Epigenetic alterations in oral cancer: tobacco as the prime culprit

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Abstract

Oral squamous cell carcinoma accounts for about 90% of the cases of oral cancer. It also remains the major contributor to cancer-related deaths worldwide. Tobacco use is a major cause, comprising more than 60 harmful chemicals that can cause cellular and molecular damage. Recent evidence indicates the induction of carcinogenic effects by tobacco via epigenetic and genetic alterations. This includes DNA methylation, histone modifications, and abnormally regulated miRNA expression. The overall disruption of these mechanisms causes further downregulation of tumor suppressor genes and activation of oncogenes. Histone-modifying enzymes and DNA methyltransferases are some essential enzymes in this process that link tobacco exposure to changes in chromatin structure and gene silencing. Additionally, these epigenetic modifications induced by tobacco and its derivatives possess the potential to serve as biomarkers for early diagnosis, prognostic prediction, and therapeutic stratification. Novel therapeutic avenues like DNA Methyltransferase and Histone deacetylase inhibitors are emerging as epigenetic modification therapies. These exhibit the ability to reverse these abnormal modifications by restoring the normal gene functioning. This comprehensive review brings together the current understanding of altered epigenetics by tobacco consumption and how important epigenetic biomarkers and epi-drugs are in fighting treatment resistance. Comprehending this interaction not only yields mechanistic insights but also facilitates translational opportunities for targeted therapy in oral carcinoma.

Keywords*: Oral squamous cell carcinoma, Tobacco consumption, Epigenetic modifications, microRNA expression, Epigenetic therapy*

Introduction

Oral squamous cell carcinoma (OSCC) is the most common form of oral carcinoma, accounting for almost 90% of all types of oral cancers [1]. OSCC affects the epithelial cells of the mouth, including the gums, floor of the mouth, the inner cheek lining, and lips [2]. GLOBOCAN 2022 has stated in a report that there were approximately 389,846 new cases of lip and oral cavity, ranking 16th, and 15th in mortality rate, leading to 188,438 deaths amongst 20 million new tumor cases recorded globally. Asia has accounted for 75.1% of deaths and 66.3% of cases worldwide, with the highest burden [3]. The precise etiology behind the progression of OSCC remains inadequately explored; however, specific risk factors can elevate an individual's susceptibility to this condition [4]. These may include tobacco consumption (either smoking or chewing), betel quid use, heavy alcohol intake, human papillomavirus infections, chronic sun exposure, weaker immunity, poor oral health, oral precancerous lesions history, as well as some genetic factors [1]. These factors synergistically contribute to the development of oral carcinoma, which ultimately helps in the initiation and progression of malignant changes in epithelial cells of the oral cavity.

The substantial risk of genetic factors in the progression of OSCC has been observed. Individuals who have hereditary deficiencies in DNA repair mechanisms or altered pathways for the metabolism of different carcinogens, especially those linked with cytochrome P450 enzymes, glutathione-S-transferases (GSTs) [5], and xenobiotic-metabolizing enzymes (XMEs) [6], are at increased risk of DNA damage associated with tobacco and alcohol consumption. TP53, CDKN2A (p16), RB1, and HRAS gene mutations are mainly observed in oral carcinomas, further affecting the pathways that include MAPK, JAK/STAT, and PI3K/AKT [7].

Furthermore, the epigenetic dysregulations that are indicated by inheritable changes in the expression of genes without changing the DNA sequence have gained more attention in the progression, treatment, and diagnosis of oral tumor phenotype [8]. Epigenetic Modifications, which include DNA methylation, histone modifications, chromatin remodeling, and microRNAs (miRNAs) functions, help in gene expression and cellular behaviour [9]. Environmental factors such as dysregulated oral microbiota, tobacco, betel quid, and alcohol cause the altered epigenetic profile in oral squamous cells [7,10]. This further results in promoter hypermethylation, histone changes, and dysregulated microRNA functions [11]. They work together to inhibit tumor suppressor genes and promote oncogenic signalling pathways, which directly help in disease progression, shaping gene expression and cell behaviour. The altered hypermethylation of tumor-suppressor genes, such as CDKN2A/p16, APC, DAPK, MGMT, CDH1, and TIMP3, has been frequently observed in OSCC, resulting in low gene activity, altered cell-cycle control, and increased tumor progression [8,12]. These key findings highlight that environmental factors mediated epigenetic modifications are significant in OSCC progression. There are multiple treatment options available for OSCC - including surgery, radiotherapy, chemotherapy, and immunotherapy- but due to the high recurrence rate, difficulty in management, treatment resistance, and late-stage diagnosis, the outcomes remain poor and suboptimal [13]. Early detection, more precise and targeted treatment options are necessary to improve the overall life and survival chances of oral carcinoma patients [14].

The importance of tobacco-mediated epigenetic dysregulations in OSCC progression, metastasis, and invasion has been discussed. Thus, there is a need to have the knowledge of the complexity of epigenetic regulation in oral carcinoma, as it helps in managing this deadly disease via finding out the potential targeted therapies for these modifications.

This review article unfolds the association between tobacco and OSCC, exploring the facet of tobacco-related epigenetics in OSCC and discussing the underlying mechanisms of epigenetic changes and their importance in the progression, initiation, and metastasis of tumor, as well as the therapeutic strategies available

Tobacco and oral cancer association

The use of both smoking and smokeless tobacco remains the leading etiological factor for OSCC globally, especially in South Asia [15]. A broad range of epidemiological and clinical studies has indicated a robust, dose-dependent correlation linking tobacco consumption with the increased risk of oral carcinoma. A Case-control study at the Kidwai Memorial Institute of Oncology in Bangalore stated the odds ratio (OR) of 3.5 for the development of oral malignancy among tobacco smokers, escalating to 8.3 for the heavy smokers who were consuming 20 bidis or cigarettes daily. This dose-response relation suggested the associated risk with tobacco use duration and intensity [16].

According to a retrospective study in India that consisted of 200 oral cavity tumor patients, identifying gutka as the most prevalent habit with 54.2%, followed by bidi smoking, 25.3%, and cigarette use, 16.9%. Gutka ingestion was strongly correlated with advanced-stage tumors, with OR = 2.8 and p = 0.002, while bidi smoking indicated a connection with nodal metastasis, with OR = 1.9 and p = 0.03, suggesting that various forms of tobacco are associated with different aggressive disease patterns [17]. In another Indian cohort study of 274 OSCC patients revealed the history of using tobacco products, gutka, and khaini was reported in 89% of patients. The most damaged regions were found to be the tongue and buccal mucosa, which are associated with tobacco use, exhibiting a strong correlation with advanced clinical and pathological stages [18]. A cross-sectional study in Finland consisting of 519 OSCC patients disclosed that the patients with tobacco and alcohol use history will have 26 times more prevalent tumors on the floor of the mouth, whereas this tobacco disease association weakens among gingival and buccal cavity tumours [19]. A population-based study of the U.S. with 19,536 cases revealed a 3.6-fold higher chance of developing oral malignancy in smokers than non-smokers. Smokeless tobacco users also had a moderate but significant elevation in risk [IRR (Incidence Rate Ratio) = 1.4, p = 0.02]. People who previously smoked and those who have ceased smoking had a much-reduced risk, indicating the need for cessation, which can help in preventing and managing the disease [20].

A Sri Lankan case-control study found that the risk of OSCC is 2.6-fold higher among smokers, and it increases to 4.3-fold for those who consume betel quid with tobacco. The very high OR of 15.3 has been yielded by the combined use of both products with carcinogenic impact [21]. The further association between these products has been validated by the analysis of 30 studies (2010-2020), suggesting a strong connection between smokeless tobacco consumption and OSCC, resulting from 21 studies, having odds ratios from 0.67 to 149 [22].

These studies conclude that tobacco (gutka, betel quid, and khaini) consumption in smoking or smokeless forms is the primary causative agent in OSCC. India and its neighbouring regions have significantly equal tobacco habits, causing a regional disease burden. The evidence of increased oral malignancy risk among populations brings forth the urgent need for effective tobacco-control strategies, public health education, and screening programs. With the advancement in molecular oncology and research, the knowledge of tobacco's role in cellular processes, which includes DNA damage and epigenetic modifications, has expanded. This offers a promising opportunity for

identifying novel biomarkers and helps in developing targeted epigenetics for tobacco-related oral malignancies.

Epigenetic modifications: a possible link between tobacco and oral carcinoma

Epigenetic alterations caused by the environmental factor tobacco, smoking or smokeless, is linked with oral carcinogenesis [7]. Epigenetic modifications refer to heritable yet reversible changes in gene expression that occur without altering the underlying DNA sequence, mainly through DNA methylation, histone modifications, and microRNA regulation [8]. Tobacco, with its derivatives, consisting of nicotine, tobacco-specific nitrosamines [N′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)], and polycyclic aromatic hydrocarbons, helps in initiating a cascade of epigenetic alterations that silence tumor suppressor genes, activate oncogenes, and promote uncontrolled cell proliferation [23]. Due to these alterations, oral epithelial cells transform to tumorous cells exhibiting a link of environmental exposure to permanent genetic changes [24]. The key component of tobacco, nicotine, binds to the nicotine acetylcholine receptors (nAChRs), EGFR, and β-AR (β-adrenergic receptor), triggering proliferative and survival signalling, thus promoting tumorigenesis. When nAChRs are activated, they activate the MAPK, AKT, and PKC pathways [25]. This makes cells multiply faster, inhibits apoptosis, and promotes angiogenesis. Nicotine-induced activation of EGFR and β-AR triggers tumor growth by acting as surrogate growth factors [26]. In OSCC cells, which have high proliferative activity and energy demands, the GLUT-1 level is reported to be overexpressed. This overexpression is mostly observed in correlation with different genetic and epigenetic factors, including tobacco as well. [27,28].

DNA methylation is the most studied epigenetic alteration affected by tobacco exposure. The reports suggested the changed expression of DNA methyltransferases induced by nicotine, such as DNMT1, DNMT3a, & DNMT3b, causing demethylation of the synuclein gamma oncogene [24]. Cigarette smoke modifies how DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) work, which causes abnormal methylation patterns all over the genome. NNK, a carcinogen found in tobacco, can turn on the AKT signaling pathway. This stops GSK3β and stabilizes DNMT1, which causes hypermethylation in the promoter regions of important tumor suppressor genes like CDKN2A (p16), DAPK, MGMT, and PTEN. The hypermethylation of these genes is frequently reported to co-occur with upregulated expression of GLUT-1 [29]

These alterations result in altered apoptosis, disturbed cell cycle control, and inhibited transcription. Conversely, global DNA hypomethylation caused by oxidative stress and reactive oxygen species (ROS), destabilizes the chromosome and turns on proto-oncogenes like EGFR, PI3K, and Cyclin D1 in an irregular way. AIM2, CEACAM1, LINE-1, PI3, and PTHLH genes are also involved in the progression of OSCC [8]. Tobacco carcinogens, such as dibenzo [def, p] chrysene, have been shown to trigger site-specific methylation in genes such as FGF3 and VAMP3, potentially functioning as early biomarkers of malignant transformation in oral mucosa [30].

Tobacco exposure also disrupts the vital component of epigenetic regulation that is histone modifications. Activation marks like H3K4me3 are raised, and the repressive marks are lowered (like H3K27me3) when carcinogens influence the patterns of histone methylation and acetylation [31]. All of these abrupt changes make it easier for the oncogenic genes to be transcribed. Histone acetylation alteration has been found to be associated in OSCC tissues invasiveness. The epigenetic silencing of pro-apoptotic genes and an increased malignant potential have caused the overexpression of histone deacetylases, especially HDAC6 and HDAC8 [32]. This connection between DNMT and

HDAC creates a feedback mechanism that keeps the phenotype stable and keeps transcriptional repression going on. Tobacco constituents significantly influence microRNAs (miRNAs), which are small non-coding RNAs that regulate gene expression post-transcriptionally. Nicotine and NNK influence miRNA expression via both methylation-dependent and independent mechanisms [33]. The upregulation of oncogenic miRNAs, such as miR-155, miR-202-3P, miR-34 FAMILY, miR-31-5p, miR-944, miR-101, miR-181b, miR-486, miR-1301, and miR-21, inhibits tumor suppressor targets (MSH2, CISH, PTEN) and activates carcinogenic pathways, consisting of PI3K/AKT, NF-κB, and STAT3, thereby promoting proliferation and inflammation [34]. Conversely, the downregulation of tumor-suppressive miRNAs, including miR-200 family, miR-29C, miR-4768-3P, miR-548AA, miR-3713, miR-30a, miR-379, miR-141, miR-429, and miR-137, facilitates epithelial-mesenchymal transition (EMT), metastasis, and resistance to therapy [35]. Both OSCC patient samples and tobacco-exposed oral keratinocyte models have confirmed the epigenetic silencing of these miRNAs.

New evidence also shows that some epigenetic changes caused by smoking last long after the person stops smoking, acting as "molecular scars" of past exposure. For example, AHRR, F2RL3, and CYP1A1 exhibit distinct hypomethylation patterns in the blood and buccal cells of smokers, persisting even decades post-cessation [36]. These persistent epigenetic signatures emphasize the enduring biological effects of tobacco and its contribution to the preservation of carcinogenic memory in oral tissues.

In summary, vital communications between DNA methylation, histone modification, and miRNA deregulation define the tobacco-induced epigenetic disruptions. These changes work together to inhibit tumor suppressor genes and activate oncogenes, thus causing less stability of the genome, therefore helps in oral carcinogenesis. Understanding these molecular mechanisms explains how tobacco products help in oral carcinogenesis and provides a basis for developing epigenetic biomarkers and therapeutic targets to counteract or mitigate these effects.

Targeting epigenetic modifications in oral cancer therapy

The reversible nature of epigenetic modifications, DNA methylation, histone modification, and non-coding RNAs makes them a promising therapeutic target for oral carcinoma management. Drugs that specifically target these mechanisms are known as “epidrugs,” including inhibitors of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs), with modulators of non-coding RNAs, and chromatin remodelers. Though in the case of OSCC, no FDA-approved epigenetic drug has been reported to date. However, recently, different FDA-approved epigenetic drugs (reported as therapy for various syndromes and other tumor types) are being repurposed in the case of OSCC. This approach has gained interest as a cost-effective and efficient approach for developing personalized oral carcinoma therapies [37].

DNMT inhibitors, including 5-azacytidine and decitabine, help in re-establishing the function of silenced tumor suppressor gene p16INK4a & MGMT via counteracting abnormal DNA hypermethylation. In preclinical models of OSCC, these inhibitors have the ability to induce apoptosis, cell cycle arrest, and differentiation [38]. Preclinical and early phase studies on OSCC cells suggested that the treatment with zebularine resulted in the inhibition of the tumor. It also enhances the efficacy of cisplatin and seems to decrease the efficacy of 5- fluorouracil when used in combination in OSCC [8,32]. Natural DNMT inhibitors like epigallocatechin gallate (EGCG), a derivative of green tea, and genistein from soy, exhibit demethylating properties and facilitate the restoration of tumor-suppressor gene expression [8]. Selective DNMT inhibitors and optimized delivery

systems are required to mitigate the challenges, including Off-target effects and toxicity, affecting clinical translation.

Histone deacetylase inhibitors (HDACis), such as vorinostat, romidepsin, trichostatin A, and entinostat, enhance histone acetylation, leading to chromatin relaxation and the reactivation of silenced tumor suppressor genes, namely p21, cyclins, and bcl-2 family. In OSCC, these agents promote apoptosis, inhibit proliferation, block the activity of HDAC enzymes, and enhance the sensitivity of resistant cells to cisplatin and radiation therapy [38]. In preclinical studies, Sodium butyrate and phenylbutyrate trigger G1/G2 cell cycle arrest, while romidepsin and apicidin exhibit anti-tumor effect. Apicidin induces apoptosis and autophagy with a significant reduction in tumor growth and proliferation in the mouse model [8,32]. In preclinical models of oral carcinoma, combination therapy consisting of HDAC inhibitor MS-275 with cisplatin has been shown to have synergistic effects on inhibition of tumor development [32].

Disruptive histone methylation, particularly mediated by EZH2, plays an important role in OSCC progression and therapeutic resistance development. Inhibitors like tazemetostat and GSK126 reduce H3K27me3 trimethylation, resulting tumor suppressor genes activation and enhanced immunological recognition. LSD1 inhibitors, such as ORY-1001 and IMG-7289, promote differentiation and suppress stemness, establishing these compounds as promising epidrugs in the management of tumors [39].

In oral carcinoma, microRNAs (miRNAs) alter the tumor-suppressive and carcinogenic pathways. Currently, this research is concentrated on methods that use antagomirs to inhibit oncogenic miRNAs and also includes miRNA mimics that restore the suppressive nature of miR-34a and miR-200. Restoring this network of tumor suppression is possible through the epigenetic reactivation of silenced miRNAs such as Mir-127 using substances like phenylbutyrate and 5-aza-2′-deoxycytidine [40]. Through increased stability and target specificity, recent advancements in viral vector and nanoparticle delivery systems are improving the therapeutic efficacy of miRNA-based epidrugs. Future directions may include multi-omics approaches, including epidrug repurposing, using integrative genomic and epigenomic profiling to identify patient-specific vulnerabilities, thereby enabling economical and personalized management of oral malignancies. To understand the tobacco-derived epigenetic modifications and their respective therapies, the following diagram (Fig.1) helps in building the knowledge about the tobacco-derived epigenetic modifications and how the epigenetic modifiers help in maintaining the genomic stability, cell proliferation, and angiogenesis.

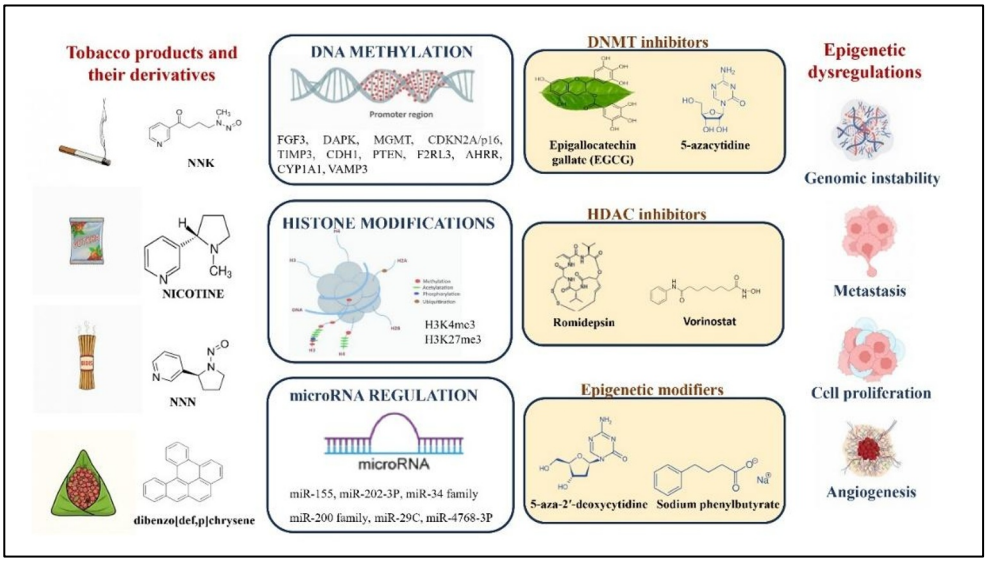

Fig.1. Tobacco and its derivatives induce some epigenetic modifications including DNA methylation, Histone modifications, and microRNA regulation which causes the suppression of tumor suppressor genes CDKN2A (p16), DAPK, MGMT, and PTEN and activation of oncogenes while dysregulating some of the microRNA which includes miR-155, 202-3P, and others. The epigenetic modifiers further help in restoring the normal functioning of tumor suppressor genes and regulate the microRNA functioning.

Conclusion

Tobacco exposure, being the major etiological factor for the genetic and epigenetic modifications in OSCC, has become a global health concern. Epigenetic modifications, including DNA methylation, histone modifications, and microRNA deregulation, work together to initiate the proliferation, invasion, metastasis, and therapeutic resistance development in tumor cells. Knowing the molecular mechanism behind the disease progression will help in the identification of epigenetic biomarkers with the advancement in personalised therapeutic interventions.

However, a number of challenges still exist. Traditional epigenetic therapies are less specific because of the heterogeneous nature of tumors and the lack of detection methods. Particularly, the inter and intra-tumor heterogeneity influence the epigenetic therapy response, which leads to differential efficacy and potential resistance across variable patient population. Future perspectives include the precise identification of molecular vulnerabilities in tobacco-linked OSCC via integration of multi-omics technologies which include metabolomics, genomes, transcriptomics, and epigenomics. Repurposing FDA-approved epigenetic agents also offers an expedited, cost-effective route to clinical application, particularly when paired with targeted and personalised therapy, immunotherapy, or a nanotechnology-based delivery system.

In summary, understanding tobacco-related epigenetic changes deepens our knowledge of OSCC development and provides a foundation for necessary treatments. Tackling these challenges in clinical and translational biology through medicine, improved epigenetic drug repurposing, and comprehensive research methods will help shape the future of oral carcinoma management.

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References:

1. T.S. Abbas, D. Mahdi, M.S. Ali, A.A. Al-Fahham, Etiology, epidemiology and pathophysiology of oral squamous cell carcinoma: a review article, International Journal of Medical Sciences and Dental Health, 11, 179–184 (2025).
2. H. Asif, A.J. Dar, M.O. Pasha, A. John, N. Iqbal, Pathology and epidemiology of oral squamous cell carcinoma, Diet Factor (Journal of Nutrition and Food Sciences), 1(2), 05–08 (2020).
3. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: A Cancer Journal for Clinicians, 71(3), 209–249 (2021).
4. Y. Tan, Z. Wang, M. Xu, B. Li, Z. Huang, S. Qin, E.D. Nice, J. Tang, C. Huang, Oral squamous cell carcinomas: state of the field and emerging directions, International Journal of Oral Science, 15(1), 44 (2023).
5. T. Dong, L. Wang, L. Liu, S. Ma, Susceptibility to oral squamous cell carcinoma: correlation with variants of CYP1A1-MspI, GSTT1, GSTM1, ALDH2, EC-SOD and lifestyle factors, Balkan Journal of Medical Genetics, 19(2), 61–70 (2016).
6. S. Irani, New insights into oral cancer-risk factors and prevention: a review of literature, International Journal of Preventive Medicine, 11, 202 (2020).
7. M.K. Mishra, S. Gupta, N. Shivangi, M. Sharma, S. Sehgal, The repertoire of mutational signatures in tobacco- and non-tobacco-induced oral cancer, Clinical and Translational Oncology, 25(12), 3332–3344 (2023).
8. H. Mesgari, S. Esmaelian, K. Nasiri, S. Ghasemzadeh, P. Doroudgar, Z. Payandeh, Epigenetic regulation in oral squamous cell carcinoma microenvironment: a comprehensive review, Cancers, 15(23), 5600 (2023).
9. N. Agarwal, A.K. Jha, DNA hypermethylation of tumor suppressor genes among oral squamous cell carcinoma patients: a prominent diagnostic biomarker, Molecular Biology Reports, 52(1), 44 (2024).
10. L. Cai, H. Zhu, Q. Mou, P.Y. Wong, L. Lan, C.W.K. Ng, P. Lei, M.K. Cheung, D. Wang, E.W.Y. Wong, E.H.L. Lau, Z.W.C. Yeung, R. Lai, K. Meehan, S. Fung, K.C.A. Chan, V.W.Y. Lui, A.S.L. Cheng, J. Yu, P.K.S. Chan, J.Y.K. Chan, Z. Chen, Integrative analysis reveals associations between oral microbiota dysbiosis and host genetic and epigenetic aberrations in oral cavity squamous cell carcinoma, NPJ Biofilms and Microbiomes, 10(1), 39 (2024).
11. D. Russo, F. Merolla, S. Varricchio, G. Salzano, G. Zarrilli, M. Mascolo, V. Strazzullo, R.M. Di Crescenzo, A. Celetti, G. Ilardi, Epigenetics of oral and oropharyngeal cancers, Biomedical Reports, 9(4), 275–283 (2018).

12. J. Hier, O. Vachon, A. Bernstein, I. Ibrahim, A. Mlynarek, M. Hier, A.M. Alaoui-Jamali, M. Maschietto, S.D. Da Silva, Portrait of DNA methylated genes predictive of poor prognosis in head and neck cancer and the implications for targeted therapy, Scientific Reports, 11(1), 10012 (2021).
13. S. Muralidharan, M. Nikalje, T. Subramaniam, J.A. Koshy, A.V. Koshy, D. Bangera, A narrative review on oral squamous cell carcinoma, Journal of Pharmacy and Bioallied Sciences, 17(Suppl 1), S204–S206 (2025).
14. P. Hsu, J. Huang, C. Tsai, Y. Lin, C. Kuo, Early molecular diagnosis and comprehensive treatment of oral cancer, Current Issues in Molecular Biology, 47(6), 452 (2025).
15. R. Mehrotra, B. Lauby-Secretan, Smokeless tobacco is a major risk factor for oral cancer in South and Southeast Asia, Oral Diseases, 31(5), 1473–1478 (2025).
16. C.N. J, H. Thapsey, N.M. S, Oral cancer and tobacco: a case control study in southern India, International Journal of Community Medicine and Public Health, 4(12), 4706–4711 (2017).
17. M.S. Ali, A.K. Chaudhary, P. Shukla, N. Purwar, P.K. Singh, Tobacco-driven oral cancer in India: clinicopathological correlates and prevention priorities, Cureus, 17(8), e89780 (2025).
18. S. Dwivedi, D. Mukherjee, N. Lohia, N. Thakur, R. Hans, R. Mohan, R. Sud, H. Darling, Association of tobacco exposure with demographic and clinicopathological factors in patients of oral squamous cell carcinoma — a single-center experience, Journal of Cancer Research and Therapeutics, 19(Suppl 2), S608–S613 (2023).
19. R. Eloranta, S. Vilén, A. Keinänen, T. Salo, A. Qannam, I.O. Bello, J. Snäll, Oral squamous cell carcinoma: effect of tobacco and alcohol on cancer location, Tobacco Induced Diseases, 22, 1–9 (2024).
20. B. Noggle, H. Cheng, M. Sarkar, Oral cancer incidence among adult males with current or former use of cigarettes or smokeless tobacco: population-based study, JMIR Cancer, 10, e51936 (2024).
21. S.T. Edirisinghe, M. Weerasekera, D.K. De Silva, I. Liyanage, M. Niluka, K. Madushika, S. Deegodagamage, C. Wijesundara, A.M. Rich, H. De Silva, H.M. Hussaini, K. De Silva, S. Yasawardene, The risk of oral cancer among different categories of tobacco smoking exposure in Sri Lanka, Asian Pacific Journal of Cancer Prevention, 23(9), 2929–2935 (2022).
22. Z. Dalirsani, A. Ghazi, A. Pakfetrat, J. Jamali, Z. Foroughi, M. Mirzaei, The relationship between smokeless tobacco and the incidence of oral cancer: a systematic review study, Addiction and Health, 17, 1522 (2025).
23. V. Polo, G. Pasello, S. Frega, A. Favaretto, H. Koussis, P. Conte, L. Bonanno, Squamous cell carcinomas of the lung and of the head and neck: new insights on molecular characterization, Oncotarget, 7(18), 25050–25063 (2016).
24. E. Nooshinfar, D. Bashash, M. Abbasalizadeh, A. Safaroghli-Azar, P. Sadreazami, M.E. Akbari, The molecular mechanisms of tobacco in cancer pathogenesis, Iranian Journal of Cancer Prevention, In Press (2017).
25. C. Schaal, S.P. Chellappan, Nicotine-mediated cell proliferation and tumor progression in smoking-related cancers, Molecular Cancer Research, 12(1), 14–23 (2014).
26. T. Nishioka, H. Tada, S. Ibaragi, C. Chen, T. Sasano, Nicotine exposure induces the proliferation of oral cancer cells through the α7 subunit of the nicotinic acetylcholine receptor, Biochemical and Biophysical Research Communications, 509(2), 514–520 (2018).
27. N. Azad, M.K. Maurya, M. Kar, M.M. Goel, A.K. Singh, M. Sagar, D. Mehrotra, V. Kumar, Expression of GLUT-1 in oral squamous cell carcinoma in tobacco and

non-tobacco users, Journal of Oral Biology and Craniofacial Research, 6(1), 25–30 (2016).
28. I. Gupta, F. Badrzadeh, Y. Tsentalovich, D. A. Gaykalova, Connecting the dots: investigating the link between environmental, genetic, and epigenetic influences in metabolomic alterations in oral squamous cell carcinoma, Journal of Experimental & Clinical Cancer Research, 43(1), 239 (2024).
29. D. Camuzi, T. De Almeida Simão, F. Dias, L.F.R. Pinto, S.C. Soares-Lima, Head and neck cancers are not alike when tarred with the same brush: an epigenetic perspective from the cancerization field to prognosis, Cancers, 13(22), 5630 (2021).
30. K. Chen, Y. Sun, Y.I. Kawasawa, A.C. Salzberg, J. Zhu, K. Gowda, C. Aliaga, H. Atkins, K. El-Bayoumy, Black raspberry inhibits oral tumors in mice treated with the tobacco smoke constituent dibenzo(def,p)chrysene via genetic and epigenetic alterations, Cancer Prevention Research, 13(4), 357–366 (2020).
31. K. Burkitt, V. Saloura, Epigenetic modifiers as novel therapeutic targets and a systematic review of clinical studies investigating epigenetic inhibitors in head and neck cancer, Cancers, 13(20), 5241 (2021).
32. A.I. Irimie, C. Ciocan, D. Gulei, N. Mehterov, A.G. Atanasov, D. Dudea, I. Berindan-Neagoe, Current insights into oral cancer epigenetics, International Journal of Molecular Sciences, 19(3), 670 (2018).
33. K. Hajiasgharzadeh, B. Naghipour, P. Shahabi, N. Dastmalchi, M.R. Alipour, The role of microRNAs in nicotine signaling, PubMed, 22, 433–450 (2023).
34. H.S. Chawra, M. Agarwal, A. Mishra, S.S. Chandel, R.P. Singh, G. Dubey, N. Kukreti, M. Singh, MicroRNA-21's role in PTEN suppression and PI3K/AKT activation: implications for cancer biology, Pathology Research and Practice, 254, 155091 (2024).
35. O. Aghiorghiesei, O. Zanoaga, A. Nutu, C. Braicu, R.S. Campian, O. Lucaciu, I.B. Neagoe, the world of oral cancer and its risk factors viewed from the aspect of microRNA expression patterns, Genes, 13(4), 594 (2022).
36. Keshawarz, R. Joehanes, W. Guan, T. Huan, D.L. DeMeo, M.L. Grove, M. Fornage, D. Levy, G. O'Connor, Longitudinal change in blood DNA epigenetic signature after smoking cessation, Epigenetics, 17(10), 1098–1109 (2021).
37. M. Montalvo-Casimiro, R. González-Barrios, M.A. Meraz-Rodriguez, V.T. Juárez-González, C. Arriaga-Canon, L.A. Herrera, Epidrug repurposing: discovering new faces of old acquaintances in cancer therapy, Frontiers in Oncology, 10, 605386 (2020).
38. M. Krishnan, S. Basappa, S. Babu, Epigenetic alterations in oral cancer: mechanisms, biomarkers, and therapeutic targets, Oral Oncology Reports, 12(10), 100681 (2024).
39. S. Feng, D.D. De Carvalho, Clinical advances in targeting epigenetics for cancer therapy, FEBS Journal, 289(5), 1214–1239 (2021).
40. R. Castilho, C. Squarize, L. Almeida, Epigenetic modifications and head and neck cancer: implications for tumor progression and resistance to therapy, International Journal of Molecular Sciences, 18(7), 1506 (2017).

Abbreviations

OSCC- Oral squamous cell carcinoma

GSTs- Glutathione-S-transferases

XMEs- Xenobiotic-metabolizing enzymes

MiRNAs- microRNAs

OR- Odds ratio

IRR- Incidence Rate Ratio

NNN- N′-nitrosonornicotine

NNK- 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

PAHs- polycyclic aromatic hydrocarbons

nAChRs- nicotine acetylcholine receptors

GLUT-1- Glucose transporters-1

HDAC- Histone deacetylase

DNMT- DNA Methyltransferase

EMT- Epithelial-mesenchymal transition

HDACis- Histone deacetylase inhibitors

AKT- Ak strain transforming

MAPK- Mitogen-Activated Protein Kinase