

Epigenetic editing and tumor-dependent immunosuppressive signaling in head and neck malignancies (Review)

SPYRIDON GOUGOUSIS¹, SAVVAS PETANIDIS², ALEXANDROS POUTOGLIDIS¹,
NIKOLAOS TSETSOS¹, PARASKEVAS VROCHIDIS³, IOANNIS SKOUMPAS⁴,
NEKTARIOΣ ARGYΡΙΟΥ⁵, THEODORA KATOPODI⁶ and KALLIOPI DOMVRI^{7,8}

¹Ear Nose Throat Department, General Hospital of Thessaloniki ‘G. Papanikolaou’, Thessaloniki GR-57010, Greece;

²Department of Pulmonology, I.M. Sechenov First Moscow State Medical University, Moscow 119992, Russian Federation;

³Ear Nose Throat Department, General Hospital of Goumenissa, Kilkis GR-61100;

⁴Ear Nose Throat Department, General Hospital of Katerini, Katerini GR-60100; ⁵Ear Nose Throat Department, General Hospital of Thessaloniki ‘G. Gennimatas’, Thessaloniki GR-54635; ⁶Department of Medicine, Laboratory of Medical Biology and Genetics, Aristotle University of Thessaloniki, Thessaloniki GR-54124;

⁷Pulmonary Department, General Hospital of Thessaloniki ‘G. Papanikolaou’, Aristotle University of Thessaloniki, Thessaloniki GR-57010; ⁸Laboratory of Histology and Embryology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, GR-54124, Greece

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Abstract. Head and neck cancer (HNC) comprises a heterogeneous variety of malignant tumors, characterized by a relatively high tumor mutation burden. Previous data have revealed that immune system dysfunction appears to serve a key role in the development and progression of HNC and established immunosuppression is vital for evading the host immune response. Despite progress in chemotherapy and radiotherapy, the survival rate of patients with HNC is still low. Therefore, the present review discusses the development of novel immunotherapy approaches based on the various immune cell signaling routes that trigger drug resistance and immunosuppression. Additionally, the present review discusses the epigenetic alterations, including DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs that drive and support HNC progression. Furthermore, the role of cancer-associated fibroblasts, tumor macrophages and myeloid cells in tumor-related immunosuppression are considered. Specifically, the molecular immune-related mechanisms in the tumor microenvironment, which lead to decreased drug sensitivity and tumor relapse, and strategies for reversing drug resistance and targeting immunosuppressive

tumor networks are discussed. Deciphering these molecular mechanisms is essential for preclinical and clinical investigations in order to enhance therapeutic efficacy. Furthermore, an improved understanding of these immune cell signaling pathways that drive immune surveillance, immune-driven inflammation and tumor-related immunosuppression is necessary for future personalized HNC-based therapeutic approaches.

Contents

1. Introduction
2. Current immunotherapy regimens
3. Impact of DNA methylation on disease pathogenesis
4. Regulation via histone modifications
5. Chromatin remodeling as a driver of cancer
6. Molecular role of lncRNAs
7. Circular RNAs (circRNAs) in HNC progression
8. CAFs in the tumor microenvironment
9. Myeloid-derived suppressor cell (MDSC)-related immunosuppression
10. Crosstalk with TAMs
11. Conclusions

1. Introduction

Head and neck cancers (HNCs) constitute a heterogeneous group of malignant tumors characterized by a high mortality rate (1). Aggressive biologic behavior and strong resistance to therapy lead to >650,000 cases and 330,000 deaths worldwide in 2020 (2).

Genetic mutations and environmental factors are associated with the genesis and progression of these malignancies.

Correspondence to: Dr Kalliopi Domvri, Pulmonary Department, General Hospital of Thessaloniki ‘G. Papanikolaou’, Aristotle University of Thessaloniki, Leoforos George Papanikolaou, Thessaloniki GR-57010, Greece

E-mail: kdomvrid@auth.gr

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Tobacco use, Epstein-Barr virus (EBV), human papillomavirus (HPV), alcohol consumption and exposure to environmental carcinogenic factors are associated with DNA mutations and lead to dysplastic lesions in the head and neck area (3,4). Over the last decades, specific genes, which are responsible for hereditary types of HNC, have been identified (5). Currently, there are several treatment options available; however, due to the high rate of relapses, the search for systemic and local alternative treatments is becoming more and more important in modern days (6,7). Entities such as familial oral squamous cell carcinoma (OSCC), nasopharyngeal cancer and familial malignant melanoma are strongly related to hereditary DNA mutations (8).

Furthermore, immune dysfunction serves a vital role in the development and progression of head and neck squamous cell carcinoma (HNSCC) (9). Previous data have revealed that immunosuppression signaling (via myeloid cells or M2 macrophages) prompts the development and progression of the disease, which is characterized by intra-tumoral heterogeneity, high mortality and poor prognosis for patients with HNSCC (10).

In the past few years, several epigenetics mechanisms have been revealed to influence the carcinogenesis of the head and neck region (11). Gene silencing by epigenetic mechanisms and hypermethylation of CpG islands are active areas of research. Deep knowledge of epigenetic pathways and mechanisms is essential for establishing innovative approaches to diagnosis and treatment of HNC (12). Thus, epigenetic editing refers to all epigenetic mechanisms that regulate genome output and the potential future application of these epigenetic editing mechanisms for the diagnosis and treatment of HNC disease.

Cancer cells employ a variety of mechanisms to avoid the immune system and secure malignant progression. Specifically, distinct ligands on the cancer cell surface interact with immune cells and activate inhibitor pathways to stop the immune response (13). Epigenetic drugs (Vorinostat, Romidepsin) are able to prevent inhibition by enhancing the expression of tumor antigens, a fundamental function that restores normal immunogenicity (14). Thus, epigenetic agents can enhance the host immune system by boosting the immune responses triggered via immunotherapy (15). This innovative and promising finding will allow a combined approach (epigenetic therapy and immunotherapy) for the treatment of several types of HNC. Overall, the present review discusses the epigenetic editing mechanisms and the various tumor-related immunosuppressive signaling routes that trigger drug resistance (chemoresistance) and immunosuppression. Additionally, the present review discusses the immune modifications and examines the role of cancer-associated fibroblasts (CAFs), tumor macrophages and myeloid cells in tumor-related immunosuppression.

2. Current immunotherapy regimens

During the last few years, novel promising therapies for HNC, including immune checkpoint inhibitors, have demonstrated encouraging results in several clinical trials (16-21). For instance, nivolumab and pembrolizumab are the main humanized antibodies specifically designed to block programmed

death-ligand 1 (PD-L1) from binding to programmed cell death-1 (PD-1), allowing T cell activation (Table I). These drugs have also been used for the treatment of patients with metastatic HNSCC (22-24). In general, immune checkpoint signaling is triggered by ligand and receptor interactions (17), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and its ligands CD80 and CD84, and PD-1 and its ligands PD-L1 and programmed cell death ligand 2 (25). Specifically, ipilimumab [Food and Drug Administration (FDA)-approved for metastatic melanoma] a monoclonal antibody that can re-activate the immune system by targeting CTLA-4, has been demonstrated to be associated with overall survival improvements among patients with squamous cell carcinoma of the head and neck (SCCHN) (26). Similarly, tremelimumab is a fully human monoclonal antibody against CTLA-4, which blocks the binding of the antigen-presenting cell ligands B7.1 and B7.2 to CTLA-4 (27). Furthermore, EGFR inhibitors are also used in HNC treatment. Cetuximab (Erbitux), a monoclonal antibody given by intravenous infusion, was approved by the FDA in 2006 as a combination with radiation therapy for the treatment of SCCHN (28). Blocking EGFR activity is vital, since EGFR expression is upregulated in 85% of all HNSCC cases and leads to tumor cell proliferation, angiogenesis, invasion, tumor recurrence and metastasis. Similarly, TNF receptor-targeted monoclonal antibodies, such as urelumab (29), target the extracellular domain of CD137, triggering activation of CD137-expressing immune cells and especially cytotoxic T cells. Furthermore, bevacizumab is a monoclonal antibody that acts as an angiogenesis inhibitor. It prevents new blood vessel formation by inhibiting vascular endothelial growth factor A (30). Similarly, ficolatuzumab is a potent hepatocyte growth factor (HGF) IgG1 antibody, which inhibits signaling through the HGF/c-Met signaling pathway and is already in phase II clinical trials (31,32). Finally, necrosis-targeted IL-12 immunocytokine is a novel immunocytokine designed for delivery of IL-12 to the tumor microenvironment, which has been demonstrated to be associated with increased overall antitumor efficacy and substantial reduction in tumor growth compared to standard chemotherapy (33).

3. Impact of DNA methylation on disease pathogenesis

DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine (34,35). Methylation usually occurs in CpG islands (Fig. 1) and characteristically acts to repress gene transcription in HNSCC (36-40). Aberrant methylation in the promoter region of tumor suppressor genes could induce their abnormal expression in order to influence carcinogenesis. Methylation of genes involved in DNA damage repair, detoxification, cell cycle regulation and apoptosis plays a key role in tumor development and progression (Table II). Previous studies have revealed that abnormal O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation can markedly increase the risk of tumorigenesis in HNC (41,42). Furthermore, promoter methylation of the MGMT, MutL homolog 1 and Ras association domain family 1 isoform A tumor suppressor genes contributes to HNSCC development (43). Similarly, somatic mutations and promoter methylation of the ryanodine receptor

Table I. List of drugs for the treatment of head and neck cancer currently undergoing clinical trials.

First author, year	Drug name	Function	Molecular target	Treatment regimen	Phase	(Refs.)
Motzer <i>et al</i> , 2019	Avelumab	Immune checkpoint	PD-1	Single agent	I	(18)
	Sunitinib	Angiogenesis	PDGF/VEGFR	Combination	II	
Janjigian <i>et al</i> , 2021	Ipilimumab	Immune checkpoint	CTLA-4	Combination	II	(26)
Ferris <i>et al</i> , 2016	Nivolumab	Immune checkpoint	PD-1	Combination	II	(23)
Burtness <i>et al</i> , 2019	Pembrolizumab	Immune checkpoint	PD-1	Single agent	I	(24)
Chu <i>et al</i> , 2021	Apatinib	Angiogenesis	VEGFR-2	Single agent	II	(19)
Garcia <i>et al</i> , 2020	Bevacizumab	Angiogenesis	VEGF-A	Combination	II	(30)
Chester <i>et al</i> , 2018	Urelumab	T cell response	CD137	Single agent	I	(29)
Mollica Poeta <i>et al</i> , 2019	Mogamulizumab	T cell signaling	CCR4	Single agent	II	(20)
Greiner <i>et al</i> , 2021	NHSIL-12	Inflammation	IL-12	Single agent	I	(33)
Pooler <i>et al</i> , 2021	LDE225	Hedgehog signaling	SMO	Single agent	I	(21)

NHSIL-12, necrosis-targeted IL-12 immunocytokine; LDE225, sonidegib; PD-1, programmed cell death-1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PDGF, platelet-derived growth factor; VEGF-A, vascular endothelial growth factor-A; CCR4, C-C motif chemokine receptor 4; SMO, smoothened.

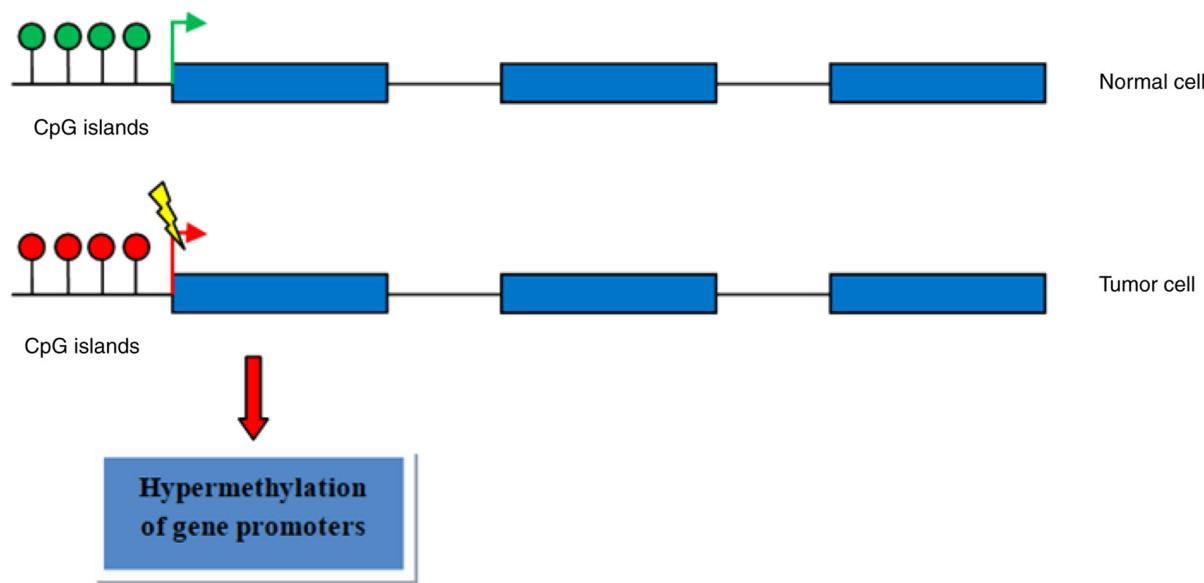


Figure 1. Schematic illustration of DNA methylation in head and neck cancer resulting in gene silencing of tumor suppressor genes, such as O-6-methylguanine-DNA methyltransferase, mutL homolog 1 and Ras association domain family 1 isoform A.

2 are responsible for the invasive pathogenesis of HNSCC (44). Long non-coding RNAs (lncRNAs) may also affect methylation levels in HNC. For instance, LNCAROD, an oncogenic lncRNA, is stabilized by m6A methylation and promotes cancer progression by forming a ternary complex with heat shock protein family A (Hsp70) member 1A (HSPA1A) and Y-box binding protein 1 (YBX1) in HNSCC (45). LNCAROD acts as a scaffold for the interaction between YBX1 and HSPA1A, preventing proteasomal degradation of YBX1 in HNSCC cells. Systematic analysis of differentially methylated expressed genes and site-specific methylation may also be used as potential prognostic markers in HNC. Specifically, abnormally differentially methylated genes, including paired box 9, serine/threonine kinase 33, G protein-coupled receptor 150, INSM transcriptional repressor 1 and epoxide hydrolase

3, have been recognized to be associated with overall survival, and this may aid the identification of diagnostic biomarkers for early-stage HNC (46). In addition, site-specific methylation patterns of galanin and galanin receptor 1/2 may serve as an important site-specific biomarker for the prediction of the clinical outcome in patients with HNSCC (47). Of note, circulating tumor DNA (ctDNA) analysis may be important in early diagnosis and eventually improve the outcomes of patients with HNSCC. Specifically, ctDNA analysis in HPV-associated oropharyngeal cancer indicated that three genes, including calmodulin like 5, Dna heat shock protein family (Hsp40) member C5γ and lymphocyte antigen 6 family member D, had a high predictive ability as emerging biomarkers and could determine patient prognosis and real-time surveillance for disease recurrences (48).

Table II. List of genes frequently altered by DNA methylation in head and neck cancer.

First author, year	Gene name	Function	Methylation status	(Refs.)
Eads <i>et al.</i> , 2001	CDKN2B (p15)	Cell cycle arrest	Hypermethylated	(37)
	TIMP3	Extracellular matrix degradation	Hypermethylated	
	ADGRE3	Cell surface receptor	Hypomethylated	
	SPP1	Cellular adhesion	Hypomethylated	
Ji <i>et al.</i> , 2016	MLH1	DNA damage repair	Hypermethylated	(42)
	RASSF1	Cytoskeleton organization	Hypermethylated	
Chen <i>et al.</i> , 2021	ATM	DNA damage repair	Hypermethylated	(38)
Zhou <i>et al.</i> , 2018	HOXA9	Cell differentiation	Hypermethylated	(39)
	AIM2	Apoptosis	Hypomethylated	
Hier <i>et al.</i> , 2021	MINT31	Chromatin remodeling	Hypermethylated	(40)
	PI3	Inflammation signaling	Hypomethylated	

CDKN2B, cyclin-dependent kinase 4 inhibitor B; TIMP3, TIMP metallopeptidase inhibitor 3; MLH1, MutL homolog 1; RASSF1, ras association domain family member 1; ATM, ataxia-telangiectasia mutated; HOXA9, homeobox A9; MINT31, methylation in gastric noninvasive neoplasia; PI3, peptidase inhibitor 3; AIM2, absent in melanoma 2; ADGRE3, adhesion G protein-coupled receptor E3; SPP1, secreted phosphoprotein 1.

4. Regulation via histone modifications

Chromatin architecture is mainly regulated by histone proteins. DNA is wound around histones to form nucleosomes. Histone modifications are mostly post translational modifications to histone proteins and include methylation, phosphorylation, acetylation, ubiquitylation and sumoylation (49). In HNC, histone modifications can affect the molecular pathogenesis of the disease. Specifically, histones may be post-translationally modified at the amino-terminal ends by acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and ADP-ribosylation. Recently, the cancer driver genes isocitrate dehydrogenase [NADP(+)] (IDH)1/2, lysine demethylase 5C (KDM5C) and KDM6A were revealed to prompt histone demethylation and hypoxic reprogramming in cancer metabolism (50). Similarly, a novel IFN α -induced lncRNA was observed to negatively regulate immunosuppression by interrupting H3K27 acetylation in HNSCC. Ectopic expression of lncMX1-215 markedly inhibits the expression of IFN α -induced, immunosuppression-related molecules, including PD-L1 and galectin-9 (51). In addition, enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), a histone-lysine N-methyltransferase enzyme that participates in histone methylation and transcriptional repression, serves a key role in HNSCC progression. Specifically, targeting EZH2 enhances antigen presentation and antitumor immunity, and circumvents anti-PD-1 resistance in HNC. This is associated with increased antigen-specific CD8 $^{+}$ T-cell proliferation, IFN γ production and tumor cell cytotoxicity (52). Of note, EZH2 inhibition reduces the histone H3K27me3 modification on the β 2-microglobulin promoter and suppresses tumor growth following combination therapy in an anti-PD-1-resistant model of HNSCC (52). Recently, the histone demethylases JARID1C/KDM5C and UTX/KDM6A and the gene-encoding metabolic enzymes IDH1/2 were identified as cancer driver genes that serve a key role in histone demethylation and hypoxic reprogramming in cancer metabolism in HNC (50).

LncRNAs may also affect the histone modification mechanism. The lncRNA PVT1 can regulate nasopharyngeal carcinoma cell proliferation via activation of the lysine acetyltransferase 2A (KAT2A) acetyltransferase and stabilization of hypoxia-inducible factor 1- α (HIF-1 α) (53). PVT1 serves as a scaffold for the chromatin modification factor KAT2A, which mediates histone 3 lysine 9 acetylation, recruiting the nuclear receptor binding protein transcriptional intermediary factor 1 β to activate nuclear factor 90 transcription, thereby increasing HIF-1 α stability and promoting a malignant phenotype in neural progenitor cells (NPCs) (53). Another lncRNA, CCAT1, can promote H3K27-acetylation and modulate the histone methylation of SPRY4 (sprouty RTK signaling antagonist 4) and homeobox B13 in the nucleus and cytoplasm, which affects cell proliferation and migration in esophageal squamous cell carcinoma (ESCC) (54).

5. Chromatin remodeling as a driver of cancer

Chromatin remodeling is vital for the rearrangement of chromatin from a condensed state to a transcriptionally active state. This allows access of genomic DNA to the regulatory transcription machinery proteins and regulates gene expression. Previous studies have demonstrated that chromatin hypoacetylation, histone 3 in HNSCC cells is hypoacetylated and that endothelial cells induce tumor acetylation (55). For instance, TP63 triggers chromatin remodeling and enhances reprogramming to epidermal differentiation and thus prompting squamous cell carcinoma development (56). In addition, zinc finger and SCAN domain containing 4 (ZSCAN4) prompts functional histone 3 hyperacetylation at the promoters of OCT3/4 and NANOG, leading to an upregulation of cancer stem cell (CSC) factors. Consistently, ZSCAN4 depletion leads to downregulation of CSC markers, a decrease in the formation of tumor spheres and inhibition of tumor growth (57). Chromatin remodeling is also initiated by lin-28 homolog B, which promotes HNC progression via regulation

of the insulin-like growth factor genes (high mobility group AT-hook 2, cyclin D2, insulin-like growth factor 1 receptor and insulin like growth factor 2 mRNA binding protein 2) (58). Similarly, lymphoid-specific helicase (LSH) promotes cancer progression by regulating the expression of fumarate hydratase (FH), a core component of the tricarboxylic acid cycle (59). LSH binds to the FH promoter, recruiting the epigenetic silencer factor histone H3 Lys 9-specific histone methyltransferase, known as G9a, to repress FH transcription. Loss-of-function mutations in SWI/SNF chromatin-remodeling subunit genes are also detected in HNSCC (60). Actin-like 6A (ACTL6A), encoding an SWI/SNF subunit linked to stem cell function, drives Yes1-associated transcriptional regulator (YAP) activation and is highly expressed together with the p53 family member p63 in HNSCC. This molecular mechanism reveals that ACTL6A and p63 collaborate as oncogenic drivers in HNSCC.

6. Molecular role of lncRNAs

LncRNAs are RNA isoforms larger than 200 nucleotides that act as regulators of gene expression (61). They are involved in numerous cellular processes, including cell proliferation, apoptosis and cellular metabolism, as well as differentiation and development (62). LncRNA MIR31HG has been reported to target HIF-1 α and P21 to facilitate HNC cancer cell proliferation and tumorigenesis by promoting cell-cycle progression (63). Furthermore, lncRNA LINC00460 enhances HNSCC cell proliferation and epithelial-mesenchymal transition (EMT)-related metastasis by facilitating peroxiredoxin-1 transfer into the nucleus and transcription of EMT-related genes, including zinc finger E-box binding homeobox 1, zinc finger E-box binding homeobox 2 and vimentin (64). On the other hand, lncRNA SLC26A4-AS1 can act as a tumor suppressor by inhibiting the migration, invasion and metastasis of tumor cells *in vitro* and *in vivo*. Of note, SLC26A4-AS1 is able to interact with DEAD-box helicase 5 (DDX5) and the E3 ligase tripartite motif containing 25, and promote DDX5 degradation through the ubiquitin-proteasome pathway (65). In particular, SLC26A4-AS1 inhibits the expression of multiple DNA double-strand break repair genes and thyroid cancer metastasis by destabilizing DDX5. In addition, other lncRNAs have been reported to function as promoters or inhibitors of cancer metastasis. Specifically, MYOSLID promotes invasion and metastasis of HNSCC by modulating partial EMT via regulation of metastasis-related genes, including Slug, podoplanin and laminin subunit β 3 (66).

7. Circular RNAs (circRNAs) in HNC progression

HNC tumor development may also be controlled by circRNAs present in the tumor microenvironment (67). CircRNAs are single-stranded, covalently closed RNA molecules that can act as microRNA (miR) sponges (68). Furthermore, specific non-coding RNA members (circRNA, lncRNA and miRNA) are also involved in various tumor-promoting mechanisms like methylation, hypoxia signaling, immune suppression and tumor-related inflammation (Fig. 2). CircRNA_102171 has been revealed to interact with catenin β interacting protein 1 to block its interaction with the β -catenin/transcription

factor 3/transcription factor 4/lymphoid enhancer binding factor 1 complex, leading to activation of the Wnt/ β -catenin signaling pathway in papillary thyroid cancer (69). Similarly, circRNA_100290 can function as a competing endogenous RNA to regulate CDK6 expression in oral cancer by sponging up miR-29b family members (70). CircPVT1 may act as an oncogene in HNSCC, where it is transcriptionally enhanced by the mut-p53/YAP/TEA domain transcription factor 1 complex and alters the expression of miR-497-5p and genes involved in regulation of cell proliferation (71). Another circRNA member, circGSK3 β , may trigger ESCC cell migration and invasion via direct interaction with GSK3 β and inhibition of GSK3 β activity (72). Certain circRNAs also serve important roles in regulating the radioresistance of ESCC (73). In particular, circRNA_100367 enhances the radioresistance of ESCC cells (KYSE-150R) via the miR-217/Wnt3 signaling pathway.

8. CAFs in the tumor microenvironment

HNC development and progression are also regulated by the CAFs signaling cascade (Fig. 3). CAFs are a group of activated fibroblasts with marked heterogeneity and plasticity in the tumor microenvironment. They secrete a variety of active factors [inside the TME, that promote extensive angiogenesis signaling (via TGF β), increased matrix deposition (via PDGF, SDF-1), and tissue remodeling (a-SMA, PGE-2, periostin)]. CAF-derived exosomal miR-196a can confer cisplatin resistance in HNC by targeting cyclin dependent kinase inhibitor 1B and inhibitor of growth family member 5, indicating that miR-196a may serve as a promising predictor of and potential therapeutic target for cisplatin resistance in HNC (74). CAFs may also affect HNC stemness inside the tumor microenvironment. For instance, periostin secreted by CAFs promotes cancer stemness in HNC via activation of the protein tyrosine kinase 7 (inactive)-Wnt/ β -catenin signaling pathway (75). Similarly, when HNSCC cells are cocultured with normal fibroblasts, this upregulates autophagy via IL6, IL8 and basic fibroblast growth factor, promoting HNC progression (76). CAF-derived IL-6 may also prompt HNC progression via osteopontin (OPN)-NF- κ B signaling, which is associated with poor patient prognosis (77). CAFs may also affect the functional polarization of tumor-associated macrophages (TAMs) in OSCC. Specifically, CAFs promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages, which is also associated with lymphatic invasion, vascular invasion, lymph node involvement and TNM stage (78).

9. Myeloid-derived suppressor cell (MDSC)-related immunosuppression

MDSCs are a heterogeneous group of immature myeloid cells implicated in the regulation of immune responses. MDSCs are directly implicated in the pathogenesis of HNC. Therefore, studies target these myeloid cell subsets in order to reverse immunosuppression (79,80). Recently, STAT3 inhibition in combination with radiation, improved tumor growth delay in HNC via downregulation of MDSCs, decrease in Tregs and upregulation of effector T cells and M1 macrophage levels (81). In addition, distinct populations of

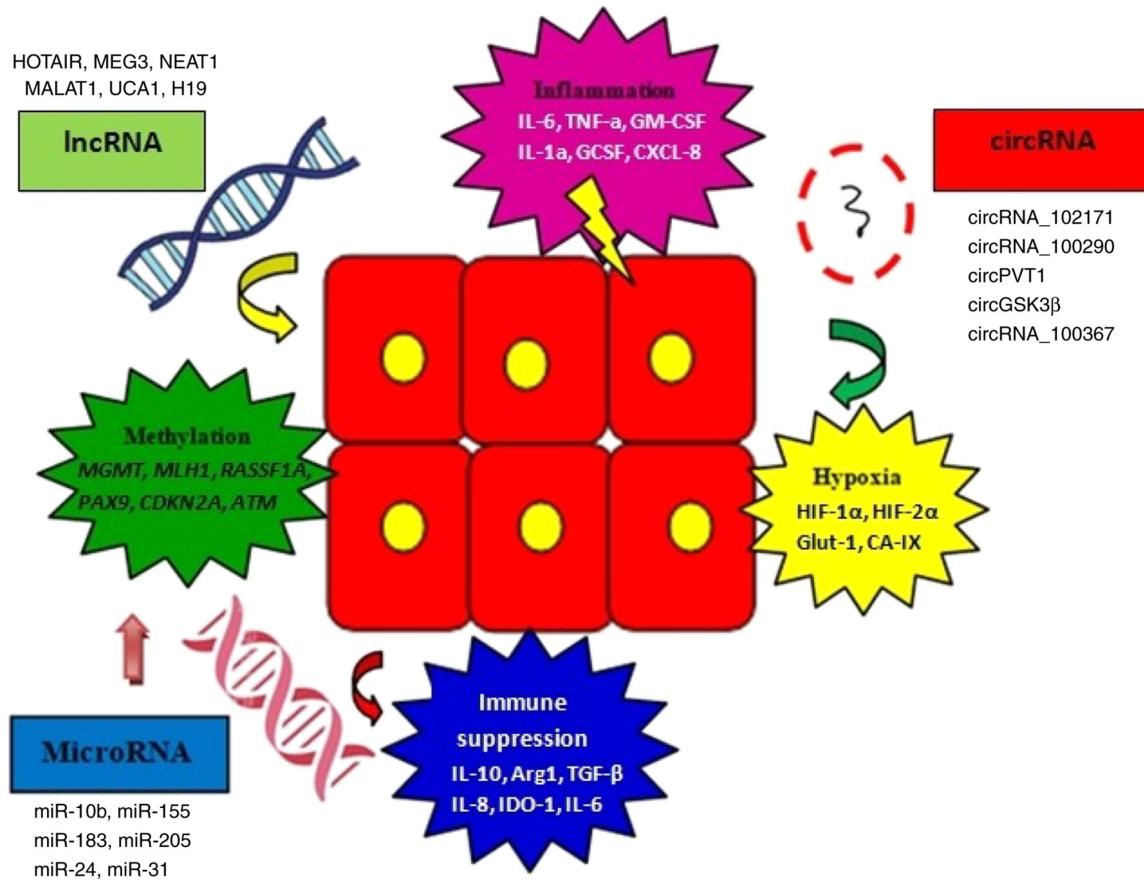


Figure 2. Specific non-coding RNAs (circRNA, lncRNA and microRNA) serve a key role in tumor-promoting mechanisms, such as methylation, hypoxia signaling, immune suppression and tumor-related inflammation. Arg1, arginase-I; ATM, ataxia-telangiectasia mutated; CA-IX, carbonic anhydrase IX; CDKN2A, cyclin-dependent kinase inhibitor 2A; circRNA/circ, circular RNA; CXCL-8, C-X-C motif chemokine ligand 8; GCSF, granulocyte colony stimulating factor; Glut-1, glucose transporter 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIF, hypoxia-inducible factor; IDO-1, indoleamine 2,3-dioxygenase 1; lncRNA, long-non coding RNA; MGMT, O-6-methylguanine-DNA methyltransferase; MLH1, MutL homolog 1; miR, microRNA; PAX9, paired box 9; RASSF1A, Ras association domain family 1 isoform A.

immune-suppressive macrophages differentiate from monocytic MDSCs via increased expression of S100A9 protein and transcription factor CCAAT/enhancer binding protein β (82). Furthermore, stimulator of interferon response cGAMP interactor (STING) serves a vital role in both differentiation and regulation of MDSC expression levels. STING represses NPC-derived MDSC induction by enhancing suppressor of cytokine signaling 1 (SOCS1) expression in both tumor cells and MDSCs (83). SOCS1 physically interacts with STAT3 through its SH2 domain to prevent STAT3 phosphorylation and dimerization, resulting in reduced MDSC induction via inhibition of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6 production (83). The expansion of MDSCs is also critical for tumor propagation. EBV latent membrane protein 1 (LMP1) may promote MDSC expansion in the tumor microenvironment by inducing extra-mitochondrial glycolysis in malignant cells, which triggers immune escape initially. Specifically, LMP1 promotes the expression of multiple glycolytic genes, including glucose transporter 1 (84). This metabolic reprogramming results in increased levels of the Nod-like receptor family protein 3 inflammasome, cyclooxygenase 2 and phosphorylated-p65 and, consequently, increased production of IL-1 β , IL-6 and GM-CSF (84). T-cell immunoglobulin mucin 3 (TIM3) may also affect MDSC

differentiation. TIM3 expression is increased in patients with recurrent HNSCC (85). CD8 $^{+}$ T cells and CD11b $^{+}$ CD33 $^{+}$ MDSCs are associated with increased TIM3 expression and effector T-cell dysfunction in human HNSCC (85).

10. Crosstalk with TAMs

Another significant factor contributing to HNC development are TAMs, which exhibit important functions in facilitating the metastatic cascade of tumor cells (86). Specifically, Snail-overexpressing cancer cells may promote M2 polarization of TAMs by delivering miR-21-abundant exosomes in HNC (87). Furthermore, high miR-21 expression is associated with release of miR-21-abundant tumor-derived exosomes and a higher level of snail family transcriptional repressor 1 and the M2 marker mannose receptor C-type 1b (87). In addition, CAFs may affect the functional polarization of TAMs. The expression of M2 biomarkers CD68, CD14, CD163, CD200R, CD206, major histocompatibility complex, class I, G, CD80 and CD86 is higher in CD14-positive cells co-cultured with the culture supernatants of CAFs in OSCC than in control cells (78). The gene expression levels of arginase-I, IL10 and TGF- β are increased in CAF-educated cells, and T cell proliferation is strongly suppressed, and the neutralization of TGF- β ,

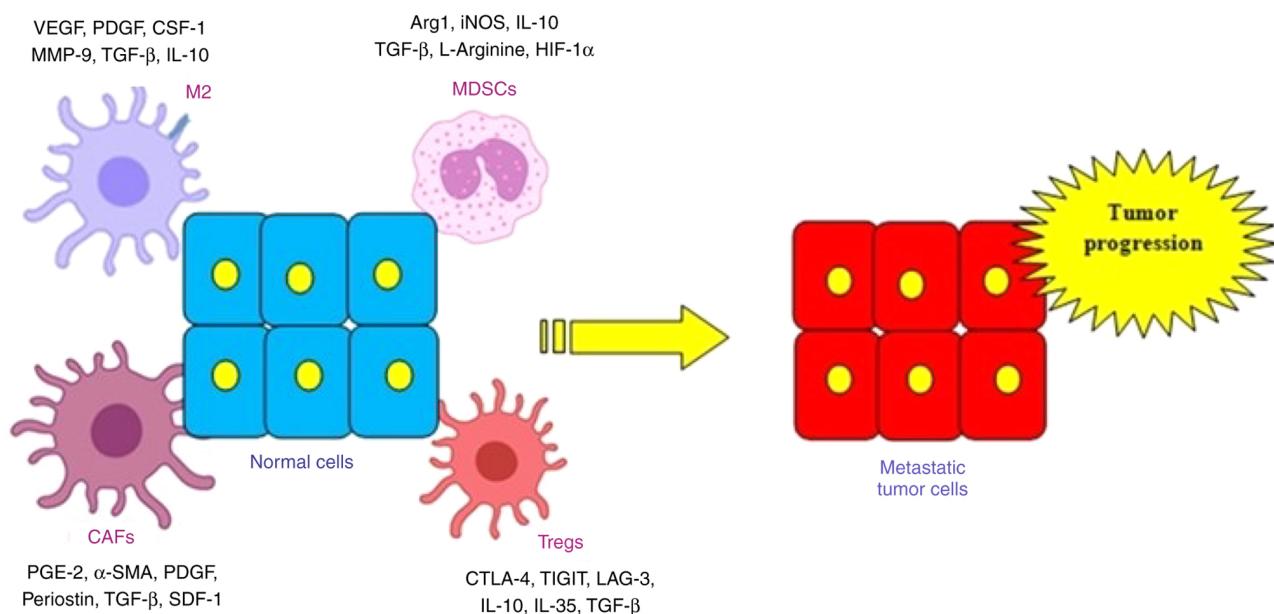


Figure 3. Tumor-related immunosuppression mediated by specific immune cells (CAFs, MDSCs, M2 macrophages and Tregs) is vital for tumor progression in head and neck cancer. M2 macrophages can secrete VEGF and PDGF, as well as Arg-1, IL-10, TGF- β and other anti-inflammatory cytokines (CSF-1) or signal peptides (MMP-9), which reduce inflammation and contribute to tumor growth and immunosuppression. MDSCs, a heterogeneous population of immature myeloid cells, regulate a number of mechanisms of tumor progression, including immune evasion, angiogenesis, pre-metastatic niche formation and epithelial-mesenchymal transition. These cells trigger suppression of immune cells via iNOS, arginase-I and IL-10 expression, and induction of hypoxia in the tumor microenvironment via HIF-1 α , TGF- β and L-Arginine upregulation. CAFs are key players in the tumour microenvironment with diverse functions, including in matrix deposition (PDGF and SDF-1), tissue remodeling (α -SMA, PGE-2 and periostin) and extensive angiogenesis signaling (TGF- β). Tregs are involved in tumor development and progression by inhibiting antitumor immunity. Tregs express the co-inhibitory molecules CTLA-4, LAG-3 and TIGIT, which specifically suppresses pro-inflammatory Th1 signaling. They are also involved in tumor immunosuppression via induction of the inhibitory cytokines IL-10, IL-35 and TGF- β . α -SMA, α -smooth muscle actin; Arg1, arginase-I; CAFs, cancer-associated fibroblasts; CSF-1, colony stimulating factor 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HIF-1 α , hypoxia-inducible factor 1 α ; iNOS, inducible nitric oxide synthase; LAG-3, lymphocyte-activation gene 3; MDSCs, myeloid-derived suppressor cells; PDGF, platelet-derived growth factor; PGE-2, prostaglandin E2; SDF-1, stromal cell-derived factor 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor.

IL-10 or arginase-I markedly restores T cell proliferation (78). Furthermore, interplay between cancer cells and M2 macrophages is necessary for miR-550a-3-5p downregulation and HPV-positive OSCC progression. MiR-550a-3-5p, downregulated by E6 oncoprotein, inhibits M2 macrophage polarization via YAP/C-C motif chemokine ligand 2 signaling, which in turn abrogates the EMT program in HPV-positive OSCC cells (88). Previous studies have revealed a novel paracrine loop between cancer cells and macrophages (89,90). M1-like TAMs activated by exosome-transferred thrombospondin 1 promote malignant migration in OSCC (91).

11. Conclusions

Understanding the epigenetic modifications and the molecular crosstalk between tumor and immune cells is vital for future therapeutic modalities against head and neck carcinogenesis. Additionally, targeting the tumor microenvironment to reverse drug resistance and inhibit immunosuppressive tumor networks emerges as a useful tool for current immunotherapeutic approaches. Targeting these aberrant patterns of signaling could provide biomarkers for early detection and diagnosis for HNSCC and will improve the therapy and extend overall survival. Furthermore, the deregulation of tumor suppressor genes and oncogenes by epigenetic mechanism is related to HNSCC tumorigenesis. Therefore, current trials are ongoing to evaluate the role of novel immune checkpoint inhibitors in order to

retain effective tumor control and improve the quality of life for patients with HNSCC. Deciphering these signaling mechanisms and their crosstalk with immune and tumor cells may bring novel perspectives for HNSCC therapeutic approaches, and thus, a number of them may be used as potential therapeutic targets or in combination with current immunotherapy regimens.

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SG, SP, AP, NT, PV, IS, NA, TK and KD contributed to manuscript drafting and to the critical revisions of the intellectual content. SG, SP and KD performed the literature search and selection and were responsible for the general study supervision. All authors read and approved the final version of the manuscript to be published. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

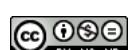
The authors declare that they have no competing interests.

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