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# Epigenetic Mechanisms in Head and Neck Cancer

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Additional information is available at the end of the chapter

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## Abstract

Head and neck cancer (HNC) is one of the most prevalent human malignancies, affecting different anatomic sites of the upper aerodigestive tract (UADT) such as the oral cavity, larynx, and naso-, oro-, and hypopharynx. HNC develops through the accumulation of multiple genetic and epigenetic alterations in a multistep process. In this issue, the aim is to describe epigenetic mechanisms behind HNC. The main mechanisms evaluated are DNA methylation, posttranslational histone modification, and noncoding RNAs.

**Keywords:** Methylation, Histone modification, microRNA, Long non-coding RNAs

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## 1. Introduction

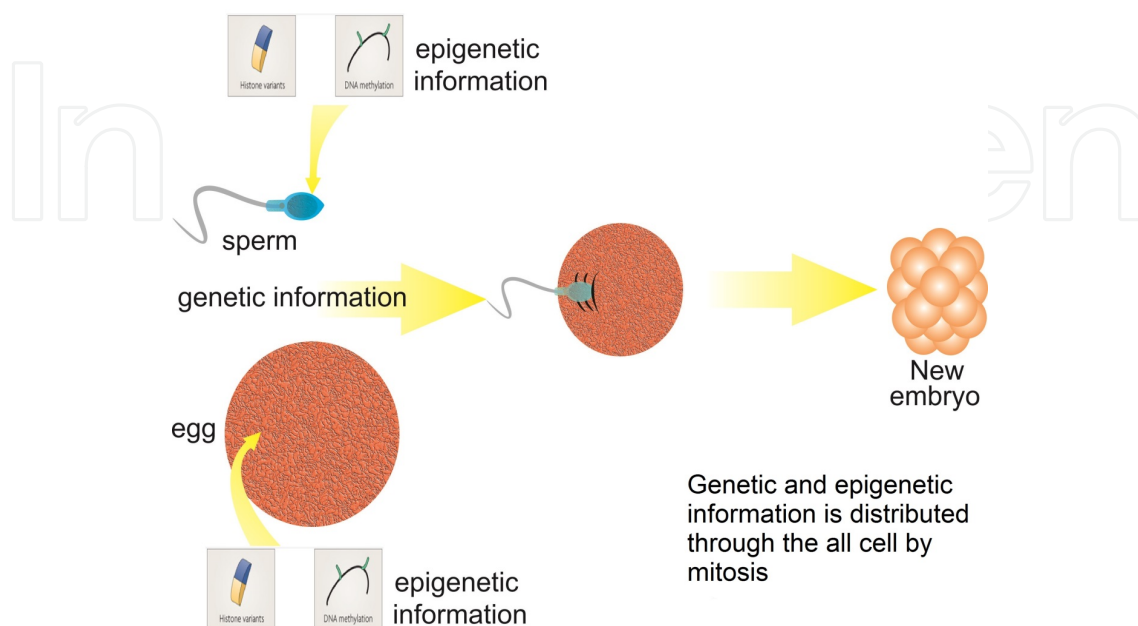
Genetic information flow (transcription, translation, and subsequent protein modification) in a normal cell represents the machine of cell gene expression.[1] Each cell has the same information, but its expression change between different types of cells. The control of gene expression is therefore at the heart of differentiation and development.[1]

In addition to inheriting genetic information, cells inherit chemical modifications that are not encoded in the nucleotide sequence of DNA, and this modification impacts the program of gene expression. This type of modification has been termed epigenetic information.[1]

Epigenetic refers specifically to the study of mitotically and meiotically heritable changes in the control of gene expression that occur without changes in the nucleotide sequence of genome and chromatin.[2]

In both genetic and epigenetic heritage, mitosis distributes genetic information or the chemical modification through the ontogeny. Meiosis distributes genetic information or chemical modification through the formation of gametes. This means that sperm and egg carry genetic

and epigenetic information. When the embryo is formed, this information is distributed through all the cells by mitosis (Figure 1). When it comes to population level, natural selection determines which genetically and epigenetically inherited information is present.

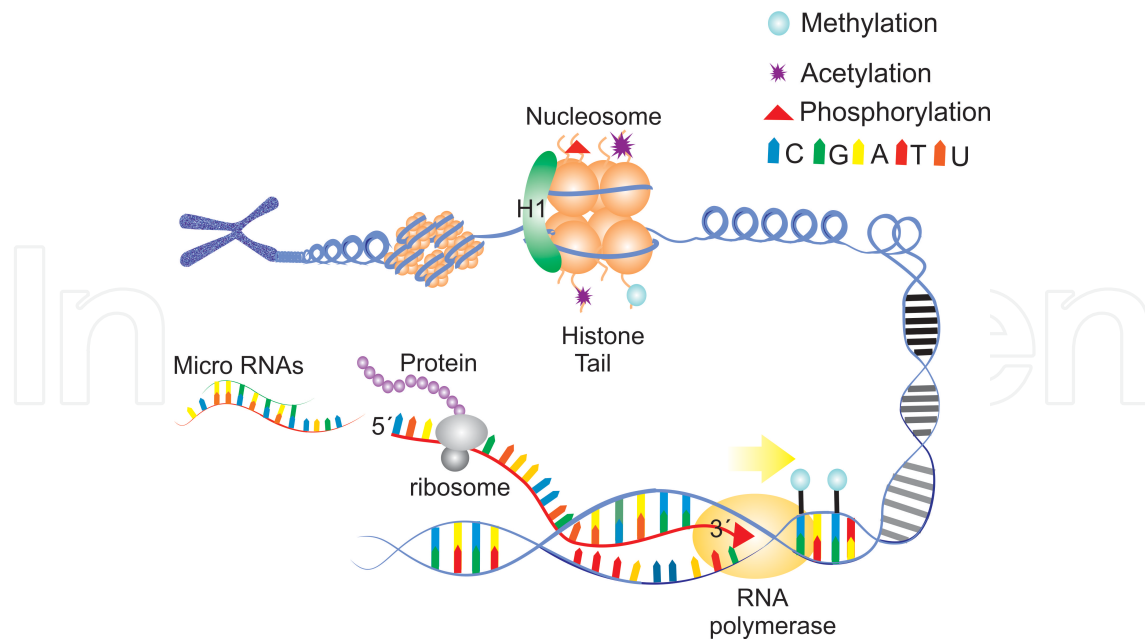


**Figure 1.** Genetic and epigenetic information in gametes and a new embryo.

Gene regulation and genome function are intimately related to the physical organization of genomic DNA and, in particular, to the way it is packaged into chromatin.[3] There are many chemical modifications affecting DNA, RNA, and proteins that create different epigenetic process.[4] These modifications are related to DNA methylation, histone modifications, chromatin remodeling factors (associated with nucleosome positioning), and noncoding RNAs.[4] The changes will influence the chromatin states and impact gene expression patterns (Figure 2).[5] Epigenetic alterations are associated with chromosomal instability and changes in transcriptional control, which influence the overall gene expression differences seen in many human malignancies.[5]

The carcinogenesis of head and neck cancer (HNC) is a human malignancy influenced by genetic factors, age, geography, and lifestyles, among which include smoking, oral hygiene, and human papillomavirus.[6, 7] In general, different types of carcinogens attack the oral mucosa through the accumulation of multiple genetic and epigenetic alterations in a multistep process.[8] The clinical appearance of HNC preoncogenic lesion of the mucosal surfaces include leukoplakia, erythroplakia, or speckled leukoplakia reflecting the presence of white, red, or mixed white/red lesion, respectively.[9]

HNC is one of the most prevalent human malignancies, affecting different anatomic sites of the upper aerodigestive tract (UADT) such as the oral cavity, larynx, and naso-, oro-, and hypopharynx.[10] The most common histologic type among the head and neck tumors are the squamous cell carcinomas (head and neck squamous cell carcinomas [HNSCCs]).[7]



**Figure 2.** Genetic and epigenetic information.

It was reported that 42,440 new cases of HNSCCs were diagnosed in the United States in 2014, and 8,390 deaths were due to this disease.[11] The mortality rate (from 2003 to 2007) was of 2.5 per 100,000 persons per year. U.S. incidence and mortality rates are about 2.5 and 2.8 in men and women, respectively.[12]

Generally, the highest HNSCC rates are found in Melanesia, South-Central Asia, and Central and Eastern Europe and the lowest in Africa, Central America, and Eastern Asia for both males and females.[13]

The incidence rates for HNSCC related to HPV infections, such as oropharynx, tonsil, and tongue base, are increasing in young adults in the United States and in some countries in Europe, which is hypothesized to be in part due to changes in oral sexual behavior.[14]

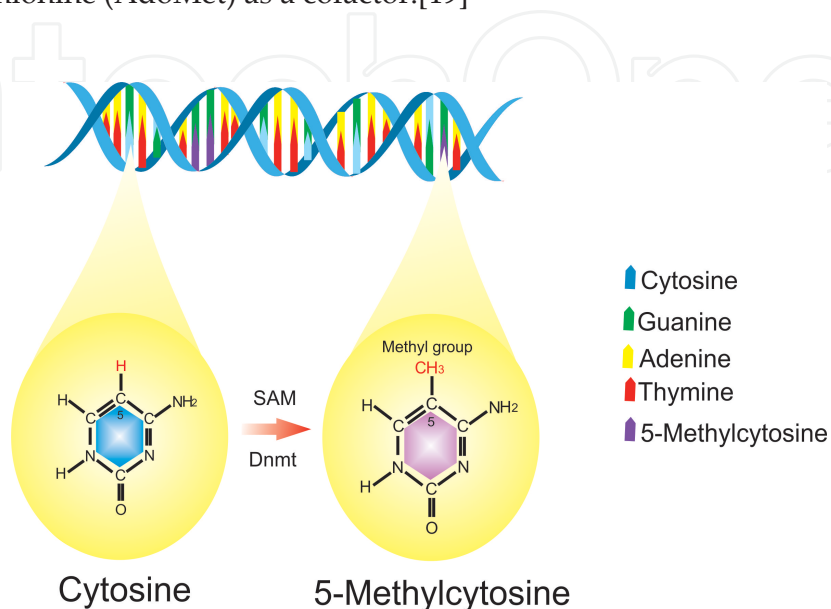
In this issue, the aim is to describe epigenetic mechanisms behind HNSCC. The main mechanisms evaluated are DNA methylation, posttranslational histone modification, and noncoding RNAs

## 2. Epigenetic mechanisms

### 2.1. DNA methylation

DNA methylation is a chemical marking system for annotating genetic information by causing gene repression through its ability to affect factor binding and chromatin structure.[14] This system has gene expression patterns that are regulated in a spatial and time-dependent manner.[5] Heritable information is carried by chemical modifications of both DNA and chromatin-associated proteins and modulates chromatin structure and DNA accessibility.[5]

DNA methylation refers to the methylation of DNA cytosine residues at the carbon 5 position. [15, 16] DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group to position 5 of a cytosine, generating 5-methylcytosine (5mC). [17] This chemical modification affects gene expression when CpG-rich areas are present inside promoter regions (figure 3). [16, 18] This is accomplished by the activities of one or more DNA methyltransferases (DNMTs), which use S-adenosylmethionine (AdoMet) as a cofactor. [19]



**Figure 3.** DNA methylation.

About 60% of human gene promoters are associated with CpG islands and are usually unmethylated in normal cells, although some of them (~6%) become methylated in a tissue-specific manner during early development or in differentiated tissues. [20]

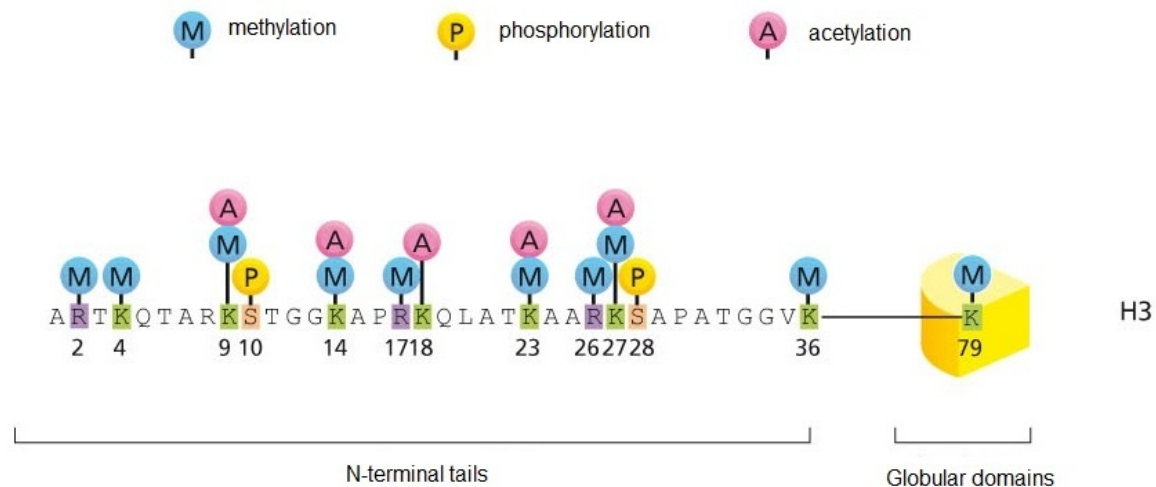
Recent findings also suggest that extensive DNA methylation changes caused by differentiation take place at CpG island “shores,” regions of comparatively low CpG density close to CpG islands. [2]

A relationship between DNA methylation and cancer has been found, given that DNA methyltransferases can be genetically altered in malignancies, that is, occur with DNMT3A20 and DNMT3B. [21] Aberrant DNA methylation is an epigenetic mechanism that contributes to the development of a wide variety of human cancers, either in the form of promoter-specific hypermethylation or genome-wide hypomethylation. [22] Hypermethylation in gene promoter regions is usually associated with expression suppression [23] and is the most common alterations in human cancers, leading to the abnormal expression of a broad spectrum of genes. [22] On the other hand, hypomethylation occurs in a large percentage in repetitive DNA elements. [24]

## 2.2. Posttranslational covalent histone modifications

Chromatin is the state in which DNA is packaged within the cell. [25] The chromatin architecture can be remodeled by a network of protein mediators called histones that play an important

role in gene regulation by compacting DNA.[26] The nucleosome is the fundamental unit of chromatin, and it is composed of an octamer of histones (H2A, H2B, H3, and H4) around which 147 base pairs of DNA are wrapped.[25] Dynamic variations in the organization of chromatin structure are mediated by histone acetylation and deacetylation (figure 4).[25]



**Figure 4.** Posttranslational covalent histone modifications.

Acetylation neutralizes the positive charge of lysine residues, weakening charge-dependent interactions between histone and nucleosomal DNA, linker DNA, or adjacent histones, thus increasing the accessibility of DNA to the transcription machinery. Histone lysine acetylation also functions in other cellular processes that require DNA access.[27]

The posttranslational modification of histones by methylation is an important and widespread type of chromatin modification that is known to influence biological processes in the context of development and cellular responses.[28] Other modifications can be performed through lysine ubiquitination, serine phosphorylation, sumoylation, and methylation of lysines and arginines.[29]

Sumoylation is a process in which proteins are functionally or structurally similar to ubiquitin and are termed ubiquitin-like proteins (UBLs). UBLs include SUMO, NEDD8, ISG15, and FAT10.[30]

Histone acetyltransferases (HAT) catalyze the transfer of an acetyl group from acetyl-CoA to  $\epsilon$ -amino group of a histone lysine residue.[31] The action of histone deacetylases (HDAC) is different because the effect is on the lysine residues. The first process involves chromatin decondensation, and the second process involves chromatin compaction. Either one or the other affects the gene transcription due to conformation changes in the chromatin.[31]

### 2.3. Noncoding RNAs

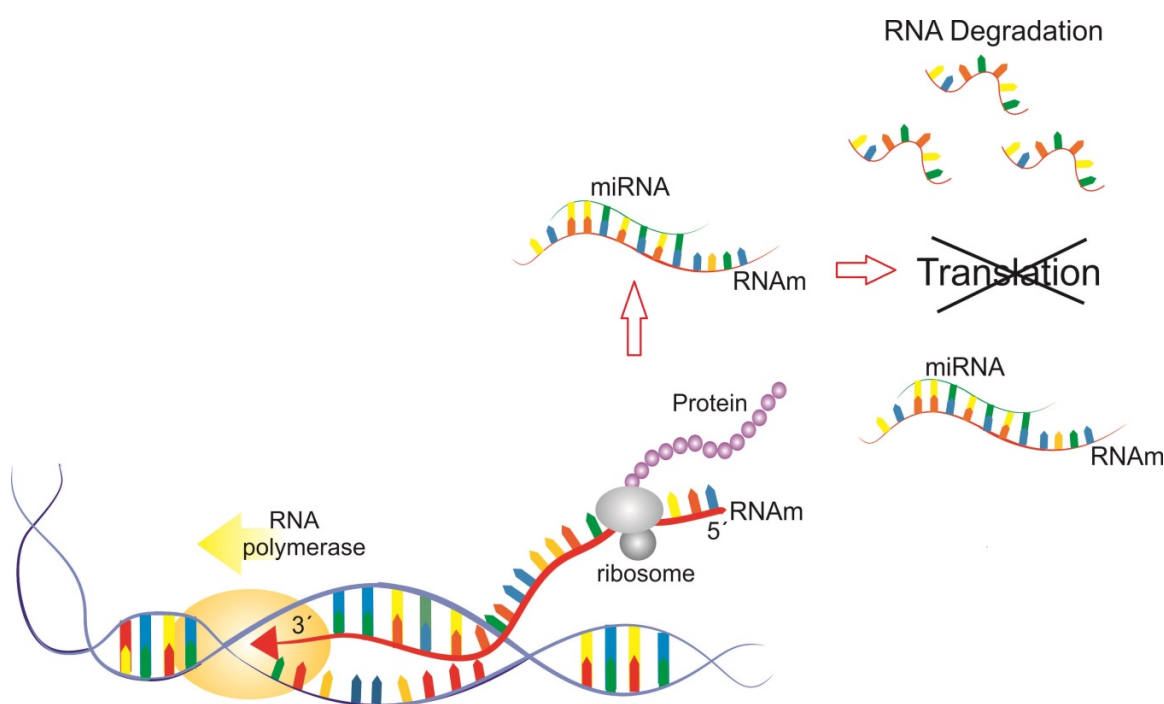
The noncoding RNAs are another level of epigenetic control for their capacity to establish other epigenetic marks and control gene expression.[4] Noncoding RNA (ncRNA) is a type of RNA



that does not code for protein but has enzymatic, structural, or regulatory function.[32] ncRNAs can be classed as either small or long ncRNA, based on their transcript length.[33, 34]

## 2.4. MicroRNAs

MicroRNAs (miRNAs) are a set of non-protein-coding RNAs that bind to partially complementary sites in the 3'-untranslated regions of their messenger RNA targets.[35] MicroRNAs are the 21–23 nucleotide single-stranded RNA molecules found in eukaryotic cells.[36] The miRNAs interfere with messenger RNA translation or cause messenger RNA degradation, thereby repressing gene expression posttranscriptionally (Figure 5).[37] This is possible through imperfect base pairing with the 3'-untranslated region (3'-UTR) of target mRNAs of protein-coding genes, leading to the cleavage of homologous mRNA or translational inhibition.[38]



**Figure 5.** RNA degradation by miRNA.

miRNAs are transcribed for the most part by RNA polymerase II as long primary transcripts characterized by hairpin structures (pri-miRNA) and are processed in the nucleus by RNase III Drosha into 70–100 nucleotide long precursor miRNAs (pre-miRNAs).[39]

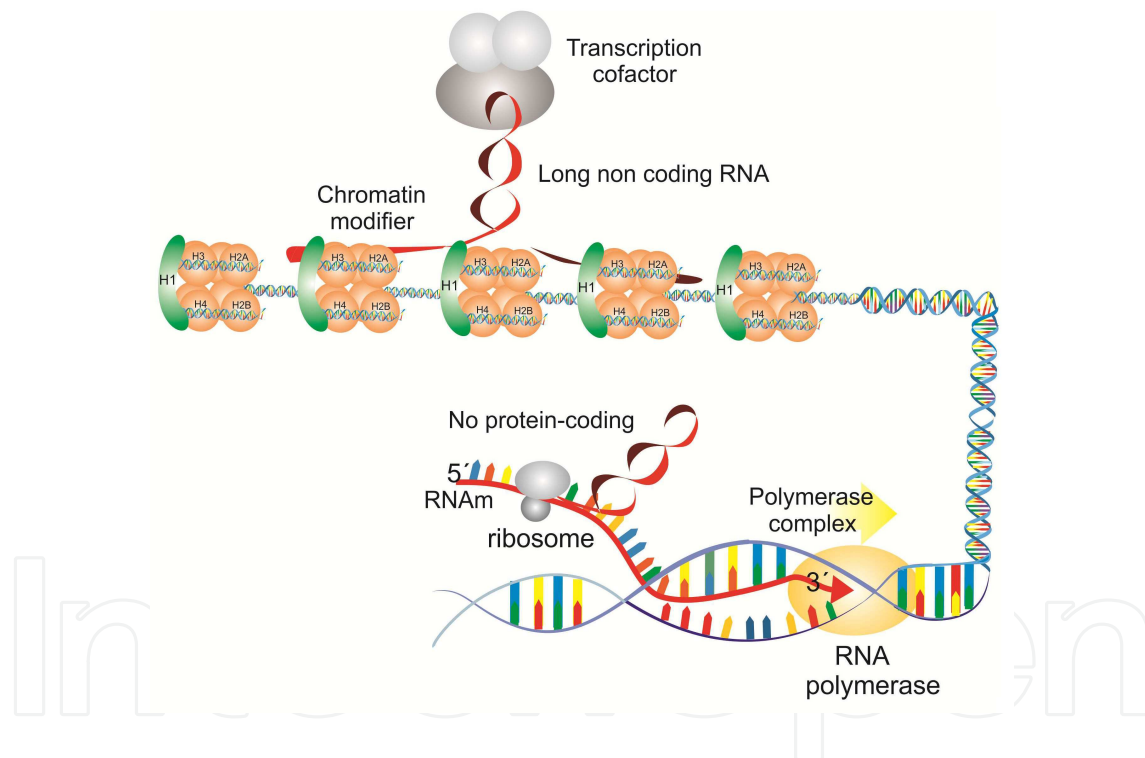
MicroRNAs are partial complementarity between their target transcripts. A single microRNA is capable of simultaneously regulating up to hundreds of genes, giving rise to an enormous modulatory potential.[40]

MicroRNAs are involved in a variety of cellular processes, including the regulation of cellular differentiation, proliferation, and apoptosis, and an aberrant expression of miRNA is known to induce various human malignancies.[41] New evidence suggests that miRNAs can act as

oncogenes or tumor suppressors, exerting a key function in tumorigenesis.[42] Recently, a new function mediating tumor metastasis in breast cancer has been assigned to miRNAs, by which this malignant step is promoted or suppressed.[42]

## 2.5. Long noncoding RNAs

Long noncoding RNAs (lncRNAs) are generated by the same transcriptional machinery as are other mRNAs. These lncRNAs have a 5' terminal methylguanosine cap and are often spliced and polyadenylated.[45] lncRNAs have length greater than 200 nucleotides without protein-coding potential but with a wide range of structural and functional roles.[34] Some of this process can include the chromatin remodeling and transcriptional control assemblies.[31] They are generally transcriptionally activated or repressed by associated transcription factors and function as molecular mediators of gene expression.[43] When the lncRNAs act as molecular scaffolds, they can guide the chromatin-modifying complexes to bind into specific genomic loci. This way, they impart a repressive or activating effect on gene expression (Figure 6).[43]



**Figure 6.** Long noncoding RNA.

According to their association with mRNA, the lncRNAs can be divided in different categories[44]: this can overlap with coding regions of a transcript on the same strand and with coding regions of a transcript on the opposite strand. This can be intronic lncRNAs from other transcript and can be intergenic lncRNAs between two genes on the same strand.[45]

The functions of the RNA in regulation of gene expression can be summarizing at transcriptional levels, RNA processing, and translation. Furthermore, they can protect genomes from foreign nucleic acids. At chromatin level, they can modulate the genome rearrangement.



Finally, the ncRNAs can operate as RNA–protein complexes, including ribosomes, snRNPs, snoRNPs, telomerase, microRNAs, and long ncRNAs.[46]

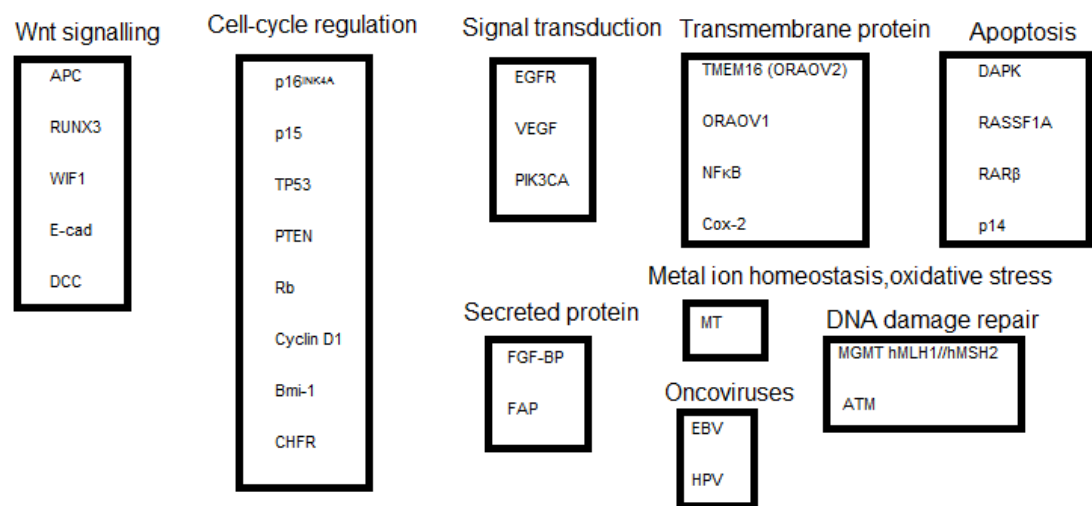
Long noncoding RNAs have also been shown to be necessary for targeting histone-modifying activities. Histone methylation is the end result of the transcription of long noncoding RNAs and the subsequent nucleation and targeting of histone modifying completes.[27]

The aberrant expression of lncRNAs has been associated with human cancers, suggesting a critical role in tumorigenesis.[47, 48] It has been demonstrated that a novel lncRNA, HOTAIR, was up-regulated and promoted cancer metastasis and predicted poor prognosis in ESCC.[34] Additionally, the association of dysregulated lncRNAs with specific developmental stages and clinical outcomes indicates their potential as strong diagnostic and prognostic predictors as well as therapeutic targets.[49]

### 3. Epigenetic changes in head and neck cancer

#### 3.1. DNA methylation in head and neck cancer

The DNA methylation events in HNSCC include genes involved in cell cycle regulation, signal transduction, secreted protein, transmembrane protein, transcription factors, prostaglandin metabolism, metal ion homeostasis, oxidative stress, and oncoviruses.[50] Additionally, HNSCCs have genes involved in DNA damage repair, apoptosis, Wnt signaling, signal transduction, tissue invasion/metastasis, tumor suppression, and others (Figure 7, Table 1). [13, 51]



**Figure 7.** Genes and DNA methylation in HNSCC. Modified from Magić et al.,[13] Polanska et al.,[50] and Kaabi et al. [51]

Major classes	Member	Function
Cell cycle regulation	p16 <sup>INK4A</sup>	A tumor suppressor gene regulating senescence and cell cycle progression
	p15	Cyclin-dependent kinase inhibitor
	TP53	A tumor suppressor gene regulating cell cycle progression and cell survival
	PTEN	A tumor suppressor gene regulating signaling pathways controlling cell proliferation and apoptosis
	Rb	A proto-oncogene regulating cell cycle progression
	Cyclin D1	Controls cell cycle and self-renewal of tissue stem cells
	Bmi-1	Transmembrane TK that acts as a central transducer of multiple signaling pathways
	CHFR	Early G2/M checkpoint
Signal transduction	EGFR	A transmembrane TK that acts as a central transducer of multiple signaling pathways
	VEGF	A transmembrane TK that promotes proliferation, migration, and survival of endothelial cells during tumor growth
	PIK3CA	A gene encoding the p110 $\alpha$ subunit of phosphoinositide 3-kinase (PI3K) $\alpha$
Secreted protein	FGF-BP	Fibroblast growth factors binding protein
	FAP	Protein secreted by cancer-associated stroma
Transmembrane protein	TMEM16 (ORAOV2)	Calcium-activated chloride channel
	ORAOV1	A regulator of cell growth and tumor angiogenesis
	NF $\kappa$ B	Proinflammatory transcription factor
	Cox-2	Catalyzes prostaglandin synthesis from arachidonic acid
Metal ion homeostasis, MT oxidative stress		Low molecular weight proteins involved in heavy metal detoxification, essential metal ion homeostasis, and cell protection against free radicals
Oncoviruses	EBV	A causative agent for most nasopharyngeal carcinomas, plasma EBV DNA load is an independent prognostic factor
	HPV	A causative agent for most oropharyngeal cancers
DNA damage repair	MGMT	DNA repair for alkylated guanine
	hMLH1/hMS	DNA mismatch repair
	H2	DNA double-strand breaks
	ATM	

Major classes	Member	Function
Apoptosis	DAPK	p53-dependent apoptosis
	RASSF1A	RAS pathway regulation and tumor suppression
	RARβ	Regulatory protein and apoptosis
	p14	Proapoptosis
Wnt signaling	APC	Wnt signaling and adhesion
	RUNX3	Wnt signaling inhibitor, TGF-β-induced tumor sup pression
	WIF1	Secreted Wnt antagonist
	E-cad	Cell adhesion, forming adherens junctions
	DCC	Cell–cell adhesion
Signal transduction	SFRP1	Antagonists of the Wnt pathway
	EDNRB	Endothelin receptor type B
	RUNX3	Wnt pathway antagonist
Tumor suppression	HIN1	Inhibitor Ras pathway
	DAPK1	Proapoptosis
	DCC	Proapoptosis
	RASSF1A/RASSF2	Negative RAS effector, proapoptotic, microtubule stabilization
Others	KIF1A	Cell division and microtubule-dependent intracellular organelle transport

**Table 1.** Genes and DNA methylation in HNSCC. Modified from Magić et al.,[13] Polanska et al.,[50] and Kaabi et al. [51]

The primary risk factors for the development of HNSCC include tobacco use, alcohol consumption, human papillomavirus (HPV) infections (mainly for oropharyngeal cancers), and Epstein–Barr virus (EBV) infections (for nasopharyngeal cancer).[52]

In promoter methylation, the p16 and p15 genes are commonly observed in human epithelial malignancies, including HNSCC. Histologically normal surgical margin epithelium of HNSCC patients with chronic smoking and drinking habits has a significantly higher prevalence of p15 methylation compared with nonsmokers and nondrinkers.[53]

Between genes that have been associated with hypermethylation, the p16 and the p14 genes undergo inactivation due to promoter hypermethylation.[13] Encoded by the CDKN2A gene, p16 inhibits cyclin-dependent kinases 4 and 6, thus blocking the promotion of cells from the G1 to the S phase of the cell cycle.[54] CDKN2A (p16) inactivation is common in lung cancer and occurs via homozygous deletions, methylation of promoter region, or point mutations.[55] CDKN2A (p16) disruption is reported as a frequent event in head and neck squamous cell

carcinomas that confers poor prognosis.[56] Other genes as DAP-K, RASSF1A, RAR $\beta$ 2, and MGMT have been reported as genes under hypermethylation promotor but with functions in DNA repair. These genes removed mutagenic (O6-guanine) adducts from DNA.[57]

DAPK and RASSF1A genes have shown methylation in HNSCC.[58] The methylation of p16 could be an initial process that might address abnormalities or deregulation of cell cycle controls.[59]

The ataxia-telangiectasia-mutated (*ATM*) gene produces a protein kinase that functions as a tumor suppressor by triggering appropriate cellular response to genome damage resulting from ionizing radiation or chemical carcinogen exposure.[60] It is currently unknown whether *ATM* is lost in HNSCCs displaying the deletion in the 11q22–23 locus.[61]

Aggressive HNSCC has been linked to expression loss of E-cadherin (*ECAD*) protein.[51] The protein *ECAD* can be inactivated by promoter hypermethylation.[62] In patients with HNSCC who are low smokers, the hypermethylation of *CDH1* occurs more commonly, suggesting that an additional factor may be driving this epigenetic alteration.[62]

Cyclooxygenase-2 (*Cox-2*) is presumed to contribute to cancer progression through its multifaceted function, and recently its inverse relationship with E-cadherin was suggested. Increased expression of *Cox-2* has been found in a variety of human malignancies, including HNSCC. [63]

The death-associated protein kinase (*DAPk*) family contains three closely related serine/threonine kinases, namely, *DAPk*, *ZIPk*, and *DRP-1*, which display a high degree of homology in their catalytic domains.[64] The methylation profile of *DAPK* in HNSCC (including oral squamous cell carcinoma) is a promising biomarker for the follow-up and early detection of head and neck cancer recurrence.[65]

The Ras association domain family protein 1A (*RASSF1A*) is arguably one of the most frequently inactivated tumor suppressors in human cancer. *RASSF1A* modulates apoptosis via the Hippo and Bax pathways but also modulates the cell cycle.[66] The epigenetic inactivation of *RASSF1A* plays an important role in the development of cancer.[67]

*TP53*, once activated, leads to apoptosis and growth arrest (either cell cycle arrest or senescence). It is clear that *TP53* mutation is common in HNSCC.[68] The p53 transcription factor stands out as a key tumor suppressor and a master regulator of various signaling pathways involved in this process.[69] Tobacco smoke is the best known and studied mutagen involved in lung carcinogenesis, and *TP53* mutational patterns differ between smokers and nonsmokers, with an excess of G to T transversions in smoking-associated cancer.[69]

The Cancer Genome Atlas has informed about smoking-related HNSCCs and its relations with the universal loss of function of *TP53* mutations and *CDKN2A*. This inactivation is accompanied with frequent copy number alterations, including amplification of 3q26/28 and 11q13/22.[70]

The phosphatidylinositol 3-kinase (*PI3K*)/*AKT*/mTOR pathway is antagonized by phosphatase and tensin homologue (*PTEN*). *PTEN* protein is a tumor suppressor frequently disrupted

in cancer, altering tumor cell growth and survival.[71] The decrease in PTEN function in HNSCC is due to several genetic and epigenetic alterations. Recently, promoter hypermethylation has been implicated in the down-regulation of PTEN in HNSCC cell lines.[72]

HNSCCs also exhibit many chromosomal abnormalities, including amplifications of the 11q13 region containing the cyclin D1 gene and the 7p11 region encoding EGFR, which lead to proto-oncogene activation.[73] The most critical point in regulation of the cell cycle is the G1 checkpoint. Cyclin D1, a G1 cyclin, has been implicated in the regulation of the G1 to S phase progression in many different cell types. Cyclin D1 forms active complexes that promote the phosphorylation of retinoblastoma protein (RB) and the activation of E2F-responsive gene.[74]

PIK3CA is a human gene that regulates various cellular functions, including proliferation and invasion. Because it is an oncogene, its activation by either gene amplification or mutation results in a cellular growth advantage contributing toward cancer formation and progression.[75] Mutations in PIK3CA have cases displayed of concurrent amplification. Additionally, some tumors (20%) contain focal amplification without evidence of mutation. The largest mutation proportions of PIK3CA are localized to hotspots that promote activation.[70] PIK3CA is an active mutation that is common in conjunction with infrequent copy number alteration, and it forms part of a subgroup of oral cavity tumors with favorable clinical outcomes.[70]

Bmi1 (B-cell-specific Moloney Murine Leukemia virus insertion site 1) is a transcription repressor for cell senescence, implicated in the self-renewal of stem cell. Bmi1 is highly expressed in the CD44+ cell population sorted from oral SCC tumors.[76]

E3 ubiquitin-protein ligase (CHFR) is a gene involved in a checkpoint regulating entry to mitosis.[77] Loss of CHFR leads to mitotic catastrophe and apoptosis due to mitotic spindle alteration. Aberrant methylation of the gene has been reported in several primary tumor genes. EGFR is a potential prognostic biomarker.[78]

Epidermal growth factor receptor (erb-B1) is a member of the erbB family of tyrosine kinase receptor proteins.[79] Previous studies have shown that EGFR is expressed or highly expressed in various human tumor cells.[79] The overexpression of EGFR is attributed to gene amplification, which is noted to be about 12 copies per cell in relation to head and neck squamous cell carcinomas. The constitutive EGFR activation caused via autocrine stimulation and through the coexpression of EGFR with its ligands, TGF $\alpha$ , has been observed and is indicative of its poor prognosis.[72] EGFR-targeted therapy is commonly used for the treatment of advanced HNSCC due to numerous findings that describe overexpression and/or high activity of EGFR in the majority of HNSCC.[80]

Various factors are known to regulate angiogenesis; for example, vascular endothelial cell growth factor (VEGF) has potent angiogenic effects. The presence of VEGF has been reported in approximately 40% of head and neck squamous cell carcinomas (HNSCCs), and its presence is associated with a poor prognosis.[72]

Other mechanisms included in the tumorous angiogenesis lie in the intake and utilization of locally stored fibroblast growth factors (FGFs).[50] FGF-1 (aFGF) and FGF-2 (bFGF) are found in most embryonic and adult normal and tumor tissues, where they are immobilized in the extracellular matrix (ECM).[81]



Fibroblast activation protein (FAP) is a member of the serine protease family that is selectively expressed in the stromal fibroblasts associated with epithelial cancers and is expressed at low or undetectable levels in the resting fibroblasts of normal adult tissues. FAP is expressed in more than 90% of epithelial carcinomas, which makes it a promising target.[82]

Oral cancer overexpressed 1 and 2 (ORAOV1-ORAOV2) overexpression was reported in HNSCC.[50, 83] The first is required for cell growth and tumor angiogenesis,[84] and the second is required in the modulation of TMEM16A activity in various epithelial tissues.[85]

Multiple signaling pathways have been linked to tumor resistance, including activation of nuclear factor kappa B (NF $\kappa$ B).[86] NF $\kappa$ B is an epigenetic modifier that plays a major role in malignant transformation, and this pathway serves as a target for epigenetic drugs.[26] The constitutive activation of NF $\kappa$ B signaling is often observed in HNSCC, suggesting a common epigenetic mechanism in HNSCC biology. Indeed, the activation of NF $\kappa$ B signaling in HNSCC induced chromatin compaction and acquisition of resistance to chemotherapy.[26]

Metallothionein (MT) is a family of cysteine-rich, low molecular weight (500–14,000 Da) proteins. MTs have been proposed to play important roles in protecting against DNA damage, apoptosis, and oxidative stress. MT is a tumor suppressor reported to show promoter hypermethylation in various cancers.[87]

Human mismatch repair genes (hMMR) have the ability to repair both mismatched bases and insertion loop errors during DNA replication.[88] Suboptimal DNA repair could result in disrupting the pattern of repeat sequences, causing chromosomal aberrations in the genome of patients suffering from instability syndromes. Significant proportions of carcinomas develop through DNA mismatch repair genes (MMR) deficiency and exhibit frequent microsatellite alteration (MA).[88]

The hypermethylated adenomatosis polyposis coli (APC) tumor suppressor gene has reduced expression levels along with loss of heterozygosity (LOH), leading to the altered functioning of the APC tumor suppressor proteins, which play a role for the integrity and function of the  $\beta$ -catenin destruction complex.[72] The APC protein, a negative regulator of this pathway, has been strongly implicated in the development of colon cancer but still has an undetermined role in the formation of oral cancer.[89]

Loss of heterozygosity of the APC gene and epigenetic events lead to the decreased expression of APC and the Wnt antagonists, the secreted frizzled-related proteins (SFRPs), Wnt inhibitory factors (WIFs), and Dickkopf family members (DKKs), primarily by promoter hypermethylation.[90] The persistent  $\beta$ -catenin signaling contributes to increased growth, metastatic potential, and resistance to chemotherapy in HNSCC and their tumor-initiating cells.[90] The methylation of WIF-1 correlated with shorter survival in oral cancer patients. The methylation of WIF1 may be considered a prognostic marker in oral cancers.[91]

Wnt pathway stimulates several intracellular signal transduction cascades (canonical and noncanonical).[92] The possible role of RUNX3 as a tumor suppressor in HNSCC has been reported. The promoter hypermethylation of antagonist's genes to Wnt (RUNX3) has been identified as a common event in cancer.[93]



Deleted in colorectal cancer (DCC) is a candidate tumor suppressor gene located at chromosome 18q21.[94] DCC promoter region hypermethylation was found in 75% of primary HNSCC. There was a significant correlation between DCC promoter region hypermethylation and DCC expression.[94] DCC is a putative conditional tumor suppressor gene that is epigenetically inactivated by promoter hypermethylation in a majority of HNSCC.[94, 95]

Secreted frizzled-related protein (SFRP1) is epigenetically silenced and functions as a tumor suppressor in oral squamous cell carcinoma (OSCC). The loss of SFRP2 expression is associated with hypermethylation of its promoter.[96]

The methylation of the *KIF1A* and *EDNRB* gene promoters is a frequent event in HNSCC.[97] *KIF1A* (kinesin family member 1A) encodes a protein that is a microtubule-dependent molecular motor involved in important intracellular functions such as organelle transport and cell division.[98] Endothelin receptor type B (*EDNRB*) is a G-protein-coupled receptor that activates a phosphatidylinositol calcium second messenger system.[97, 99]

Runt domain transcription factors (RUNXs) are homologous to products encoded by the *Drosophila* segmentation genes *runt* and *lozenge*. [100] The *RUNX3* gene is located on human chromosome 1p36, a region that has long been suspected to harbor one or more suppressors of various tumors.[101] Inactivation of *RUNX3*, which is caused mainly by epigenetic alteration, is closely associated with bladder tumor development, recurrence, and progression.[100, 101]

HIN-1 (high in normal-1) is a putative cytokine with growth inhibitory activities and is down-regulated by aberrant methylation in breast cancers.[102] Evidence suggests that HIN-1 is a potential tumor suppressor gene in non-small cell lung cancer (NSCLC), silenced by promoter hypermethylation and negatively regulated by AKT signaling pathway.[103] Silencing of HIN-1 expression and methylation of its promoter occurs in multiple human cancer types, suggesting that the elimination of HIN-1 function may contribute to several forms of epithelial tumorigenesis.[104]

### 3.2. Histone modifications in head and neck cancer

Aberrant regulation of the demethylases controlling H3K9me3 and H4K20me3 levels could contribute to the oncogenic potential. For instance, levels of H3K4me2 and me3 are significantly different in oral squamous cell carcinoma in comparison with cells of the healthy tissues; the level of H3K4me2 is increased while that of H3K4me3 is decreased.[102, 105]

A similar trend was observed in tongue squamous cell carcinoma (SCC) cells where the levels of the H3K27me3 marks at chromatin near homeobox genes were inversely correlated with the transcript levels in nontumorigenic, immortalized human oral keratinocytes (OKF6-TERT1R) and tumorigenic oral SCC-9 cells.[25] This investigation found that the levels of the H3K27me3 marks at chromatin near homeobox genes were inversely correlated with the transcript levels in nontumorigenic, immortalized human oral keratinocytes (OKF6-TERT1R) and tumorigenic oral SCC-9 cells.[25]

The emerging importance of the regulation of the H3K27me3 mark as a driver of squamous differentiation suggests that SCCs may harbor defects in the epigenetic regulation of squamous differentiation.[106] The dysregulation of squamous differentiation is fundamental to the development of SCC and has been reported to occur early in premalignant lesions.[107]

On the other hand, serine phosphorylation plays an important role in assembling the DNA damage response complex by identifying DNA double-strand breaks (DSB) in the chromatin. Upon DSB, ATM induces the phosphorylation of  $\gamma$ H2AX at serine 139, resulting in the recruitment of BRCA1, BRCA2, Rad51, Mre11, NBS1, FANCD2, and p53 repair proteins to sites of DNA damage.[108]

In addition, the phosphorylation of serine 536 involved in the phosphorylation of RELA has also been reported. RELA, also known as p65, is an REL-associated protein involved in NF- $\kappa$ B heterodimer formation, nuclear translocation, and activation.[109] The phosphorylation of serine 468 is also associated with RELA.[109]

Regarding sumoylation, there are also desumoylating enzymes called SENPs, which remove the SUMO residues from the sumoylated proteins. Two molecules involved in the SUMO pathway, Ubc9 and SENP5, are up-regulated in SCCs. The up-regulation of SENP5 is found in oral SCCs, and strong SENP5 expression is correlated with poor prognosis.[110]

Finally, the lysine methylation of HSP90AB1 is important for its homodimerization and its interaction with stress-induced phosphoprotein 1 (STIP1) and cell division cycle 37 (CDC37), which are co-chaperones of HSP90AB1 in human cancer cells, resulting in enhancement of cancer cell growth.[111]

### **3.3. miRNAs in head and neck cancer**

DNA copy number variations or deregulation of miRNA expression has been shown to contribute to carcinogenesis, including carcinogenesis of HNSCC.[112] Several studies have reported global miRNA expression changes in carcinogenesis of HNSCC, using various samples sizes, anatomical sites, and profiling methodologies.[113]

It has been demonstrated the differential expression patterns of miRNAs in numerous types of cancer. MiR-92a, miR-103/107, miR-21, miR143, miR145, miR-205 and miR-296, among others, have been confirmed to be involved in the development of esophageal squamous cell carcinoma (ESCC).[34]

It has been reported that members of the miR-17-92 cluster were deregulated in 15 patients with OSCC (tongue and floor of the mouth) and 35 patients with HNSCC. The miR-19a and the miR-19b were strongly up-regulated. The miR-17-3p/miR-17-5p and the miR-92b were moderately up-regulated. Evidence has been found that the miR-17-92 cluster is up-regulated in many cancer types.[114] Furthermore, the miR-196a and the miR-10b, not previously associated with HNSCC, may play an oncogenic role in this disease through the deregulation of cell proliferation.[114]

Other studies informed that miR-21, miR-31, miR-504, and miR-10b are target tumor suppressor genes. These miRNAs are involved in HNSCC.[115] Many studies have confirmed the

tumor suppressor roles of the let-7 family (the miR-99 family, miR-107, miR-133a, miR-137, miR-138, and miR-375). Other miRNAs such as miR-21, let-7, miR-107, miR-138, and miR-200c are involved in regulating stemness or the epithelial–mesenchymal transition of tumor cells. [115]

It has also been evaluated that miR-34a is significantly down-regulated in HNSCC tumors and cell lines.[116] The ectopic expression of miR-34a in HNSCC cell lines significantly inhibited tumor cell proliferation, colony formation, and migration. Tumor samples from HNSCC patients showed an inverse relationship between miR-34a and survival as well as between miR-34a and E2F3 levels.[116]

In silico analysis identified three putative microRNA-107 (miR-107) binding sites in the 3'-untranslated region (UTR) of PKC $\epsilon$ . [117] An inverse relationship was revealed between miR-107 and PKC $\epsilon$  in HNSCC cell lines. These data demonstrated that PKC $\epsilon$  is directly regulated by miR-107 and, moreover, suggest that miR-107 may be a potential anticancer therapeutic for HNSCC.[117]

New computational approach strategies complementary to microRNA profiling are capable of simultaneously predicting tumor suppressor microRNAs as well as their functional targets from gene expression.[118] It provided a plausible mechanism that loss of the tumor suppressor function of miR-204 as a result of allelic imbalance at 9q21.1–q22.3 may significantly increase genetic susceptibility to HNSCC oncogenesis and progression.[118] The complete suppression of miR-204 and its host gene TRPM3 has become possible that the mRNA expression may serve as a marker the expression status in HNSCC.[118]

### 3.4. lncRNAs in head and neck cancer

lncRNAs have been linked to essential growth-promoting activities, and their deregulation contributes to tumor cell survival. A prominent example is the Hox transcript antisense intergenic lncRNA, HOTAIR.[119] The HOTAIR gene controls gene expression, and its expression is deregulated in a spectrum of cancers. Furthermore, HOTAIR expression correlates with patient survival.[119, 120]

HOTAIR serves as a scaffold for at least two distinct histone modification complexes. A 5' domain of HOTAIR binds Polycomb Repressive Complex 2 (PRC2), while a 3' domain of HOTAIR binds the LSD1/CoREST/REST complex. lncRNAPCAT-1, a target gene of polycomb repressive complex 2, has been implicated in disease progression by promoting cell proliferation.[121] The ability to link two distinct complexes enables RNA-mediated assembly of PRC2 and LSD1 and coordinates targeting of PRC2 and LSD1 to chromatin for coupled histone H3 lysine 27 methylation and lysine 4 demethylation.[122]

In prostate cancer, the up-regulation of antisense noncoding RNA in the INK4 locus (ANRIL) is required for the expression of the tumor suppressors INK4a/p16 and INK4b/p15.[121]

Some examples of lncRNAs that has a role in chromatin remodeling include *XIST*, which acts by recruiting the PRC2 complex to initiate X-chromosome inactivation as well as *MALAT* and *NEAT1*, both of which play a role in mRNA processing and nuclear organization.[123]

The expression of LINC00312 in nasopharyngeal carcinoma has a tumor-suppressive function. Under physiological conditions, LINC00312 inhibits proliferation in nasopharyngeal epithelium by preventing cell cycle passage from the G1 into S phase but increases cell adhesion, motility, and invasion by down-regulating the expression of estrogen receptor alpha (ER $\alpha$ ). [124]

Other genes regulated by lncRNAs that have been implicated in cancer include NDM29, BACE1AS, and *Drosophila* hsr- $\omega$  gene.[125] Neuroblastoma differentiation Marker 29 (NDM29) is an RNA polymerase (pol) III-transcribed noncoding (nc) RNA whose synthesis drives neuroblastoma (NB) cell differentiation to a nonmalignant neuron-like phenotype.[126] BACE1 plays a pivotal role in the accumulation of  $\beta$ -amyloid plaques and has been shown to regulate the expression of BACE1 by increasing BACE1 mRNA stability.[127] The heat shock RNA omega (hsr $\omega$ ) gene of *Drosophila melanogaster* is inducible by cell stress and provides structural base for sequestering diverse RNA-processing/regulatory proteins.[128]

#### 4. Clinical applications in head and neck cancer

To date, four epigenetic inhibitors have been approved by the U.S. Food and Drug Administration (FDA) for cancer treatment.[129] The DNMT inhibitors as 5-aza-cytidine and 5-aza-2'-deoxycytidine are widely used in in vitro in research. The cytosine analogs are converted to deoxynucleotide triphosphates inside the cell and then incorporated into the DNA during replication in the original C positions.[130]

Other inhibitors of HATs approved by FDA are p300, lysine methyltransferases (H3K79 methyltransferase DOT1L, or the polycomb complex member EZH2), and lysine demethylases (LSD1). Furthermore, small molecule inhibitors targeting the histone reader, BRD4, have also shown promise as therapeutic agent in many cancer types.[131]

The effects of an epigenetic inhibitor as lysine residues on histone tails are HDAC inhibitors that counteract the global overexpression of HDACs in cancer and reinstate a more permissive nucleosome structure for transcription.[132] Vorinostat (a pan-HDAC inhibitor) and romidepsin (a class I HDAC inhibitor) have each shown >30% response rates against cutaneous T-cell lymphoma (CTCL) in phase II trials.[132]

Cetuximab is an inhibitor of the epidermal growth factor receptor (EGFR) that is used in radiation therapy. It was found to enhance HNSCC patient survival compared with radiation therapy alone.[133] The FDA has approved cetuximab to treat HNSCC; the drug has a response rate of about 10% when used as a single agent in recurrent/metastatic disease.[134] However, despite approval of cetuximab, improvement in patient survival with the use of this agent has been only modestly incremental.[133]

Advances in oncogenomics have also identified mutations in epigenetic-associated genes that encode histones and their linkers, proteins associated with the recruitment of DNA-binding proteins, HDAC I and II interacting proteins, corepressor proteins, and transcriptional activators and coactivators.[135]

The understanding of which gene mutations, DNA methylation, posttranslational histone modification, and noncoding RNAs drive the carcinogenesis may help us understand how tumor susceptibility guides the development of new combination therapies. However, it is important remember that not all cancers are equally susceptible to epigenetic therapies. The biology underpinning this observation urgently warrants our attention if epigenetic therapies are to be more widely applicable.[136]

## 5. Conclusions

Understanding the complexity of the epigenome, the different dynamics, and the different subunits is complex and intimidating. Gene mutations, DNA methylation, posttranslational histone modification, and noncoding RNAs are actors involved in modulating its interactions with genomic sequences, and this is fundamental for health and disease.

Only our hope and desire can continue creating new ways to interact with the epigenome, and it will be possible to build a new world where the HNSCC and other types of cancer will be able to have new therapies and new opportunities for a better life.

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