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Genomic Instability And Dna Damage Response In Oral Squamous Cell Carcinoma: Molecular Mechanisms, Tumor Evolution, And Therapeutic Vulnerabilities

Dr Arpita Kabiraj[1*], Prof (Dr) DHEERAJ SHARMA[2], Prof (Dr) Urvashi Tomar[3], Dr. Arunee Garg[4], Dr Ankit Goyal[5], Dr Ashima Bansal[6]

[1*]Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, arpita.kabiraj82@gmail.com, https://orcid.org/0000-0003-4382-0351
[2]Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, drdheeraj25@gmail.com,
[3]Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, urvashitomar87@yahoo.com,
[4]Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, dr_arunee@yahoo.com,
[5]Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, ankit.goyal1408@gmail.com,
[6]Private Practitioner, Kurukshetra, Haryana, gargashima220@gmail.com,
*Corresponding Author: Dr Arpita Kabiraj
*Department of Oral Pathology and Microbiology, Index Institute of Dental Sciences, Indore, arpita.kabiraj82@gmail.com, https://orcid.org/0000-0003-4382-0351

Abstract
This review comprehensively examined the molecular mechanisms of genomic instability and DNA damage response (DDR) in oral squamous cell carcinoma (OSCC), emphasizing tumor evolution and therapeutic vulnerabilities. It highlighted how genotoxic agents induced DNA strand breaks, microsatellite instability, and chromosomal aberrations, with key mutations identified in TP53, CDKN2A, and NOTCH1. The DDR network involving ATR, ATM, PARP, and BRCA proteins was outlined, revealing its role in maintaining genomic integrity and its disruption contributing to OSCC progression. The review discussed oxidative stress and ROS-mediated oncogenic pathways, along with epigenetic alterations, cGAS/STING signaling, and immune evasion strategies. Biomarkers like homologous recombination deficiency (HRD) scores, mutation signatures, and extracellular markers were identified as diagnostic and prognostic tools. Lastly, therapeutic targets including PARP, ATR, CHK1, and WEE1 inhibitors were analyzed, underscoring their clinical potential in sensitizing OSCC to chemoradiotherapy and immunotherapy.

Keywords: *Chromosomal Aberrations, Epigenetic Alterations, Homologous Recombination Deficiency, Microsatellite Instability, Oxidative Stress, Parp Inhibitors.*

INTRODUCTION

The Global Cancer Statistics (GLOBOCAN) database of the World Health Organization (WHO), reveals OSCC ranks 15th in global mortality [1.8 lakhs deaths annually, male-to-female ratio being 2.26] (1). Early detection of cancerous lesions by detecting genetic biomarkers like altered cell cycle, unstable DNA strands, microsatellite defects, and changes in regulatory genes helps to arrest the disease in early stages, resulting in a drastic improvement in functionality and survival rate. Most of the contemporary research is based on common cancer types- cervical, breast, respiratory, or gastrointestinal lesions. Fewer articles provide insight on head and neck neoplasms, and OSCC occupies a smaller area within that niche. This review presents a comprehensive reading on the mechanism of DDR, altered cellular response in oxidative stress, epigenetic modifications, and clinical implications in cancer, focusing on OSCC, along with related therapeutic insight.

Genomic Instability in OSCC

Genotoxic agents like harmful UV or ionizing radiations disrupt covalent bonds between nucleotides, resulting in single or double strand breaks (DSBs), helix-distorting bulky lesions, or cross-linked DNA strands, that are repaired by distinct pathways according to the causal agent and mode of damage incurred. The cell cycle undergoes various checkpoints of genetically predetermined molecules, which result in repair and recovery of DNA or apoptosis. The cellular response to damaged nucleotides is called DNA damage response (DDR), and altered DDR is a hallmark of carcinogenesis (2). Loss of heterozygosity (LOH) and microsatellite instability (MSI) are common in DNA mismatch repair (MMR), marked by hMSH2 and hMLH1 genes (3). Lin SC et al. (4) state that MSI, detected in 35% of OSCC cases, displays maximum mutation in locus 4q21.2, and is strongly linked to lymph node metastasis. In a systematic review by Bozdag LA et al. (2024), the LOH and microsatellite defects were highest in chromosome region 9p (22.2%) followed by 3p (16.7%), 17p (11.1%), 8p (7.4%), and 4p (5.5%), respectively (5). According to another study, D3S966 nucleotide marker (mutation of hMLH1 MMR genes) on 3p showed the highest LOH and MSI (42.8%). The tumor stage and differentiation grades varied significantly with MSI (p values were 0.002 and 0.035, respectively) (3). Next-generation sequencing (NSG) analysis requires minimal DNA, tracing MSI at low levels for minimal residual disease detection, copy-number variation, and tumor-mutational-burden determination (6). In a recent study on the comparison between NSG and immunohistochemistry, NSG displayed sensitivity of 95.8%, specificity of 99.4%, positive predictive value of 94.5%, and negative predictive value of 99.2% for the detection of MSI successfully (7).

There are several types of genetic mutations, out of which point mutation for TP53, chromosomal rearrangements such as amplification and translocation of CCND1, deletion of CDKN2A and NOTCH1, and aneuploidy (abnormal number of chromosomes) are commonly seen in OSCC. 30 % of TP53 mutations are missense type, decapacitating its transcription abilities, and others are nonsense, frameshift, and splice-site (8). Main actions of TP53 are regulating MDM2 (Mouse Double Minute 2) protein, CDK (Cyclin-Dependent Kinase Inhibitor 1A) inhibitor p21, BAX (Bcl-2-associated X protein), PUMA (p53 Upregulated Modulator of Apoptosis), and GADD45 (Growth Arrest and DNA-Damage-inducible 45). Mutation and deletion of CDKN2A, which encodes the p16INK4a protein, is also frequently noted (8,9). Deletions of CDKN2A were found in a significant percentage of OSCC cases, according to a study (10). Neurogenic locus notch homolog protein 1 (NOTCH1) mutations lead to aggressive tumor behaviour in the early stages of OSCC (11). PTEN (Phosphate and TENsin homolog deleted on chromosome 10) alterations are associated with poor prognosis in OSCC patients (12). Overexpression of Cyclin D1 (CCND1) results in uncontrolled proliferation in OSCC (13). A study on tongue-SCC stated that apart from TP53, Dystonin (DST) and Ring Finger Protein 213 (RNF213) were frequently mutated along with CDKN2A and NOTCH1 (14). The Rat Sarcoma gene (RAS) interacts with MAPK/ERK and the PI3K/AKT pathways. Mutations of codons 12,13, and 61 cause uncontrolled activation in cancer, causing resistance to tyrosine kinase inhibitor-based therapy, commonly used in the treatment of OSCC (8).

DNA Damage Response (DDR) Network: Molecular Architecture

Genotoxic agents like radiation and reactive oxygen species (ROS) are major causes for triggering the tumor micro-environment (TME). DDR is exhibited by homologous recombination (HR), base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), and DNA mismatch repair (MMR). Oncogenic stress disintegrates mitochondria, releases caspase, and triggers apoptosis (2). TP53, phosphatidylinositol 3-kinase-related kinase (PIKK) family molecules like ataxia telangiectasia mutated (ATM) and RAD-3 related (ATR) detect changes in DNA structure and mediate downstream protein phosphorylation events to facilitate DDR (15). BER for single strand break (SSB), NER for bulky adducts, NHEJ and HR for double strand breaks (DSBs), and DNA mismatch repair (MMR) for base-pair mismatches are common pathways (2).

ATR, ATM, and replication protein A (RPA) bind to checkpoint molecules CHK1 and CHK2T that inhibit further cyclin-dependent kinase (CDK) activities, resulting in arrest /inhibition of G1-S, intra-S, and G2-M "cell checkpoints", increasing chances of repair before mitosis. ATR/ATM pathways also stimulate DNA repair proteins transcriptionally or post-transcriptionally, by modulating their phosphorylation, acetylation or ubiquitylation. If DDR is compromised, neoplasia progresses (16-18). The different DDR-pathways, checkpoints, epigenetic changes, and genome-related diagnostics are listed in Table I. While normal cells overcome replication

stress by repairing via multiple pathways, cancer cells often fail to regulate even a single pathway, leading to uncontrolled proliferation and genetic instability. PARP proteins, aided by BRCA 1 and BRCA 2 genes, cause HRR. Failure of HRR leads to secondary, compensatory, and incomplete repair by other means like NHEJ, which results in aberrant chromosomes and genomic instability (16,18-19). PARP-1 inhibition does not impact chromosomes with one strand of wild BRCA genes (normal) and another strand of mutated/absent BRCA, as cell repair is unimpeded, but arrests future replication in case both alleles are mutated. PARP-1 inhibitors are often used as therapeutic medication to curb further cancer growth (20). Figure 1 illustrates how the DNA damage response coordinates nuclear and mitochondrial pathways to repair lesions, enforce cell cycle checkpoints, or direct cells toward apoptosis or malignant transformation when repair fails.

Oxidative Stress and Reactive Oxygen Species (ROS) in OSCC Pathogenesis

Reactive oxygen species (ROS) are free radicals generated from cellular metabolites, radiation, hazardous chemicals, etc, which impact phagocytosis, prostaglandin synthesis, and cytochrome P-450 respiration. Examples are hydroxyl radicals, superoxide anion radicals, hydrogen peroxide, singlet oxygen, hypochlorite, nitric oxide radicals, and peroxynitrite radicals. During cancer initiation, ROS modulates key transcription factors like nuclear factor-κB (NF-κB), hypoxia-inducible factor (HIF), and p53, the major molecules of cell signalling, even in minimal quantity, resulting in inhibition of antiproteases, upregulation of matrix-metalloproteinases (MMPs), and enhanced angiogenesis (21). Overexpression of enzymes in cyclooxygenase (COX), lipoxygenase (LOX), and NADPH oxidase pathways (NOX) also creates excess ROS in OSCC, resulting in altered kinases/phosphatase activities and transcriptional errors [Gao et al. (22)]. The wingless type 1 (WNT-1) and canonical WNT/ β-catenin pathways are activated by binding to Frizzled receptors and LRP5/6 co-receptors, activating target genes like C-Myc, cyclin D1, c-jun, fra-1, and u-PAR. A Study done by Ionescu et al. (2024) stated that WNT dysregulation correlates with highly dysplastic and highly aggressive variants of OSCC (21). Undesired oral habits, like arecanut chewing, cause upregulation of MKP-1, indirectly enhancing transcription factor SNAIL in OSCC, and smoking increases oxidative stress, inducing changes in JNK/P 38 and MAP signaling. Radiotherapy-induced apoptosis leads to secondary iatrogenic redox ROS accumulation (23). Ramundo et al. (2021) stated that ROS modulates transforming growth factor β (TGF β), aggravating the epithelial-mesenchymal transition (EMT) via suppressor of mothers against decapentaplegic (SMAD) pathway (24). Cancer stem cells (CSCs) are another important biomarker, eg, NANOG, OCT-4, and SOX 2. Increased NANOG expression correlated with poor prognosis and therapeutic resistance in OSCC (25). Other examples of CSCs are CD44, CD24, CD133, Musashi-1, ALDH1, and RAS (Renin-Angiotensin System) (26). Table 1 summarizes different types of DNA damage, their repair pathways, cell cycle checkpoints, and how these mechanisms are altered in cancer with related diagnostic markers. Table 2 presents the studies (2022–2025) associating genetic alterations and DNA repair mechanisms in OSCC with clinical outcomes, prognostic markers, and therapeutic implications.

CROSSTALK BETWEEN DDR AND OTHER ONCOGENIC PATHWAYS

GMP-AMP synthase and stimulator of interferon genes (cGAS/ STING) is a 522-amino-acid cytosolic protein, which binds to DNA and is inactive under normal conditions. In case of DNA exposure, cGAS is bound to the cell membrane by the N-terminal and fragmented DNA, which activates the cGAS/STING pathway. The cGAS dimer catalyses ATP / GTP into cyclic AMP-GMP, activating STING and TANK-binding kinase 1 (TBK1). This promotes interferon regulatory factor 3 (IRF3), interferon 1 (INF-1), and NFκB translocation for further transcription (27). According to Yu et al., damaged mitochondria release DNA via mitochondrial permeability transition pore (mPTP) and voltage-dependent anion channel 1 (VDAC1) oligomers, thereby stimulating cGAS/STING pathway and causing hyperinflammation (28). cGAS triggers the antigen-presenting cells (APC), exposing the tumor cells to CD8 T cells and natural killer (NK) cells, thereby clearing the TME. The cancer cells block the pathway by abnormal methylation and improper post-translational modifications in order to thrive in TME. Altered catabolism of the amino acid tryptophan by indoleamine 2,3 dioxygenase (IDO) enzyme suppresses T cells, and conversion of arginine into L-ornithine by arginase (ARG1) and nitric oxide synthetase (NOS) causes dysregulation of M2 macrophages and myeloid-derived suppressor cells (MDSCs). Thus, cGAS/STING induces immunosuppression in TME (27,29). Interestingly, PD-1, a peculiar transmembrane protein of activated lymphocytes, binds to programmed cell death ligands (PD-L1and PD-L2), resulting in reduced TME cytokine

levels and suppressed function of lymphocytes. This action "hides" the tumor cells from immune recognition (30). Endothelial-mesenchymal Transition (EMT) is controlled by three families of EMT-Transcription factors- the Zinc finger E-box binding proteins (ZEB 1 & 2), the SNAI family (SNAIL & SLUG), and the TWIST family (TWIST 1). The activated ZEB 1 directly modulates Ubiquitin-Specific protease 7 (USP 7), resulting in stabilization of CHK 1 and an increase of HRR (31). ZEB 1 works with ATM in a positive feedback loop, increasing DRR in radio and chemotherapy (32). In OSCC, activation of the AKT pathway resulted in EMT, characterized by down-regulation of E-cadherin, desmoplakin, and beta-catenin and up-regulation of vimentin. This phenomenon is important for cancer survival in metastases (33).

Well-documented epigenetic alterations in OSCC include DNA methylation, histone modification, and non-coding RNA expression. Aberrant hypomethylation leads to activation of proto-oncogenes, causing malfunction of Cyclins, EGFR, AIM2, CEACAM1, LINE-1, PI3, and PTHLH genes. Hypermethylation silences tumor suppressor genes (TSGs) and causes cancer. Common hypermethylated genes in OSCC are CDKN2A, O6-methylguanine-DNA methyltransferase (MGMT), Death Associated Protein Kinase 1 (DAPK1), TIMP3, TFPI2, SOX17, and GATA4 (34). TME witnesses several histone modifications like methylation, acetylation, ubiquitination, phosphorylation, sumoylation, and others. According to a study, OSCC and oral lichen planus (OLP) displayed a higher level of inactivated modified H3K4me2 histone. Histone deacetylation of HIF-1α, mediated by the enzyme HDAC2, and acetylation of protein H3K14 accelerate tumor growth and proliferation. Modification of small non-coding RNAs (ncRNA) like microRNA (miRNA) is responsible for cellular proliferation, metastasis, and genomic instability. In a study by Cervigne et al., it was seen that progressive leukoplakia and OSCC displayed 109 types of miRNAs when different lesions were compared (34-37).

THERAPEUTIC TARGETING OF DDR PATHWAYS IN OSCC

The ATR/ATM sensors attach to undefended strands and trigger the recruitment of PARP molecules. Tumor cells multiply by one pathway, while others are impaired. Blocking that pathway by therapeutic means results in tumor cell death and is known as synthetic lethality. Common examples are using PARP inhibitors in BRCA mutant tumors. The goal is to cause the maximum amount of DNA damage by synthetic lethality in G1 and S phases of the cell cycle, and then prevent DNA repair during G2 (18). According to a recent review, ATR and CHK1 activate the intra-S checkpoint response by inhibiting CDK through degradation or inhibition of CDC25 phosphatases. Phosphorylated CHK1 activates WEE1 kinase, thereby directly inhibiting CDK activity, allowing time to repair DNA damage. Inhibition of ATR, CHK1, and WEE1 forces tumor cells to enter M phase without repair, followed by cellular catastrophe and death (38). Pre-clinical research states that ceralasertib (ATR inhibitor) increases the sensitivity of HNSCC to radiation; the combination of olaparib or niraparib (both PARP inhibitors) enhances the radiosensitivity of OSCC cells; Olaparib combined with radiotherapy increases DNA damage and reduces the proliferation of SMAD4-deficient HNSCC cells (39-41). In a meta-analysis, combined therapy like niraparib (PARP inhibitor) and dostarlimab (PD-1 inhibitor), and olaparib with cisplatin or durvalumab (PD-L1 inhibitor) showed promising results (15% patients responded to DDR inhibitor and PD-L1 therapy) (42). A few ATM/ATR inhibitors are Wortmannin, KU-55933, AZD0156, AZD1390, and WSD 0628 (43). One major mechanism of treatment resistance is PARP re-mutation (PARP-trapping) and WEE protein dephosphorylation, causing malfunctioning of the DNA-PK pathway (38,44).

BIOMARKERS OF GENOMIC INSTABILITY AND DDR DEFICIENCY IN OSCC

Extracellular and cytosolic biomarkers of genetic instability are matrix metalloproteinases (MMPs 2/9), tight junction proteins (Claudin, E-Cadherin), transmembrane glycoproteins (CD44/133), and apoptosis inhibitors (Bcl-2). Nuclear biomarkers include mutated TSGs (p53, p63, p16), distorted cell-cycle checkpoints (CCD-1, MDM2), transcriptional regulators (SOX-2/OCT-4), and DNA repair regulators (Htert, MutSα, MutLα) (45). Signature mutation (SM), a recent diagnostic aid, is the analysis of a large sample of mutated nucleotides that is specific for a group, which can predict progression and age of the tumor (46). A recent International Cancer Genome Consortium, The Cancer Genome Atlas, and Pan-Cancer Analysis of Whole Genomes Network identified mutational signatures utilizing data from over 23,000 cancer patients, for different groups of tumors (47). South AP et al. found tobacco smoke yields distinct signature mutations in lung and HNSCC- the catalogue of somatic mutations in cancer 4 (COSMIC 4) and COSMIC 5; the latter occurring more in aged patients. Other

SMs were COSMIC signatures 1 and 5, endogenous deaminases of the AID (activation-induced cytidine deaminase) and APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide) family. Amongst all types, larynx SCC displayed the greatest proportion of COSMIC 4 and 5 [46]. Another reliable marker is the homologous recombination deficiency (HRD) score. BRCA 1 /2 are key drivers of HRD. According to Zhou et al., patients with no-HRD exhibited higher recurrence [hazard ratio (HR)-1.43] and mortality rates [HR- 26.04] when compared to the HRD-positive group of nasopharyngeal SCC (48). Wang et al. stated that esophageal-SCC patients with high HRD score undergoing therapy have overall increased survival rate than low HRD counterpart, but non-therapy high HRD cohort displayed shorter disease-free period and reduced overall survival period compared to therapy receiving group (49). Amongst epigenetic markers, γH2AX is important as it is directly linked to the ATR/ATM pathway. Increased H2AX indicates higher cell repair capacity, whereas knockdown displays decreased cell proliferation. Higher H2AX miRNA levels were observed in metastatic clones and tissues, highlighting its potential as a target in cancer therapeutics (50). For successful sample collection, liquid biopsy of cell-free DNA (cfDNA)- a mixture of carcinogenic and healthy nucleotides has been introduced in recent years. For OSCC, two types of cfDNA-the circulating cfDNA (ccfDNA) and the salivary cfDNA (scfDNA) are important genomic biomarkers (51). Kinane DF et al. proposed various advantages of this technique in OSCC eg, non-invasive collection, simultaneous screening of various dysregulated proteins suspended in TME, as an initial screening tool, and for regular monitoring of tumor progression, and detection of metastases (52). According to a comprehensive review, liquid biopsy successfully detected lung metastasis in the mouth apart from OSCC and HNSCC lesions. The study also reported altered microbiome and genetic mutations in saliva samples collected by liquid biopsy as a hallmark of lung carcinoma (53). A few studies regarding genetic analysis in OSCC are presented in Table II.

FUTURE DIRECTIONS AND CLINICAL TRANSLATION

Extensive immune evasion and re-stabilization of TME have posed major challenges to curb metastasis and recurrence in OSCC. Genomic analysis helps in differentiating and staging tumors accurately, predicting future metastasis, and administering targeted medicine based on synthetic lethality. Apoptosis of cancer cells without harming normal cells by precision medicine will be the game-changer in the future. Further research is needed to study unknown pathways of therapy resistance, DNA escape routes, and an in-depth analysis of the mutated genome.

CONCLUSION

The different mechanisms of DNA repair and harmful outcomes in case of DNA damage are being researched globally. Detecting the differences between the normal pathways and TME pathways is the key to formulating an exact treatment plan for the patient. Knowing the DDR, genetic modifications, and epigenetic changes helps in a detailed assessment of tumor stage, progression, and future prognosis. Genomic analysis enables clinicians to target radiotherapy or chemotherapy towards the defective niche only, sparing the normal tissue. This endeavour will strengthen the post-treatment revival, curb future recurrences, and prevent further metastases. Instead of blindly damaging healthy tissue, only targeted destruction of tumor cells is the backbone of precision medicine.

REFERENCES

1. Wu J, Chen H, Liu Y, Yang R, An N. The global, regional, and national burden of oral cancer, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. J Cancer Res Clin Oncol. 2025 Jan 28;151(2):53. doi: 10.1007/s00432-025-06098-w. PMID: 39875744; PMCID: PMC11775039.
2. Alhmoud JF, Woolley JF, Al Moustafa AE, Malki MI. DNA Damage/Repair Management in Cancers. Cancers (Basel). 2020 Apr 23;12(4):1050. doi: 10.3390/cancers12041050. PMID: 32340362; PMCID: PMC7226105.
3. Ashazila MJ, Kannan TP, Venkatesh RN, Hoh BP. Microsatellite instability and loss of heterozygosity in oral squamous cell carcinoma in Malaysian population. Oral Oncol. 2011 May;47(5):358-64. doi: 10.1016/j.oraloncology.2011.03.005. Epub 2011 Mar 29. PMID: 21450513.

4. Lin SC, Chang MF, Chung MY, Chang CS, Kao SY, Liu CJ, Chang KW. Frequent microsatellite alterations of chromosome locus 4q13.1 in oral squamous cell carcinomas. J Oral Pathol Med. 2005 Apr;34(4):209-13. doi: 10.1111/j.1600-0714.2004.00296.x. PMID: 15752255.
5. Arslan Bozdag L, Inan S, Elif Gultekin S. Microsatellite Instability and Loss of Heterozygosity as Prognostic Markers in Oral Squamous Cell Carcinoma: Molecular Mechanisms, Detection Techniques, and Therapeutic Strategies. Genes Chromosomes Cancer. 2024;63(10):e70002
6. Yu F, Leong KW, Makrigiorgos A, Adalsteinsson VA, Ladas I, Ng K, Mamon H, Makrigiorgos GM. NGS-based identification and tracing of microsatellite instability from minute amounts DNA using inter-Alu-PCR. Nucleic Acids Research. 2021;49(4):e24-.
7. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. Cancer Med. 2018;7(3):746-756. PMID: 29436178; PMCID: PMC5852359. DOI: 10.1002/cam4.1372.
8. Cabral LGS, Martins IM, Paulo EPA, Pomini KT, Poyet JL, Maria DA. Molecular Mechanisms in the Carcinogenesis of Oral Squamous Cell Carcinoma: A Literature Review. Biomolecules. 2025;15(5).
9. Jambhekar A, Ackerman EE, Alpay BA, Lahav G, Lovitch SB. Comparison of TP53 mutations in myelodysplasia and acute leukemia suggests divergent roles in initiation and progression. Blood Neoplasia. 2024;1(1):100004.
10. Gadhikar MA, Zhang J, Shen L, Rao X, Wang J, Zhao M, Kalu NN, Johnson FM, Byers LA, Heymach J, Hittelman WN. CDKN2A/p16 deletion in head and neck cancer cells is associated with CDK2 activation, replication stress, and vulnerability to CHK1 inhibition. Cancer research. 2018;78(3):781-97.
11. Wu-Chou YH, Hsieh CH, Liao CT, Lin YT, Fan WL, Yang CH. NOTCH1 mutations as prognostic marker in oral squamous cell carcinoma. Pathology-Research and Practice. 2021;223:153474.
12. Rajendran P, Sekar R, Dhayasankar PS, Ali EM, Abdelsalam SA, Balaraman S, Chellappan BV, Metwally AM, Abdallah BM. PI3K/AKT signaling pathway mediated autophagy in oral carcinoma-A comprehensive review. International Journal of Medical Sciences. 2024;21(6):1165.
13. Ramos-Garcia P, Gil-Montoya JA, Scully C, Ayén A, González-Ruiz L, Navarro-Triviño FJ, González-Moles MA. An update on the implications of cyclin D1 in oral carcinogenesis. Oral diseases. 2017;23(7):897-912.
14. Vettore AL, Ramnarayanan K, Poore G, Lim K, Ong CK, Huang KK, Leong HS, Chong FT, Lim TK, Lim WK, Cutcutache I. Mutational landscapes of tongue carcinoma reveal recurrent mutations in genes of therapeutic and prognostic relevance. Genome medicine. 2015;7: 1-5.
15. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Molecular cell. 2010;40(2):179-204.
16. Matsuoka S, Ballif BA, Smogorzewska A, McDonald III ER, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, Shiloh Y. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. science. 2007;316(5828):1160-6.
17. Campisi J, di Fagagna Cellular FD. senescence: When bad things happen to good cells. Nat Rev Mol Cell Biol. 2007;8(9):729-40. PMID: 17667954.DOI: 10.1038/nrm2233
18. O'Connor MJ. Targeting the DNA damage response in cancer. Molecular cell. 2015;60(4):547-60.
19. Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends in cell biology. 2016;26(1):52-64.
20. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature. 2005;434(7035):913-7.
21. Ionescu C, Kamal FZ, Ciobica A, Halitchi G, Burlui V, Petroaie AD. Oxidative stress in the pathogenesis of oral cancer. Biomedicines. 2024;12(6):1150.
22. Gào X, Schöttker B. Reduction–oxidation pathways involved in cancer development: a systematic review of literature reviews. Oncotarget. 2017;8(31):51888.
23. Manjunathan R, Jayaraman S, S R, Ileng Kumaran R, Chandrakesan P, Rajagopal P, Sambandam Y. Reactive oxygen species in oral squamous cell carcinoma progression and importance of stem cells in cancer therapeutics. In: Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. Singapore: Springer; 2022. 1-24.

24. Ramundo V, Giribaldi G, Aldieri E. Transforming growth factor-β and oxidative stress in cancer: a crosstalk in driving tumor transformation. Cancers. 2021;13(12):3093.
25. Baillie R, Tan ST, Itinteang T. Cancer stem cells in oral cavity squamous cell carcinoma: a review. Frontiers in Oncology. 2017;7:112.
26. Chiou SH, Yu CC, Huang CY, Lin SC, Liu CJ, Tsai TH, Chou SH, Chien CS, Ku HH, Lo JF. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clinical cancer research. 2008;14(13):4085-95.
27. Shen R, Liu D, Wang X, Guo Z, Sun H, Song Y, Wang D. DNA damage and activation of cGAS/STING pathway induce tumor microenvironment remodeling. Frontiers in cell and developmental biology. 2022;9:828657.
28. Yu CH, Davidson S, Harapas CR, Hilton JB, Mlodzianoski MJ, Laohamonthonkul P, Louis C, Low RR, Moecking J, De Nardo D, Balka KR. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. Cell. 2020;183(3):636-49. PMID: 33031745; PMCID: PMC7599077. DOI: 10.1016/j.cell.2020.09.020.
29. Lemos H, Mohamed E, Huang L, Ou R, Pacholczyk G, Arbab AS, Munn D, Mellor AL. STING promotes the growth of tumors characterized by low antigenicity via IDO activation. Cancer research. 2016;76(8):2076-81.
30. Kciuk M, Kołat D, Kałuzińska-Kołat Ż, Gawrysiak M, Drozda R, Celik I, Kontek R. PD-1/PD-L1 and DNA damage response in cancer. Cells. 2023;12(4):530.
31. Moyret-Lalle C, Prodhomme MK, Burlet D, Kashiwagi A, Petrilli V, Puisieux A, Seimiya H, Tissier A. Role of EMT in the DNA damage response, double-strand break repair pathway choice and its implications in cancer treatment. Cancer Science. 2022;113(7):2214-23.
32. Zhang X, Zhang Z, Zhang Q, Zhang Q, Sun P, Xiang R, Ren G, Yang S. ZEB1 confers chemotherapeutic resistance to breast cancer by activating ATM. Cell death & disease. 2018;9(2):57.
33. Krisanaprakornkit S, Iamaroon A. Epithelial-mesenchymal transition in oral squamous cell carcinoma. International Scholarly Research Notices. 2012;2012(1):681469.
34. Mesgari H, Esmaelian S, Nasiri K, Ghasemzadeh S, Doroudgar P, Payandeh Z. Epigenetic regulation in oral squamous cell carcinoma microenvironment: a comprehensive review. Cancers. 2023;15(23):5600.
35. Mancuso M, Matassa D, Conte M, Colella G, Rana G, Fucci L, Piscopo M. H3K4 histone methylation in oral squamous cell carcinoma. Acta Biochimica Polonica. 2009;56(3):405-10.
36. Arif M, Vedamurthy BM, Choudhari R, Ostwal YB, Mantelingu K, Kodaganur GS, Kundu TK. Nitric oxide-mediated histone hyperacetylation in oral cancer: target for a water-soluble HAT inhibitor, CTK7A. Chemistry & biology. 2010;17(8):903-13.
37. Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, Pintilie M, Jurisica I, Perez-Ordonez B, Gilbert R, Gullane P. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Human molecular genetics. 2009;18(24):4818-29.
38. Kono T, Ozawa H. A comprehensive review of current therapeutic strategies in cancers targeting DNA damage response mechanisms in head and neck squamous cell cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2024:189255.
39. Dok R, Glorieux M, Bamps M, Nuyts S. Effect of ATR inhibition in RT response of HPV-negative and HPV-positive head and neck cancers. International Journal of Molecular Sciences. 2021;22(4):1504.
40. Molkentine JM, Molkentine DP, Bridges KA, Xie T, Yang L, Sheth A, Heffernan TP, Clump DA, Faust AZ, Ferris RL, Myers JN. Targeting DNA damage response in head and neck cancers through abrogation of cell cycle checkpoints. International journal of radiation biology. 2021;97(8):1121-8.
41. Hernandez AL, Young CD, Bian L, Weigel K, Nolan K, Frederick B, Han G, He G, Devon Trahan G, Rudolph MC, Jones KL. PARP inhibition enhances radiotherapy of SMAD4-deficient human head and neck squamous cell carcinomas in experimental models. Clinical Cancer Research. 2020;26(12):3058-70.
42. Botticelli A, Cirillo A, Strigari L, Valentini F, Cerbelli B, Scagnoli S, Cerbelli E, Zizzari IG, Rocca CD, D'Amati G, Polimeni A. Anti–PD-1 and anti–PD-L1 in head and neck cancer: a network meta-analysis. Frontiers in immunology. 2021;12:705096.

43. Sharma R, Mishra A, Bhardwaj M, Singh G, Indira Harahap LV, Vanjani S, Pan CH, Nepali K. Medicinal chemistry breakthroughs on ATM, ATR, and DNA-PK inhibitors as prospective cancer therapeutics. Journal of Enzyme Inhibition and Medicinal Chemistry. 2025;40(1):2489720.
44. Patterson-Fortin J, Bose A, Tsai WC, Grochala C, Nguyen H, Zhou J, Parmar K, Lazaro JB, Liu J, McQueen K, Shapiro GI. Targeting DNA repair with combined inhibition of NHEJ and MMEJ induces synthetic lethality in TP53-mutant cancers. Cancer research. 2022;82(20):3815-29.
45. Ranganath K, Feng AL, Franco RA, Varvares MA, Faquin WC, Naunheim MR, Saladi SV. Molecular biomarkers of malignant transformation in head and neck dysplasia. Cancers. 2022;14(22):5581.
46. South AP, den Breems NY, Richa T, Nwagu U, Zhan T, Poojan S, Martinez-Outschoorn U, Johnson JM, Luginbuhl AJ, Curry JM. Mutation signature analysis identifies increased mutation caused by tobacco smoke associated DNA adducts in larynx squamous cell carcinoma compared with oral cavity and oropharynx. Scientific reports. 2019;9(1):19256.
47. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, Boot A, Covington KR, Gordenin DA, Bergstrom EN, Islam SA. The repertoire of mutational signatures in human cancer. Nature. 2020;578(7793):94-101.
48. Zhou X, Ying H, Sun Y, Zhang W, Luo P, Zhu S, Zhang J. Homologous recombination deficiency (HRD) is associated with better prognosis and possibly causes a non-inflamed tumour microenvironment in nasopharyngeal carcinoma. The Journal of Pathology: Clinical Research. 2024;10(5):e12391.
49. Wang Y, Ding B, Tao Y, Huang L, Zhu Q, Gao C, Feng M, Han Y. Homologous recombination deficiency score is an independent prognostic factor in esophageal squamous cell carcinoma. J Pathol Clin Res. 2024;10(6):e70007. PMID: 39469984; PMCID: PMC11519826. DOI: 10.1002/2056-4538.70007.
50. Prabhu KS, Kuttikrishnan S, Ahmad N, Habeeba U, Mariyam Z, Suleman M, Bhat AA, Uddin S. H2AX: A key player in DNA damage response and a promising target for cancer therapy. Biomedicine & Pharmacotherapy. 2024;175:116663.
51. Rapado-González Ó, Rodríguez-Ces AM, López-López R, Suárez-Cunqueiro MM. Liquid biopsies based on cell-free DNA as a potential biomarker in head and neck cancer. Japanese Dental Science Review. 2023;59:289-302.
52. Kinane DF, Gabert J, Xynopoulos G, Guzeldemir-Akcakanat E. Strategic approaches in oral squamous cell carcinoma diagnostics using liquid biopsy. Periodontology 2000. 2024;96(1):316-28.
53. Ma L, Guo H, Zhao Y, Liu Z, Wang C, Bu J, Sun T, Wei J. Liquid biopsy in cancer current: status, challenges and future prospects. Signal Transduction and Targeted Therapy. 2024;9(1):336.
54. Hang B. Formation and repair of tobacco carcinogen-derived bulky DNA adducts. J Nucleic Acids. 2010;2010:709521. PMID: 21234336; PMCID: PMC3017938. DOI: 10.4061/2010/709521.
55. Schormann N, Ricciardi R, Chattopadhyay D. Uracil-DNA glycosylases—structural and functional perspectives on an essential family of DNA repair enzymes. Protein science. 2014;23(12):1667-85.
56. Petruseva IO, Evdokimov AN, Lavrik OI. Molecular mechanism of global genome nucleotide excision repair. Acta Naturae. 2014;6(1 (20)):23-34.
57. Choudhury A, Zhao H, Jalali F, Al Rashid S, Ran J, Supiot S, Kiltie AE, Bristow RG. Targeting homologous recombination using imatinib results in enhanced tumor cell chemosensitivity and radiosensitivity. Molecular cancer therapeutics. 2009;8(1):203-13.
58. Lempiäinen H, Halazonetis TD. Emerging common themes in regulation of PIKKs and PI3Ks. The EMBO journal. 2009;28(20):3067-73.
59. Orans J, McSweeney EA, Iyer RR, Hast MA, Hellinga HW, Modrich P, Beese LS. Structures of human exonuclease 1 DNA complexes suggest a unified mechanism for nuclease family. Cell. 2011;145(2):212-23.
60. Ramakrishnan P, Sujatha S, Kundu P. Methylation status of p16INK4a and p14ARF gene in saliva of smokeless tobacco users: A pilot descriptive study. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2021;33(2):221-6.
61. Strzelczyk JK, Krakowczyk Ł, Owczarek AJ. Methylation status of SFRP1, SFRP2, RASSF1A, RARβ and DAPK1 genes in patients with oral squamous cell carcinoma. Archives of oral biology. 2019;98:265-72.
62. Yang H, Jin X, Dan H, Chen Q. Histone modifications in oral squamous cell carcinoma and oral potentially malignant disorders. Oral Diseases. 2020;26(4):719-32.

63. Zheng M, Cao MX, Luo XJ, Li L, Wang K, Wang SS, Wang HF, Tang YJ, Tang YL, Liang XH. EZH2 promotes invasion and tumour glycolysis by regulating STAT3 and FoxO1 signalling in human OSCC cells. Journal of Cellular and Molecular Medicine. 2019;23(10):6942-54.
64. Naeem, W., Nawab, F., Sarwar, M.T., Khalil AT, Gaber DA, Ahmad H, Fazeel M, Alorini M, Khan IA, Irfan M, Khan M, Khurram SA, Ali A. Profiling genetic mutations in the DNA damage repair genes of oral squamous cell carcinoma patients from Pakistan. Scientific Reports. 2025;15(1):7896
65. Kumar S, Rangarajan A, Pal D. Post-translational regulation of stemness under DNA damage response contributes to the gingivobuccal oral squamous cell carcinoma relapse and progression. Oral Oncology Reports. 2025:100730.
66. Halder M, Sardar J, Mondal P. Evaluation of DNA Damage in Oral Squamous Cell Carcinoma by Comet Assay. Journal of Primary Care Dentistry and Oral Health. 2024;5(1):18-23.
67. Na L, Meijie Z, Wenjing Z, Bing Z, Yanhao D, Shanshan L, Yongle Q. HMG20A was identified as a key enhancer driver associated with DNA damage repair in oral squamous cell carcinomas. BMC Oral Health. 2022;22(1):473.
68. Gupta R, Strbenac D, Satgunaseelan L, Cheung VK, Narayanappa H, Ashford B, Mitchell J, Thind A, Palme CE, Ch'ng S, Low TH. Comparing genomic landscapes of oral and cutaneous squamous cell carcinoma of the head and neck: quest for novel diagnostic markers. Modern Pathology. 2023;36(8):100190.

FIGURE LEGEND:

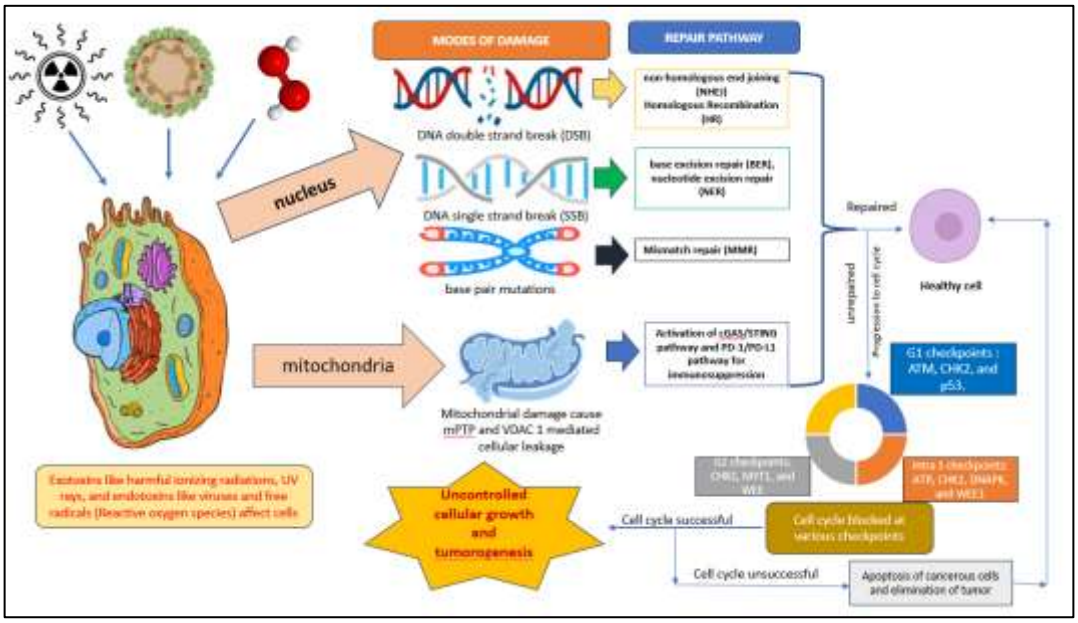

Figure. 1 DNA Damage Response (DDR) pathway: Exogenous stress induces nuclear and mitochondrial damage. Nuclear DNA lesions DSB, SSB, base mutations are repaired via NHEJ, HR, NER, and MMR. Cell cycle checkpoints (G1, S, G2) arrest damaged cells for repair. Successful repair restores healthy cells; failure triggers apoptosis or cancer cell proliferation if checkpoints are bypassed. mPTP and VDAC1 regulate mitochondrial involvement.

Table 1: DDR pathways, targets, and related diagnostic aids

DNA Damage Response (DDR) in Healthy Cells	Cancer Proliferation and Tumor Microenvironment (TME)	Diagnostic Aids
Types of DNA Damage	Repair Pathways	Cellular Checkpoints

Single Strand Break (SSB): One DNA strand break, intact another strand. Causes: ROS, irradiation, topoisomerase I poisons (Camptothecin, Irinotecan, Topotecan) (18).	**Direct Repair (DR):** Pyrimidine dimers corrected by MGMT, ALKBH enzymes (2).	**G1-S:** Delays replication for repair via NHEJ; ATM, CHK2, p53 involved.
Double Strand Break (DSB): Both DNA strands break simultaneously or progressively. SSB can progress to DSB (18).	**Base Excision Repair (BER):** Removes uracil via AP endonuclease, DNA ligase restores DNA (55).	**Intra-S:** Delays origin firing, halts replication. DDR targets ATR, CHK1, DNAPK, WEE1.
Bulky Adducts: Large nucleotide binding blocking replication; sources include tobacco smoke, rubber incineration (54).	**Nucleotide Excision Repair (NER):** XPC proteins remove defects and restore DNA (56).	**G2-M:** Last repair stage; CDK1 phosphorylation delays mitosis. DDR targets: CHK1, MYT1, WEE1.
Base Pair Mutations: Deletion, inversion, translocation, point mutation, frame shift mutation.	**Homologous Recombination (HR):** Controlled by p53, BRCA1/2, ATM. RAD51 phosphorylation and ABL1 inhibitor release enable repair (18, 57).	
	Non-Homologous End Joining (NHEJ): Uses microhomologies; KU70, KU80, DNA-PKcs, XRCC4-XLF, ligase 4. ATM, ATR, mTOR involved (58).	
	Mismatch Repair (MMR): hExo1 binds MutS/MutL proteins, cuts defective DNA, polymerase and ligase complete repair (59).	

Footnote: MGMT: O6-methylguanine -DNA methyl transferase; ALKBH: alpha-keto gluterate dioxygenase B homolog; XRCC: X-ray repair cross complimenting protein; ABL: Abelson gene; Mut: mutator gene

Table 2: Few important studies and their results regarding genetic analysis of the past 4 years (2022-2025)

Author and Year	Parameters	Outcomes	Method/Technology Used	Key Conclusion/Clinical Relevance
Wafaa N et al., 2025 (64)	Analysis of five genes—TP53, ATR, ATM, CHEK1, CHEK2 in OSCC	TP53 highest mutation; recurrence rates: TP53 (77.7%), ATR (74.04%), CHK1 (100%)	NGS, Bioinformatics Tools	TP53, ATR, and CHK1 mutations predict recurrence risk in OSCC; potential for targeted surveillance and treatment strategies.
Sachendra Kumar et al., 2025 (65)	Mutation analysis for bucco-gingival SCC in cured vs. relapsed patients	Loss-of-function in MAPKAP1 promotes stemness, relapse in Indian OSCC patients	AI Deep Learning, RNA-seq	MAPKAP1 mutation identified as a novel marker for relapse risk; important in precision oncology for Indian OSCC cases.
Halder M. et al., 2024 (66)	DNA damage-tumor grade correlation	Max DNA damage in poorly differentiated (29.9 μm), followed by moderately (25.60 μm) and well-differentiated (23.28 μm) OSCC	Comet Assay	DNA damage level correlates directly with OSCC histopathological grading; assists in tumor aggressiveness assessment.
Zhou X et al., 2024 (48)	BRCA1/2 mutations, HRD status, clinical molecular characteristics	HRD-negative patients had higher recurrence (HR: 1.43) and mortality (HR: 26.04)	HRD Assay, Clinical Molecular Profiling	HRD status serves as a prognostic biomarker in OSCC, guiding therapy choice (e.g., PARP inhibitors).
Gupta R. et al., 2023 (68)	Mutation signature analysis, tumor mutation burden in head and neck/oral cavity/cutaneous SCC	P53, PIK3CA, MUC4 most common; MS in COSMIC 1, 2, 13 frequent in OSCC	NGS, Tumor Mutation Burden Analysis	MS profiling refines OSCC classification and helps in risk stratification; important for therapeutic decisions.
Na L et al., 2022 (67)	Mutation analysis in 72 OSCC patients	HMG20A regulates DNA repair; novel therapeutic target in OSCC	RT-PCR (Two Methods)	HMG20A is a potential target for DNA repair modulation in OSCC, offering new therapeutic avenues.

Footnote: PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, involved in cell growth and survival pathways, MUC4: Mucin 4 gene, associated with epithelial cell protection and signaling, HMG20A: High Mobility Group 20A, a regulator of DNA repair mechanisms in oral squamous cell carcinoma (OSCC), COSMIC: Catalogue of Somatic Mutations in Cancer, HRD: Homologous Recombination Deficiency