REVIEW ARTICLE

Cytogenetics in Oral Cancer: A Comprehensive Update

Sowmya S Venkatesiah[1], Dominic Augustine[2], Vanishri C Haragannavar[3], Elham A Khudhayr[4], Marwah H Matari[5], Wahba A Elagi[6], Neethi Gujjar[7], Shankargouda Patil[8]

Abstract

Aim: To evaluate the application of cytogenetic techniques in determining the diagnosis, prognosis, and therapeutics in oral cancer.

Background: Genetic aberrations that play an important role in oral oncogenesis demand for substantial research for in-depth characterization of the tumor. Cytogenetic techniques have the potential to detect these aberrations. This review highlights about various cytogenetic approaches in cancer and how the cytogenetic findings support its application in the field of oral oncology.

Methods: Google scholar search was done for articles on cancer cytogenetics, and in particular, PubMed database was queried for articles published from 2015 to 2020 using keywords cytogenetics, chromosomal aberrations, conventional cytogenetics, karyotyping, banding techniques, molecular cytogenetics, fluorescent *in situ* hybridization, spectral karyotyping, comparative genomic hybridization, multiplex ligation probe analysis, and next-generation sequencing (NGS) in oral cancer. Abstracts were reviewed, and relevant full text was accessed to extract the cytogenetic findings in oral cancer.

Results: Data regarding various cytogenetic approaches from conventional to molecular techniques have been published in oral cancer. They convey a highly complex cytogenetic finding from gross chromosomal aberrations to specific gene mutations in oral cancer.

Conclusion: Crucial information in the development and progression of oral cancer is achieved through cytogenetic findings in particular with the molecular cytogenetic techniques. Novel technologies like NGS have emerged in recent years that hold promise in the detection of these alterations more efficiently.

Clinical significance: An appraisal of cytogenetic analysis in oral cancer helps to determine the diagnosis and the most important prognosticators. It assists in building targeted therapies for patient benefit.

Keywords: Conventional cytogenetics, Fluorescent *in situ* hybridization, Microarray techniques, Molecular cytogenetics, Next-generation sequencing, Oral cancer.

The Journal of Contemporary Dental Practice (2021): 10.5005/jp-journals-10024-3223

Introduction

Cancers are a result of genetic variations and the chromosomal aberrations are considered as a key feature of oncogenesis.[1] Any irregularity or abnormality of chromosome distribution, number, structure, or arrangement is referred as chromosomal aberration.[2] They can be numerical (aneuploidy) or structural aberrations (translocations, deletions, etc).[3] Chromosomal aberrations lead to the amplification or deletion of genes and are commonly observed in tumors.[4,5] These genetic changes may appear way prior than the actual clinical manifestation and can serve as prognostic biomarkers. Therefore, knowledge of genetic changes and chromosomal instability is of high significance as it aids in better understanding of disease etiology.[6] It is also a modern era of precision medicine; hence, in-depth characterization of the tumors adds value in cancer therapeutics. Cytogenetics and cytogenomic technologies have the potential to detect the aberrations in the cancer cells.[7] Morphology, structure, pathology, function, and behavioral study of chromosomes during somatic cell division (mitosis) and germ cell division (meiosis) and their influence on phenotype is known as cytogenetics.[8,9] Cytogenetic techniques are mainly categorized into conventional (karyotyping) and molecular cytogenetics.[7,10] The history of cytogenetics goes back to the era of the 1840s where Nageli first described transitory cytoblasts, Waldeyer coined the term "chromosome,"[9] and Mendel's laws explained the behavior of chromosomes in germ cells.[11] These earlier genetic studies were mostly confined to plant and animal species. The emergence of human cytogenetics began late in the 1950s with the discovery of exact number of human chromosome (46 chromosomes) by Tijo and Levan.[12] Based on the technological evolution, modern human cytogenetics are classified into three eras.[13–15] Flowchart 1 represents different eras of human cytogenetics. At present, cytogenetics is widely employed in genetic testing and counseling, prenatal diagnosis, genotoxicity studies, hematopoietic disorders, and in the field of oncology.[16,17] Crucial diagnostic and prognostic information of specific abnormalities associated with cancer can be envisioned through cytogenetic findings, more precisely by molecular cytogenetics.[17,18] The present narrative review emphasizes the application of cytogenetics in the field of oral cancer. The main aim of this review is (1) to elucidate the methodological aspects of cytogenetics in cancer, (2) to gather data and provide information

[1–3,7]Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India

[4–6]Jazan University, Jazan, Saudi Arabia

[8]Department of Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia

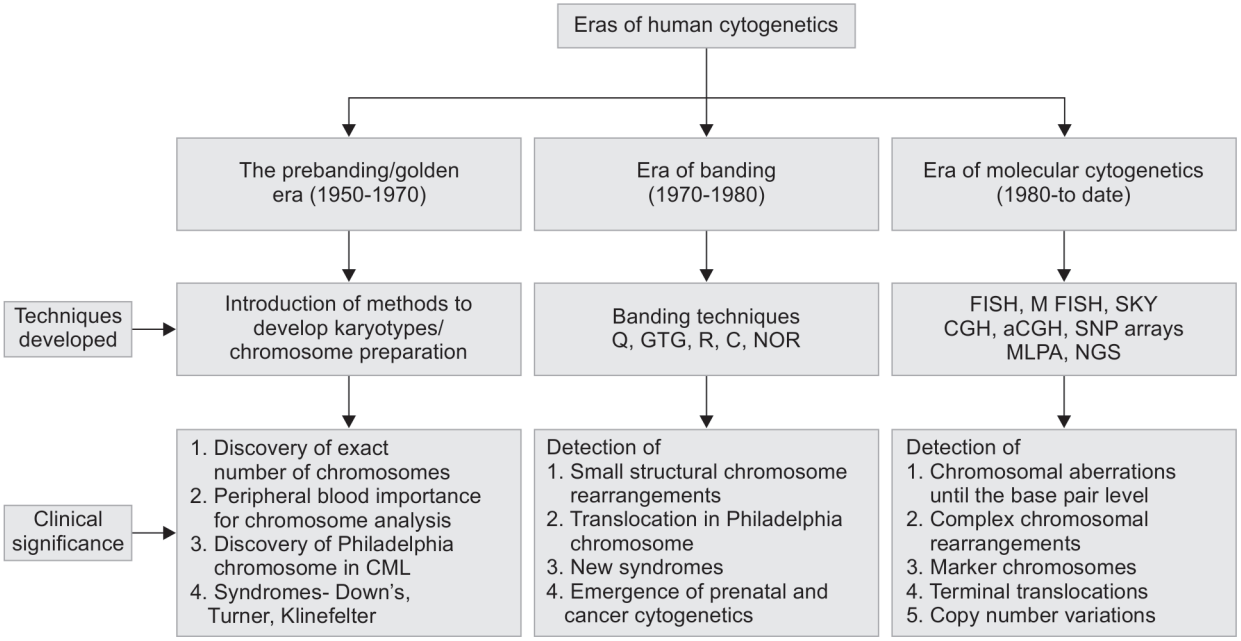
Corresponding Author: Shankargouda Patil, Department of Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia, Phone: +966507633755, e-mail: dr.ravipatil@gmail.com

How to cite this article: Venkatesiah SS, Augustine D, Haragannavar VC, *et al*. Cytogenetics in Oral Cancer: A Comprehensive Update. J Contemp Dent Pract 2021;x(x):xx–xx.

Source of support: Nil

Conflict of interest: None

Flowchart 1: Summary of the eras in human cytogenetics



on cytogenetic alterations in oral cancer, and also (3) to evaluate how beneficiary is cytogenetics in determining the therapeutics and prognosis in oral cancer patients.

Methodological Approaches in Cancer Cytogenetics

Chromosomal abnormalities are exhibited in most malignant solid tumors, and utilization of this data can refine the histopathologic diagnosis of many tumors.[19,20] The ease with which chromosome preparations can be obtained enables cytogenetic techniques to be routinely employed in leukemias. They are also considered as mandatory investigations to diagnose, classify, and determine the prognosis of leukemia patients. This is followed by its application in lymphomas and solid tumors.[21–23] An explosion of technological advances has been observed in clinical research and cancer diagnostics in the past decade. Identification of Philadelphia chromosome in chronic myeloid leukemia (CML) by conventional cytogenetics in 1960[21] to the fusion gene identification by high-throughput sequencing techniques in various cancers in 2020[24] is the paradigm of this technological advancement. Conventional cytogenetics by banding techniques was introduced early with quinacrine mustard stain or quinacrine hydrochloride on chromosomes that yielded characteristic Q bands. Quenching of the fluorescent stain limited the routine use of quinacrine banding technique. GTG banding technique was developed shortly after that, where metaphase chromosomes were treated with enzyme trypsin followed by Giemsa stain. Permanent preparations, better resolution, and fluorescent microscopy being avoided made GTG banding as a commonly employed technique in the clinical settings. Reverse of G banding or R-banding uses heat application before Giemsa staining and is rarely used owing to the complicated procedures. C-banding identifies the constitutive heterochromatin regions, whereas NOR identifies the active nucleolar organizer regions on chromosomes using silver nitrate stain. C banding and NOR staining are applied to analyze polymorphism of donor and recipient cells and to evaluate the outcome of bone marrow transplantation in leukemia patients. Although banding techniques are considered as the gold standard at every cytogenetic laboratory, cancer karyotypes show complex rearrangements of different chromosomal origin. Moreover, low mitotic index, inferior quality metaphases, and demand of technique expertizations may be detrimental to karyotype the tumor cells. The advent of molecular cytogenetics, microarray-based technologies, and next-generation sequencing (NGS) has expanded the approach and has enabled to detect aberrations that could have escaped by traditional karyotyping.[9,19,25]

Unprecedented access of genomic DNA using either interphase nuclei, metaphase spread, tissue sections, or living cells is achieved by fluorescent *in situ* hybridization (FISH).[19] This method employs probes, which are fragments of genomic DNA. The fluorochrome-labeled probes are complementary to specific sequences in the human genome. They are hybridized to fixed metaphase chromosomes or interphase nuclei, and the signals obtained are then visualized using a fluorescence microscope.[26,27] Centromeric probes may help to detect numerical aberrations. It consists of chromosome-specific DNA repeats (satellite DNA). Whole chromosome painting probes participate in structural aberration detection and are capable of binding to the entire length of specific metaphase chromosome. Locus/gene-specific probes are utilized to detect recurrent structural abnormalities by hybridizing to particular sequences within individual genes.[25] Multiplex FISH (M FISH) and spectral karyotyping (SKY) allow for the simultaneous identification of all 24 human chromosomes in different colors by a single hybridization. An enormous progress in understanding the complexity of cancer karyotypes has been achieved through these techniques. Nevertheless, limited resolution and the requirement for high-quality metaphase chromosome spreads remain a challenge.[19,28] Comparative genomic hybridization (CGH) involves competitive

hybridization of differentially labeled cells with fluorescent dyes on metaphase spreads. In this technique, equal amounts of control DNA from normal karyotype and sample DNA from study are differentially labeled with red and green fluorochromes and are cohybridized. CGH allows for genome-wide screening of cells. The major drawback of CGH is resolution, and to overcome this, array-based CGH (aCGH) was developed. Replacement of metaphase chromosome by increasingly shorter normal genomic DNA fragments like bacterial artificial chromosomes (BACs) or oligonucleotides yielded a higher resolution of chromosomes.[19] Improvisations in array technologies, known as single nucleotide polymorphism (SNP) arrays, resulted in hybridization efficiency of two DNA fragments that differed in a single nucleotide. The amenability of any cancer specimen to DNA extraction is an added advantage of CGH and SNP arrays. However, these techniques demand for at least 60–70% tumor purity to identify single-copy genomic alterations; contamination with normal and noncancerous cells is problematic.[19,29] Multiplex ligation probe analysis (MLPA) is a polymerase chain reaction (PCR)-based technique in which probes hybridized to DNA sample are amplified using single PCR primer. Requirement of multiple techniques is avoided in MLPA and genetic aberrations such as changes in copy number, methylation, or the presence of point mutations can be easily detected. The technique is robust and cost-effective. Since they are unable to detect unknown point mutations and distinguish polyploidy from diploidy or haploidy, balanced translocations or inversions are some of the major drawbacks of this technique.[30,31] The field of genomics is revolutionized by NGS technique, also referred as massive parallel sequencing. First-generation low-throughput sequencing was developed by Frederick Sanger in 1977, which underwent revolution over decades giving rise to high-throughput second- and third-generation NGS technologies.[2] Whole-genome, exome sequencing, tumor-specific gene panels can be easily elucidated through this approach.[33] Commercially available second-generation sequencing technologies involve Roche 454, Illumina (Miseq, Hiseq etc.), and Ion torrent, which are the short-read sequencers. To overcome the limitations of short read sequencers, third-generation sequencing techniques evolved eventually. The third-generation sequencing technologies include single-

Table 1: Conventional and molecular cytogenetic techniques with principle, advantages, and disadvantages

Techniques	*Principle*	*Advantages*	*Limitations*
Banding • Q—Quinacrine fluorescent stain • GTG—Trypsin treatment and Giemsa stain • R—Denaturing in hot acidic saline before Giemsa stain • C—Denaturation with sodium hydroxide, incubation in saline and Giemsa stain • NOR—Ammoniacal silver solution, Silver nitrate stain	Protein digestion and/or special dye generate banding pattern specific for each chromosome	Low cost for reagents and instrumentation Simple and robust procedures	Low resolution Dependent on chromosome condensation Requires mitotic cells and well-spread chromosomes Low efficacy in highly rearranged karyotypes
FISH—Three types of probes • Whole chromosome painting • Centomere specific • Gene/locus specific	Small-labeled DNA fragment is used as a probe to search for homologous target sequences in DNA	Rapid Simple and robust procedure	Conclusions limited to the tested targets Reagents cost more
Multicolor karyotyping M FISH SKY	Hybridization with 24 differentially labeled chromosome-specific probes allows painting of every chromosome in distinct color	Accurate origin identification of all segments in complex rearrangements	Requires mitotic cells and well-spread chromosomes Less accuracy in detecting intrachromosomal, breakpoints
CGH	Competitive hybridization of differentially labeled cells with fluorescent dyes on metaphase chromosomes	Cell culture not required	Dedicated instrument required Low resolution Dependent on chromosome condensation
aCGH/SNP arrays	Hybridization performed on matrix or microarray instead of metaphase chromosomes	High resolution	Expensive
MLPA	A PCR-based technique in which probes hybridized to the sample DNA are amplified using only one PCR primer pair	Simple Fast Inexpensive	Cannot detect unknown point mutations, differentiate polyploidy
NGS Roche 454 Illumina/Solexa Ion torrent Pacific biosciences Oxford nanopore	Sequencing of DNA by pyrosequencing/synthesis/ligation/enzyme/nanoscaled pore	High throughput High accuracy	Expensive

molecule real-time (SMRT) sequencing by Pacific biosciences and Oxford nanopore sequencing technologies. They provide longer sequencing reads but have a major issue of high error rate.[34,35] Understanding the genetic basis of tumor initiation and progression can be easily achieved by the advances in NGS. This makes them an attractive platform to better guide personalized precision medicine. These technologies have a higher coverage rate of detecting aberrations in comparison with microarray-based techniques. However, the routine use of these technologies is limited due to high cost and long processing time.[19] Table 1 discusses various techniques involved in cancer cytogenetics with advantages and disadvantages, respectively. Flowchart 2 illustrates the relationship of cytogenetics application with various methodologies in cancer.

Oral Cancer Cytogenetics

A broad spectrum of genomic imbalances from gross chromosomal aberrations (polysomy, aneuploidy, intrachromosomal rearrangements) to specific gene alterations (amplifications, point mutations etc.) is observed in oral squamous cell carcinoma (OSCC). These imbalances can drive to specific abnormal karyotypes by oncogene activation and silencing of tumor suppressor genes.[36] Progressive transformation of oral oncogenesis also involves the epigenetic changes, which include promoter methylations and miRNA deregulations.[37] All of these genetic events are responsible for deregulation of normal cell genome, desynchronizing the cell cycle, leading to malignant transformation.[38] They also cause aggressive phenotype due to elevated metastatic potential and recurrence rates. Enhanced examination of these alterations by considering specific markers is required for understanding the development and progression of OSCC.[39] Therefore, cytogenetic analysis has an important role to play in the comprehensive workup of OSCC.

Methodology of Screening Data in Oral Cancer Cytogenetics

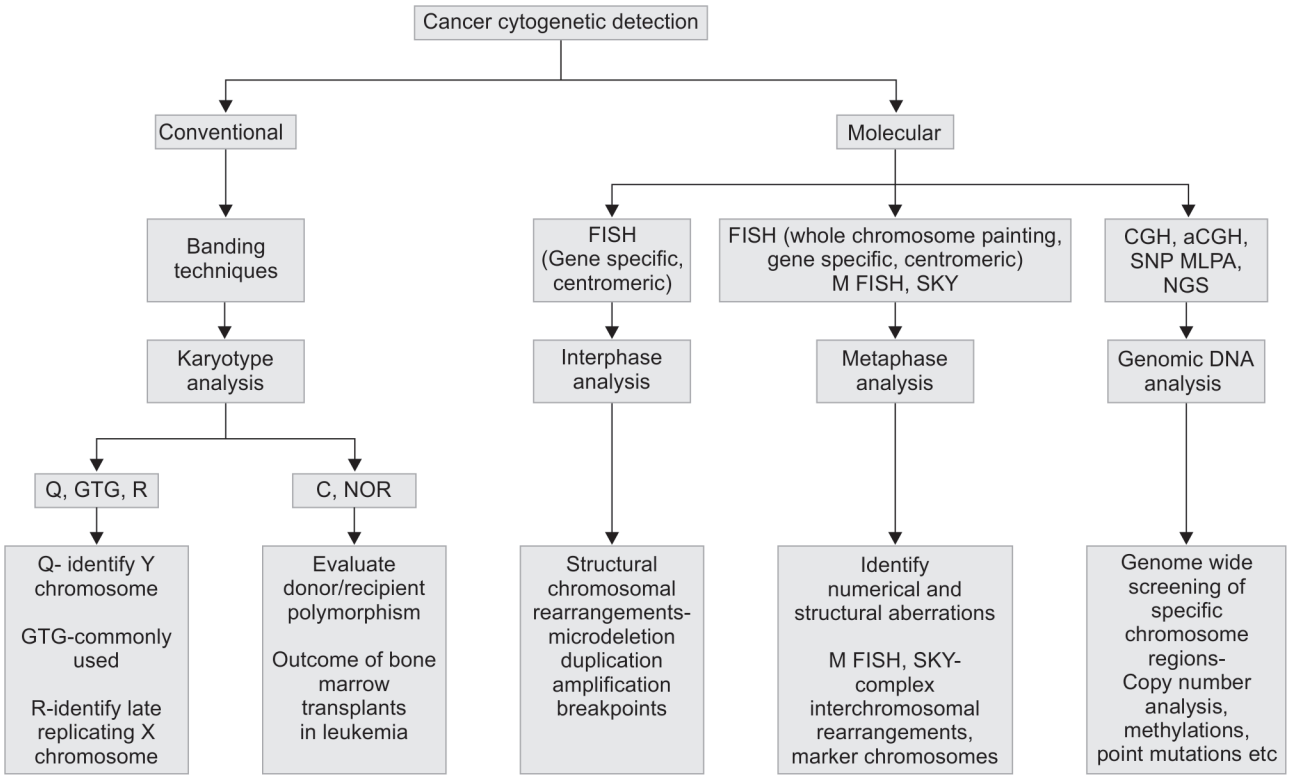
A web-based search is performed via the PubMed database with the keywords cytogenetics, chromosomal aberrations, conventional cytogenetics, karyotyping, banding techniques, molecular cytogenetics, fluorescent *in situ* hybridization, spectral karyotyping, comparative genomic hybridization, multiplex ligation probe analysis, and NGS in oral cancer. Original research studies, reviews, and case reports published from 2015 to 2020 are included to evaluate the diagnostic, prognostic, and therapeutic cytogenetic findings in oral cancer. Lastly, future perspective of cytogenetics in oral cancer is emphasized.

Role of Various Techniques in Oral Cancer Cytogenetics

Diagnostic and Prognostic Significance

Banding techniques: Banding is usually combined or redefined with other cytogenetic techniques. In a study by Ribeiro et al., HSC-3 tongue cell line with lymph node metastasis (LNM) was used to characterize the cytogenetic, genomic, and epigenetic involvement. GTG banding technique was done on metaphase chromosomes with other techniques like MFISH, aCGH, and MLPA. Several simple rearrangements involving two chromosomes to complex rearrangements involving multiple chromosomes, isochromosomes, aneuploidies, loss, and partial deletions in chromosomes were determined by GTG banding in the cell line, which reflects the OSCC signature.[40]

Flowchart 2: Summary of various techniques and cancer cytogenetic application



FISH, MFISH, and SKY: In the clinical setting, FISH has become an essential tool in the diagnosis and management of a variety of solid tumors, including OSCC. FISH technique can also be employed in noninvasive procedures like the detection of oral cancer through micronuclei in buccal epithelial cells.[41] Wangsa et al. utilized a multiple FISH marker to predict the prognosis of oral tongue squamous cell carcinoma (OTSCC) independent of the tumor stage. They analyzed oncogenes TERC on 3q26, EGFR on 7p12, CCND1 on 11q13, and TP53 on 17p13, respectively, and suggested that a diverse distribution of copy number changes is associated with poor prognosis.[42] CCND1 copy number analysis by FISH was significantly correlated with increased nuclear cyclin D1 and occult nodal metastasis in early floor of the mouth (FOM) and tongue cancers in a study by Noorlag et al.[43] According to Kakuya et al., copy number changes and ACTN4 gene amplification by FISH revealed significantly shorter overall survival time and were considered as significantly independent risk factors for death in patients with stage I/II oral tongue cancer.[44] Investigation of epidermal growth factor receptor (EGFR) gene amplification by FISH was associated with advanced clinical stage regardless of the age of patients by Costa et al.,[45] and EGFR (7p11.2), CCND1 (11q13.3) copy number gains were associated with OSCC progression and LNM in a study by Chien et al.[46] According to a study done by Cierpikowski et al., an aggressive behavior of OSCC was seen due to the PDGFRα/HER2 and PDGFRα/p53 co-expression.[47] Chromogenic *in situ* hybridization (CISH) was implemented to analyze chromosome 7 status by Mastronikolis et al. and chromosome 17 numerical status by Chrysovergis et al. in OSCC tissues. It was observed that they were correlated with a progressive dedifferentiation of the malignant tissues and chromosome 7 polysomy was observed more frequently in non-human papilloma virus (HPV) cases.[48,49] It is also reported that loss of heterozygosity (LOH) on chromosome 17 (17p13 band) leads to p53 overexpression correlating with advanced stage and positive LNM as analyzed by Zedan et al.[50] M FISH results by Ribeiro et al. in the HSC-3 cell line with LNM had a complex karyotype with multiple chromosomal aberrations.[40] Wang et al. established a novel OTSCC cell line designated as UCSF-OT-1109 from a never-smoking patient where SKY analysis revealed numerical and structural chromosomal abnormalities. Copy number aberration analysis showed cell line losses in chromosome 3p and 9p and lacked the amplification of 3q and 11q.[51]

CGH, aCGH, and SNP arrays: The molecular basis of oral carcinogenesis can be determined by genome-wide screening approach like CGH. da Silva et al. investigated metastatic and non-metastatic tongue tumors to analyze genes potentially contributing to OSCC progression to metastasis by aCGH. Predominant amplifications of chromosomal regions that encompass the RAB5, RAB7, and RAB11 genes (3p24-p22, 3q21.3, and 8p11–12, respectively) in metastatic OSCC were detected.[52] Chen et al. analyzed genome-wide LOH and DNA copy number aberration and their associations with risk factors, tumor characteristics, and oral cancer-specific mortality with HPV-negative OSCC through SNP arrays. 4q, 8p, 9p, and 11q regions played an important role in oral cancer and survival from this disease in their assay.[53] Meta-analysis by Chong et al. found high-frequency gains in chromosomes 5p, 14q, 11q, 7p, 17q, 20q, 8q, and 3q, and high-frequency losses in chromosomes 3p, 8p, 6p, 18q, and 4q through aCGH in OSCC. These chromosomes contain multiple cancer-related genes like CCDN1 (11q13), EGFR (7p12), V-Myc avian myelocytomatosis (MYC) viral oncogene homolog (8q24), telomerase RNA component (3q24), fragile histidine triad (3p14.2), and p16 (9p21) that might be altered during oral carcinogenesis.[54]

MLPA: Multiple parallel analysis of quantitative genetic alterations from small quantities of fragmented tumor DNA is efficiently achieved by MLPA in OSCC. In a study by van Kempen et al., copy number status was correlated with HPV status in oropharyngeal squamous cell carcinoma (OPSCC), with occult LNM in OSCC, and with patient survival by MLPA. They found that gain of the 11q13 region (CCND1 gene) was significantly correlated with LNM in stage I–II OSCC.[55] Ribeiro et al. analyzed DNA copy number alteration and methylation status using methylation-specific MLPA in OSCC. They observed better prognosis with WT1 gene promoter methylation and that of MSH6 and GATA5 gene promoter methylation served as worst prognostic predictors. Shorter survival rate was significantly associated with GATA5 gene promoter methylation. It was also observed that PAX5 gene promoter methylation was significantly associated with tongue tumors.[56]

NGS: In diagnostic clinical settings, targeted NGS are widely accepted and play a crucial role in novel discoveries but are not yet fully reported in OSCC. A systematic review published by Sharma et al. showed a total of 28 loci that were validated to be associated with oral cancer by candidate gene studies, genome-wide association studies, and NGS approaches. The loci detected were 14q32.33 (AKT1), 5q22.2 (APC), 11q22.3 (ATM), 2q33.1 (CASP8), 11q13.3 (CCND1), 16q22.1 (CDH1), 9p21.3 (CDKN2A), 1q31.1 (COX-2), 7p11.2 (EGFR), 22q13.2 (EP300), 4q35.2 (FAT1), 4q31.3 (FBXW7), 4p16.3 (FGFR3), 1p13.3 (GSTM1-GSTT1), 11q13.2 (GSTP1), 11p15.5 (H-RAS), 3p25.3 (hOGG1), 1q32.1 (IL-10), 4q13.3 (IL-8), 12p12.1 (KRAS), 12q15 (MDM2), 12q13.12 (MLL2), 9q34.3 (NOTCH1), 17p13.1 (p53), 3q26.32 (PIK3CA), 10q23.31 (PTEN), 13q14.2 (RB1), and 5q14.2 (XRCC4).[57] According to Nakagaki et al. and Ma et al., TP53, NOTCH1, CASP8, CDKN2A, PIK3CA, HRAS, MET, STK11, and CDH1 were the most frequently mutated genes in OSCC patients as detected by NGS techniques.[58,59] A review by Kim et al. also reveals that p53, CDKN2A, PIK3CA, and HRAS are the most common genes pertaining to the development and progression of OSCC as detected by NGS techniques Ion torrent, Illumina, etc.[60]

Therapeutic Significance

OSCC exhibits tumor heterogeneity, which remains a major challenge for treating this malignancy. Growth factor receptors, signal transduction or transcription activation key molecules and genes involved in proliferation and metastasis of cancer cells are some of the cancer-specific genetic targets. Targeting these molecules has an advantage of increasing the therapeutic index and reducing the toxicity of the drugs in oral cancer patients. Several drug-targeted gene therapies are utilized and under further evaluation for treating OSCC. p53 is the most common mutated gene in OSCC, and p53-targeted therapy reactivates the transcriptional activity of wild-type 53 by restoring p53 to its wild type. PRIMA-1, MIRA-1, STIMA-1, and COTI-2 are the p53-targeting drugs. Depending on targeting mechanism, EGFR-targeting drugs consist of two subgroups. Function as monoclonal antibodies against EGFR is obtained by drugs cetuximab and nimotuzumab, and the EGFR tyrosine kinase inhibitors are gefitinib, erlotinib, and afatinib, which are currently under clinical trials for treating OSCC. Vascular endothelial growth factor (VEGF) receptors and their inhibitors include monoclonal antibodies bevacizumab or multikinase inhibitors like sorafenib and vandetanib. Mammalian target of rapamycin (mTOR) inhibitors reduce the tumor growth by making them radiation sensitive and sensitive to EGFR inhibitors. Agents targeting the programmed cell death receptor 1 (PD-1) like pembrolizumab and nivolumab are in various stages of clinical

trials in treating oral cancer.[60–62] There are many more therapeutic approaches which are rapidly evolving for effectively treating oral cancer. da Silva et al. highlight pan-Rab inhibitors as a potential therapeutic approach for invasive OSCC in patients exhibiting amplifications of chromosomal regions encoding RAB5, RAB7, and RAB11 genes, which were detected by aCGH in their study.[52] A study by Koole et al. has shown that FGFR3 gene copy numbers as determined by FISH in OSCC and OPSCC may serve as an interesting therapeutic target for FGFR3-directed therapies.[63] The development of these drugs is possible only due to the exploration of the molecular mechanism involved in oral cancer. In this context, findings through cytogenetic and cytogenomic techniques can make a major contribution to enhance novel discoveries and help develop targeted therapies. Table 2 summarizes various targeted therapies which are currently available and are under various stages of clinical trials for treating OSCC.

Review of publications (2015–2020) have also revealed that the above-discussed cytogenetic techniques were usually coupled with other techniques like quantitative polymerase chain reactions (qPCR), immunohistochemistry (IHC) etc. to arrive at the diagnosis or to validate the results obtained by a particular technique. It was observed that LOH in chromosomes 3p, 9p, 11q, and 17p was consistent in most oral cancer cases and amplifications of genes in these regions were associated with advanced stages and poor

Table 2: Targeted gene therapies currently used and under evaluation in the treatment of OSCC

Targeted therapies	*Mechanism of action*	*Drugs*
p53 targeted	Reactivating the transcriptional activity of wild-type p53, induce apoptosis by caspase activation	PRIMA-1, MIRA-1, STIMA-1, COTI-2
EGFR targeted	Stabilize EGFR protein through the ubiquitin/proteasome pathway	Cetuximab, nimotuzumab, gefitinib, erlotinib
VEGF targeted	Act as monoclonal antibodies and multikinase inhibitors against VEGF	Bevacizumab, sorafenib, vandetanib
mTOR inhibitors	Regulate P13K/AKT signal transduction pathway	Rapamycin, everolimus, sirolimus
PD-1 targeted	Immune checkpoint inhibitors	Pembrolizumab, nivolumab, durvalumab, atezolizumab

Table 3: Cytogenetic alterations in OSCC and their outcomes detected by various cytogenetic techniques (2015–2020)

References	*Sample type*	*Technique*	*Chromosomal region/aberrations*	*Gene involved*	*Alterations*	*Outcome of studies*
Zedan et al.[50]	Paraffin-embedded tissue sections	FISH, IHC	17 trisomy	p53	Amplification	
			17monosomy	p53	Deletion	Aggressive tumors with poor prognosis
Chen et al.[53]	Peripheral blood	SNP array	4p, 8q, 9p, 11q	MYEOV, CCND1, ORAOV1, FGF19, FGF4, FADD, etc.	Amplification	Points heterogeneity and genomic complexity of OSCC
da Silva et al.[52]	Paraffin-embedded tissue sections	aCGH, IHC	3p24-p22, 3q21.3, 8p11-12	RAB5, RAB7, RAB11	Amplification	OSCC progression, prognostic markers
Kempen et al.[55]	Paraffin-embedded tissue sections	MLPA	11q13	CCND1, FGF4, FADD, CTTN	Amplification	Biomarker for predicting occult LNM in stage I–II OSCC.
Wangsa et al.[42]	Paraffin-embedded tissue sections	FISH	3q26, 7p12, 11q13, 17p13	TERC, EGFR CCND1, TP53		Poor prognosis in OTSCC
Chong et al.[54]		aCGH, qPCR	3q, 5p, 7p, 8q, 9p, 10p, 11q	CCND1, EGFR, TPM2, LRP12, CTTN, FADD, etc.	Amplification	3q amplifications-advanced stage 11q13-poor prognosis 3 and 8 CNAs—poor prognosis
			3p and 8p		Deletion	
Ribeiro et al.[56]	Fresh frozen sections	MS-MLPA		WT1, MSH6 GATA5, PAX5	Promoter methylation	MSH6,GATA5-poor prognosis PAX5 associated with tongue tumors
Noorlag et al.[43]	Paraffin-embedded tissue sections	FISH, IHC	11q13	CCND1, FADD	Amplification	Marker for occult nodal metastasis in early FOM cancers

Kakuya et al.[44]	Tissue sections	FISH, IHC		ACTN4	Amplification	Prognostic marker for overall survival in stage I/II OTSCC
Wang et al.[51]	Cell line	SKY, Tp53 targeted sequencing WES	Cell line losses 3p, 9p	Tp53, CDKN2A, SPTBN5, NOTCH2, FAM136A		A novel OTSCC cell line (UCSF-OT-1109) from a never-smoking patient was established
			19p		Focal amplification	
			3q and 11q		No amplification	
Riberio et al.[40]	Cell line	GTG banding, MFISH, aCGH, MS MLPA	Gains-1, 3q, 5p, 7p, 8q, 9q, 10, 11p, 11q13, 12, 13, 14, 17, 18p, 20, Yp, Xq	TP73, GATA5	Methylation and gain	HSC-3 cell line is a complex karyotype—help develop therapeutics in advanced stages
			Loss-18q	RARB, ESR1, CADM1	Methylation and loss	
Costa et al.[45]	Paraffin-embedded tissue sections	FISH, IHC		EGFR	Amplification	Advanced stage regardless of the age of the patient
Cierpikowski et al.[47]	Paraffin-embedded tissue sections	FISH, IHC		PDGRFα/HER2 or PDGRFα/p53	Co-expression	Poorly differentiated OSCC, invasion, aggressive behavior
Mastronikolis et al.[48]	Paraffin-embedded tissue sections	CISH	7 polysomy			Rare, advanced stage, observed in non-HPV cases
Chien et al.[46]	Frozen tissue sections, Peripheral blood	SNP array, FISH, qPCR	7p11.2, 11q13.3 3p14.2–p12.1, 4q35.1 etc.	EGFR, CCND1 FHIT, FAT1, CDKN2A, ATM	Amplification	OSCC progression, lymph node metastasis
Chrysovergis et al.[49]	Paraffin-embedded tissue sections	CISH	17 polysomy, sporadic monosomy	HER2	Amplification	Progressive dedifferentiation of malignant tissue
				p53	Deletion	
Sharma et al.[57] Kim et al.[60]		NGS-Ion torrent, Illumina	17p13.1, 11q13.3, 9q34.3, 3q26.32 9p21.3, 11p15.5	p53, CCNDI, NOTCH1, PIK3CA, CDKN2A		p53-most common NOTCH1-poor survival PIK3CA—seen in stage 4 OSCC

prognosis.[64] The above-discussed cytogenetic alterations, their outcomes depicted in Table 3, and the therapeutic approaches mentioned are just few examples of the complexity involved in OSCC. Further research and larger validation studies must be carried out for in-depth characterization of the molecular basis in OSCC, and more targeted therapies should be employed for the benefit of the patient. Table 3 summarizes various cytogenetic techniques and their respective findings published from 2015 to 2020 in OSCC.

Future Perspective

Recently, improvements in high-throughput technologies (HTS) have led to profile the molecular basis of many tumors, including OSCC. The HTS platforms belong to NGS technologies and have helped to understand the omic mechanisms in cancers more efficiently.[65] From second-generation sequencing, HTS platforms Illumina Solexa[66] and Ion torrent (Thermo Fisher),[67] to recent third-generation sequencing techniques like SMRT by Pacific biosciences,[68] minion by Oxford nanopore technologies,[69] have been carried out in both clinical and research settings to evaluate the efficiency of these techniques in oral cancer patients. These platforms are rapidly developing either by bioinformatics tools or by computational methods and have greatly enhanced the analysis of sequenced DNA and RNA fragments. The most diverse microbial community is the oral microbiome, which is crucially important to study human oral cancers, and HTS, in particular, have been applied to characterize the oral microbiome.[70] Tumor heterogeneity of OSCC possesses as one of the major challenges, which could be revealed through single-cell sequencing. Single-cell analysis can be used to characterize genetic and nongenetic mechanisms, identify minimal residual disease and tumor microenvironment, and determine the disease progression by identifying the cell subpopulations but in a routine clinical practice, this technology is still unreachable. The ongoing revolution of these technologies can effectively help in achieving regenerative therapy and targeted therapy in OSCC patients in the near future.[71,72]

Conclusion

Cytogenetic techniques have evolved enormously, and deciphering the cancer genomes has provided insights into the diagnosis, prognosis, and therapeutics in numerous cancers, including OSCC. Molecular techniques like FISH, aCGH, and MLPA have outshone traditional conventional cytogenetic techniques by providing unprecedented access to oral cancer genome. In the future, with advancements and cost-effectiveness, NGS has a great potential to detect the molecular basis of oral cancer precisely. Through these technologies, targeted therapies can be pursued although some challenges regarding cost and practical

applicability for the benefit of patient needs to be overcome. Multidisciplinary approach between clinicians, researchers, and cytogeneticists with modern technological advances can play a crucial role to interpret the results and achieve better therapeutics in OSCC patients.

References

1. Ali J, Sabiha B, Jan HU, et al. Genetic etiology of oral cancer. Oral Oncol 2017;70:23–28. DOI: 10.1016/j.oraloncology.2017.05.004.
2. Jain AK, Singh D, Dubey K, et al. Chromosomal aberrations. In: Mutagenicity: assays and applications. Academic Press; 2018. p. 69–92.
3. Janssen A, van der Burg M, Szuhai K, et al. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. Science 2011;333(6051):1895–1898. DOI: 10.1126/science.1210214.
4. Ramos-García P, Ruiz-Ávila I, Gil-Montoya JA, et al. Relevance of chromosomal band 11q13 in oral carcinogenesis: an update of current knowledge. Oral Oncol 2017;72:7–16. DOI: 10.1016/j.oraloncology.2017.04.016.
5. Albertson DG. Gene amplification in cancer. Trends Genet 2006;22(8):447–455. DOI: 10.1016/j.tig.2006.06.007.
6. Hovhannisyan G, Harutyunyan T, Aroutiounian R. Micronuclei and what they can tell us in cytogenetic diagnostics. Curr Genet Med Rep 2018;6(4):144–154. DOI: 10.1007/s40142-018-0149-6.
7. Ribeiro IP, Melo JB, Carreira IM. Cytogenetics and cytogenomics evaluation in cancer. Int J Mol Sci 2019;20(19):4711. DOI: 10.3390/ijms20194711.
8. Ponnuraj KT. Cytogenetic techniques in diagnosing genetic disorders. In: Advances in the study of genetic disorders. Croatia InTech; 2011. p. 45–64.
9. Kannan TP, Zilfalil BA. Cytogenetics: past, present and future. Malays J Med Sci MJMS 2009;16(2):4. PMID: 22589651.
10. Giersch ABS. Introduction to cytogenetics. 2014. p. 3304–3310.
11. Ferguson-Smith MA. History and evolution of cytogenetics. Mol Cytogenet 2015;8(1):1–8. DOI: 10.1186/s13039-015-0125-8.
12. Wan TS. Cancer cytogenetics: An introduction. Cancer Cytogenetics. 2017:1–10. DOI: 10.1007/978-1-4939-6703-2_1.
13. Dutta UR. The history of human cytogenetics in India—a review. Gene 2016;589(2):112–117. DOI: 10.1016/j.gene.2016.01.052.
14. Hassold T. Human cytogenetics and human chromosome abnormalities. In: Encyclopedia of genetics, genomics, proteomics and bioinformatics. 2004.
15. Liehr T, Othman MA, Rittscher K, et al. The current state of molecular cytogenetics in cancer diagnosis. Expert Rev Mol Diagn 2015;15(4):517–526. DOI: 10.1586/14737159.2015.1013032.
16. Mark HF, Jenkins R, Miller WA. Current applications of molecular cytogenetic technologies. Ann Clin Lab Sci 1997;27(1):47–56. PMID: 8997457.
17. Sandberg AA, Meloni-Ehrig AM. Cytogenetics and genetics of human cancer: methods and accomplishments. Cancer Genet Cytogenet 2010;203(2):102–126. DOI: 10.1016/j.cancergencyto.2010.10.004.
18. Das K, Tan P. Molecular cytogenetics: recent developments and applications in cancer. Clin Genet 2013;84(4):315–325. DOI: 10.1111/cge.12229.
19 Grade M, Difilippantonio MJ, Camps J. Patterns of chromosomal aberrations in solid tumors. In: Chromosomal instability in cancer cells. 2015. p. 115–142.
20. Cooley LD, Wilson KS. The cytogenetics of solid tumors. In: The principles of clinical cytogenetics. Springer, New York, NY; 2013. p. 371–411.
21. Wan TS, Ma ES. Molecular cytogenetics: an indispensable tool for cancer diagnosis. Chang Gung Med J 2012;35(2):96–110. DOI: 10.4103/2319-4170.106161.
22. Mrózek K, Harper DP, Aplan PD. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Hematol Oncol Clin North Am 2009;23(5):991–1010. DOI: 10.1016/j.hoc.2009.07.001.
23. Nanjangud G, Amarillo I, Rao PN. Solid tumor cytogenetics: current perspectives. Clin Lab Med 2011;31(4):785–811. DOI: 10.1016/j.cll.2011.07.007.
24. Stangl C, de Blank S, Renkens I, et al. Partner independent fusion gene detection by multiplexed CRISPR-Cas9 enrichment and long read nanopore sequencing. Nat Commun 2020;11(1):1–4. DOI: 10.1038/s41467-020-16641-7.
25. Wang N. Methodologies in cancer cytogenetics and molecular cytogenetics. Am J Med Genet 2002;115(3):118–124. DOI: 10.1002/ajmg.10687.
26. Varella-Garcia M. Molecular cytogenetics in solid tumors: laboratorial tool for diagnosis, prognosis, and therapy. Oncologist 2003;8(1):45–58. DOI: 10.1634/theoncologist.8-1-45.
27. Ratan ZA, Zaman SB, Mehta V, et al. Application of fluorescence in situ hybridization (FISH) technique for the detection of genetic aberration in medical science. Cureus 2017;9(6):e1325. DOI: 10.7759/cureus.1325.
28. Bayani JM, Squire JA. Applications of SKY in cancer cytogenetics. Cancer Invest 2002;20(3):373–386. DOI: 10.1081/cnv-120001183.
29. Mao X, Young BD, Lu YJ. The application of single nucleotide polymorphism microarrays in cancer research. Curr Genomics 2007;8(4):219–228. DOI: 10.2174/138920207781386924.
30. Hömig-Hölzel C, Savola S. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. Diagn Mol Pathol 2012;21(4):189–206. DOI: 10.1097/PDM.0b013e3182595516.
31. Kozlowski P, Jasinska AJ, Kwiatkowski DJ. New applications and developments in the use of multiplex ligation-dependent probe amplification. Electrophoresis 2008;29(23):4627–4636. DOI: 10.1002/elps.200800126.
32. Kamps R, Brandão RD, Bosch BJ, et al. Next-generation sequencing in oncology: genetic diagnosis, risk prediction and cancer classification. Int J Mol Sci 2017;18(2):308. DOI: 10.3390/ijms18020308.
33. Rizzo G, Black M, Mymryk JS, et al. Defining the genomic landscape of head and neck cancers through next-generation sequencing. Oral Dis 2015;21(1):e11–e24. DOI: 10.1111/odi.12246.
34. Sakamoto Y, Sereewattanawoot S, Suzuki A. A new era of long-read sequencing for cancer genomics. J Human Genet 2020;65(1):3–10. DOI: 10.1038/s10038-019-0658-5.
35. Liu L, Li Y, Li S, et al. Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012;2012:251364. DOI: 10.1155/2012/251364.
36. Kyrodimos E, Chrysovergis A, Mastronikolis N, et al. Impact of chromosome 9 numerical imbalances in oral squamous cell carcinoma: a pilot grid-based centromere analysis. Diagnostics 2020;10(7):501. DOI: 10.3390/diagnostics10070501.
37. Hema KN, Smitha T, Sheethal HS, et al. Epigenetics in oral squamous cell carcinoma. J Oral Maxillofac Pathol JOMFP 2017;21(2):252. DOI: 10.4103/jomfp.JOMFP_150_17.
38. Papanikolaou VS, Kyrodimos E, Tsiambas E, et al. Chromosomal instability in oral squamous cell carcinoma. J BUON 2018;23(6):1580–1582. PMID: 30610780.
39. Bavle RM, Venugopal R, Konda P, et al. Molecular classification of oral squamous cell carcinoma. J Clin Diagn Res 2016;10(9):ZE18. DOI: 10.7860/JCDR/2016/19967.8565.
40. Ribeiro IP, Rodrigues JM, Mascarenhas A, et al. Cytogenetic, genomic, and epigenetic characterization of the HSC-3 tongue cell line with lymph node metastasis. J Oral Sci 2018;60(1):70–81. DOI: 10.2334/josnusd.16-0811.
41. Jyoti S, Naz F, Khan S, et al. Detection of aneugenicity and clastogenicity in buccal epithelial cells of pan masala and gutkha users by pan-centromeric FISH analysis. Mutagenesis 2015;30(2):263–267. DOI: 10.1093/mutage/geu067.
42. Wangsa D, Chowdhury SA, Ryott M, et al. Phylogenetic analysis of multiple FISH markers in oral tongue squamous cell carcinoma suggests that a diverse distribution of copy number changes is associated with poor prognosis. Int J Cancer 2016;138(1):98–109. DOI: 10.1002/ijc.29691.
43. Noorlag R, Boeve K, Witjes MJ, et al. Amplification and protein overexpression of cyclin D1: predictor of occult nodal metastasis in early oral cancer. Head Neck 2017;39(2):326–333. DOI: 10.1002/hed.24584.

44. Kakuya T, Mori T, Yoshimoto S, et al. Prognostic significance of gene amplification of ACTN4 in stage I and II oral tongue cancer. Int J Oral Maxillofac Surg 2017;46(8):968–976. DOI: 10.1016/j.ijom.2017.03.001.
45. Costa V, Kowalski LP, Coutinho-Camillo CM, et al. EGFR amplification and expression in oral squamous cell carcinoma in young adults. Int J Oral Maxillofac Surg 2018;47(7):817–823. DOI: 10.1016/j.ijom.2018.01.002.
46. Chien HT, Cheng SD, Liao CT, et al. Amplification of the EGFR and CCND1 are coordinated and play important roles in the progression of oral squamous cell carcinomas. Cancers 2019;11(6):760. DOI: 10.3390/cancers11060760.
47. Cierpikowski P, Lis-Nawara A, Gajdzis P, et al. PDGFRα/HER2 and PDGFRα/p53 co-expression in oral squamous cell carcinoma. Anticancer Res 2018;38(2):795–802. DOI: 10.21873/anticanres.12286.
48. Mastronikolis NS, Tsiambas E, Fotiades PP, et al. Numerical imbalances of chromosome 7 in oral squamous cell carcinoma. Anticancer Res 2018;38(4):2339–2342. DOI: 10.21873/anticanres.12480.
49. Chrysovergis A, Papanikolaou V, Mastronikolis N, et al. Chromosome 17 In situ hybridization grid-based analysis in oral squamous cell carcinoma. Anticancer Res 2020;40(7):3759–3764. DOI: 10.21873/anticanres.14365.
50. Zedan W, Mourad MI, Abd El-Aziz SM, et al. Cytogenetic significance of chromosome 17 aberrations and P53 gene mutations as prognostic markers in oral squamous cell carcinoma. Diagn Pathol 2015;10(1):1–9. DOI: 10.1186/s13000-015-0232-1.
51. Wang SJ, Asthana S, van Zante A, et al. Establishment and characterization of an oral tongue squamous cell carcinoma cell line from a never-smoking patient. Oral Oncol 2017;69:1–10. DOI: 10.1016/j.oraloncology.2017.03.020.
52 da Silva SD, Marchi FA, Xu B, et al. Predominant Rab-GTPase amplicons contributing to oral squamous cell carcinoma progression to metastasis. Oncotarget 2015;6(26):21950. DOI: 10.18632/oncotarget.4277.
53. Chen C, Zhang Y, Loomis MM, et al. Genome-wide loss of heterozygosity and DNA copy number aberration in HPV-negative oral squamous cell carcinoma and their associations with disease-specific survival. PLoS One 2015;10(8):e0135074. DOI: 10.1371/journal.pone.0135074.
54. Vincent–Chong VK, Salahshourifar I, Razali R, et al. Immortalization of epithelial cells in oral carcinogenesis as revealed by genome-wide array comparative genomic hybridization: a meta-analysis. Head Neck 2016;38(S1):E783–E797. DOI: 10.1002/hed.24102.
55. van Kempen PM, Noorlag R, Braunius WW, et al. Clinical relevance of copy number profiling in oral and oropharyngeal squamous cell carcinoma. Cancer Med 2015;4(10):1525–1535. DOI: 10.1002/cam4.499.
56. Ribeiro IP, Caramelo F, Marques F, et al. WT1, MSH6, GATA5 and PAX5 as epigenetic oral squamous cell carcinoma biomarkers-a short report. Cell Oncol 2016;39(6):573–582. DOI: 10.1007/s13402-016-0293-5.
57. Sharma V, Nandan A, Sharma AK, et al. Signature of genetic associations in oral cancer. Tumor Biol 2017;39(10):1010428317725923. DOI: 10.1177/1010428317725923.
58. Nakagaki T, Tamura M, Kobashi K, et al. Targeted next-generation sequencing of 50 cancer-related genes in Japanese patients with oral squamous cell carcinoma. Tumor Biol 2018;40(9):1010428318800180. DOI: 10.1177/1010428318800180.
59. Ma J, Fu Y, Tu YY, et al. Mutation allele frequency threshold does not affect prognostic analysis using next-generation sequencing in oral squamous cell carcinoma. BMC Cancer 2018;18(1):758. DOI: 10.1186/s12885-018-4481-8.
60. Kim S, Lee JW, Park YS. The application of next-generation sequencing to define factors related to oral cancer and discover novel biomarkers. Life 2020;10(10):228. DOI: 10.3390/life10100228.
61. Liu L, Chen J, Cai X, et al. Progress in targeted therapeutic drugs for oral squamous cell carcinoma. Surg Oncol 2019;31:90–97. DOI: 10.1016/j.suronc.2019.09.001.
62. Ketabat F, Pundir M, Mohabatpour F, et al. Controlled drug delivery systems for oral cancer treatment—current status and future perspectives. Pharmaceutics 2019;11(7):302. DOI: 10.3390/pharmaceutics11070302.
63. Koole K, van Kempen PM, Swartz JE, et al. Fibroblast growth factor receptor 3 protein is overexpressed in oral and oropharyngeal squamous cell carcinoma. Cancer Med 2016;5(2):275–284. DOI: 10.1002/cam4.595.
64. Karunakaran K, Muniyan R. Genetic alterations and clinical dimensions of oral cancer: a review. Mol Biol Rep 2020;47(11):9135–9148. DOI: 10.1007/s11033-020-05927-0.
65. Park SJ, Saito-Adachi M, Komiyama Y, et al. Advances, practice, and clinical perspectives in high-throughput sequencing. Oral Dis 2016;22(5):353–364. DOI: 10.1111/odi.12403.
66. Tseng HH, Tseng YK, You JJ, et al. Next-generation sequencing for microRNA profiling: microRNA-21-3p promotes oral cancer metastasis. Anticancer Res 2017;37(3):1059–1066. DOI: 10.21873/anticanres.11417.
67. Jayaprakash C, Varghese VK, Jayaram P, et al. Relevance and actionable mutational spectrum in oral squamous cell carcinoma. J Oral Pathol Med 2020;49(5):427–434. DOI: 10.1111/jop.12985.
68. Singh N, Sahu DK, Tripathi RK, et al. Differentially expressed full-length, fusion and novel isoforms transcripts-based signature of well-differentiated keratinized oral squamous cell carcinoma. Oncotarget 2020;11(34):3227. DOI: 10.18632/oncotarget.27693.
69. Aminuddin A, Ng PY, Leong CO, et al. Mitochondrial DNA alterations may influence the cisplatin responsiveness of oral squamous cell carcinoma. Sci Rep 2020;10(1):1–7. DOI: 10.1038/s41598-020-64664-3.
70. Ramos RT, Sodré CS, de Sousa PM, et al. High-throughput nucleotide sequencing for bacteriome studies in oral squamous cell carcinoma: a systematic review. Oral Maxillofac Surg 2020;24(4):387–401. DOI: 10.1007/s10006-020-00873-4.
71. Qi Z, Barrett T, Parikh AS, et al. Single-cell sequencing and its applications in head and neck cancer. Oral Oncol 2019;99:104441. DOI: 10.1016/j.oraloncology.2019.104441.
72. Huang LY, Hsieh YP, Wang YY, et al. Single-cell analysis of different stages of oral cancer carcinogenesis in a mouse model. Int J Mol Sci 2020;21(21):8171. DOI: 10.3390/ijms21218171.