamous Cell Carcinomas, Nature 517 (2015) 576–582.
[43] C.R. Leemans, P.J.F. Snijders, R.H. Brakenhoff, The Molecular Landscaoe Of Head And Neck Cancer, Nat. Rev. Canc. 18 (2018) 269–282.
[44] A.W.Y. Chai, K.P. Lim, S.C. Cheong, Translational Genomics And Recent Advances In Oral Squamous Cell Carcinoma, Semin. Canc. Biol. 61 (2020) 71–83.
[45] A.W.Y. Chai, P.S. Yee, S. Price, S.M. Yee, H.M. Le3e, V.K.H. Tiong, E. Goncalves, F.M. Behan, J. Bateson, J. Gilbert, A.C. Tan, U. Mcdermott, M.J. Garnett,
[46] S. C. Cheong, Genome-Wide Crispr Screens Of Oral Squamous Cell Carcinoma Reveal Fitness Genes In The Hippo Pathway, Elife 9 (2020), E57761, Https://Doi.Org/ 10.7554/Elife.57761.
[47] C.S. Farah, Molecular, Genomic And Mutational Landscape Of Oral Leukoplakia,Oral Dis. 27 (2021) 803–812.
[48] Prime Ss, Cirillo N, Cheong Sc, Prime Ms, Parkinson Ek. Targeting The Genetic Landscape Of Oral Potentially Malignant Disorders Has The Potential As A Preventative Strategy In Oral Cancer. Cancer Letters. 2021 Oct 10;518:102-14.
[49] You L, Tong R, Li M, Liu Y, Xue J, Lu Y. Advancements And Obstacles Of Crispr-Cas9 Technology In Translational Research. Molecular Therapy – Methods &Clinical Development 2019; 13: 359–370
[50] Foulkes Al, Soda T, Farrell M, Giustirodríguez P, Lázaro-Muñoz G. Legal And Ethical Implications Of Crispr Applications In Psychiatry. North Carolina Law Review 2019; 97(5): 1359–1398.