Epigenetics of oral and oropharyngeal cancers (Review)

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Abstract. Oral and oropharyngeal cancers represent the two most common malignancies of the head and neck region. The major risk factors for these cancers include alcohol consumption, tobacco use (via smoking or chewing) and high-risk human papillomavirus infection. The transition from normal epithelium to premalignant tissue and finally carcinoma is in part caused by a summation of genetic and epigenetic modifications. Epigenetic refers to modifications in the way the genome is expressed in cells. The most common examples of epigenetic control of gene expression are DNA methylation, histone modification and regulation by small non-coding RNAs. The aim of the current paper was to review the recent studies on the main epigenetic changes that have been suggested to serve a role in the carcinogenesis process and progression of oral and oropharyngeal cancers. Furthermore, it is discussed how the epigenetic changes may be used as potential predictive biomarkers and how recent findings in the field may impact the personalized cancer therapy approach for these tumors.

Contents

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1. Introduction

Oral and oropharyngeal cancers are the most common malignancies of the head and neck region, with squamous cell carcinoma being the most common histotype (1,2). Reportedly, ~300,000 new cases of oral cancers are diagnosed worldwide annually, of which ~140,000 are oropharyngeal cancers, with a male: female ratio that varies between 2:1 and 4:1 (3). Oral squamous cell carcinoma (OSCC) and oropharyngeal squamous-cell carcinoma (OPSCC) are often listed together and, accounted as a single entity, represent the sixth most common malignancy worldwide (4-6).

Knowledge of these cancers has improved substantially over recent decades, but the therapeutic outcome has remained mostly unchanged, with a five year survival rate of ~50% (7). The more advanced the stage of cancer at diagnosis, the lower the response rate to therapy; furthermore, patients diagnosed with high stage OSCC or OPSCC have a high risk of developing metastases (both lymph node and distant) and relapses (7).

Oral carcinogenesis is caused by environmental and endogenous factors; categorized in the first group are regular tobacco and alcohol use and persistent high-risk human papillomavirus (HPV) infection. HPV-related OPSCC constitutes a different biological entity compared with HPV-negative cases (8). In particular, HPV-related OPSCC differs significantly in epidemiology from HPV-negative cases and those of the oral cavity: HPV-negative head and neck squamous cell carcinoma (HNSCC) mostly affects individuals of more than 60 years in age, and its incidence is decreasing; conversely, the incidence of HPV-positive HNSCC is increasing, and is often diagnosed in young adults (9,10). HPV status also significantly impacts on prognosis; namely, HPV-positive OPSCC is associated with better prognosis when compared with HPV-negative cases and OSCC (9,11).

Endogenous factors are generally linked to genetic and epigenetic alterations. Genetic alterations refer to changes

in the DNA sequence including deletions, amplifications and mutations; these changes are not reversible. Epigenetic alterations are a group of heritable modifications in DNA expression that manifest without direct DNA modification. In the present review, the main epigenetic alterations described recently in the literature on oral and oropharyngeal cancers are discussed.

2. OSCC and OPSCC carcinogenesis

'Field cancerization' (also known as the field effect), is a concept first introduced by Slaughter *et al* (12) in 1953 for oral cancers. They described field cancerization as being a process in which an area of epithelium has been preconditioned by an, at that time, unknown carcinogenic agent, enabling epidermoid carcinoma of the oral stratified squamous epithelium to originate. Some of the carcinogenic agents involved in neoplastic transformation of oral epithelium have been established, among which tobacco and alcohol are considered as principal risk factors; however, the mechanisms of field cancerization in HPV-related HNSCC remain unknown (9). Tobacco and alcohol seem to cause an anaplastic transformation that involves an area or 'field' of mucosa rather than single cells (13). This would explain the tendency of OSCC and OPSCC to recur (10-30% of cases) in different areas relative to the original location, even following surgical removal with tumor free margins (13).

Aside from in HNSCC, the field effect has been also described for cancers of other anatomical regions, including the esophagus (14), colon (15), urinary tract (16), skin (17), breast (18), uterine cervix (19) and airway epithelium (20).

HPV-related carcinogenesis. HPV is an established causative factor in~25% of global cases of HNSCCs. The oropharynx is the anatomical location in which HPV-related cancers are more frequently observed; they amount to ~36% of the total, ~87% of which are related to HPV 16. In the oral cavity, ~23% of cancers are HPV-related, ~69% of which are HPV 16-related. HPV 18 is the second most common papilloma virus type in HNSCC, amounting for ~8% of OSCCs and ~1% of OPSCCs (21). Certain locations appear to have higher HPV-related cancer incidence: For instance, tonsil and base of the tongue cancers have been identified to be HPV-linked in 50-80% of cases (22-24).

E6 and E7 are considered the most important HPV proteins involved in carcinogenesis; E6 protein has several functions, with a key and well-established function being to induce the degradation of p53, a widely studied oncosuppressor (25-27). E6 binds the ubiquitin-protein ligase, E6AP; the E6/E6AP heterodimer can then bind p53, which is ubiquitinated and degraded in proteasomes (26,27).

E7 binds retinoblastoma protein (pRb). The main function of pRb is to negatively regulate several transcriptional factors, including E2F1-3 (25). When E7 binds pRb, E2F transcriptional factors are no longer inhibited, and have the possibility to activate the transcription of cell cycle-related genes, including cyclin A and cyclin E (25).

Several biomarker expression studies performed in HPV-positive OPSCC have established a clear distinction between HPV-active and HPV-inactive cancers (28-34). HPV-active tumors contain and express HPV sequences, are positive for p16 immunohistochemistry as well as for HPV RNA (35) and exhibit gene expression profiles that suggest a 'HPV signature', dominated by changes in genes that control the cell cycle, proliferation and mitosis (36). Numerous differences have been described in gene expression profiles between HPV-active and HPV-negative cancers, the latter being dominated by changes in genes that control cell motility, angiogenesis and epithelial-mesenchymal transition, associated with aggressive clinical behavior (36). The gene expression profiles of HPV-inactive tumors lack the characteristic 'HPV signature' and are notably more similar to HPV-negative tumors, although some differences can be detected, the clinical significance of which remains to be determined (36,37).

Tomar *et al* (37), proposed that HPV-inactive cancers may originate as HPV-active lesions that progressively become independent of E6/E7 for proliferation and tumorigenic potential. Methylation mechanisms have been demonstrated as responsible for the silencing of HPV sequences in cells that have acquired mutations that may 'replace' E6/E7 function (38). HPV methylation has been investigated in a variety of cancers and cancer cell lines (38); however, the role of HPV methylation and other mechanisms leading to a possible progression from HPV-dependence to HPV-independence in head and neck cancer remains to be determined (37,39).

Tobacco-related carcinogenesis. Tobacco consumption is among the most important risk factors in numerous cancers. Expectedly, it has been widely demonstrated that tobacco consumption is a major risk factor for OSCC and OPSCC (40,41).

Among smoker patients, the risk for the development of oral cancer is greater than three times higher than in non-smokers; for pharyngeal cancers the risk becomes greater than six times higher for smokers (42). The risk of oral and oropharyngeal cancers returns to baseline when smoking is stopped for at least 20 years (43).

Additionally, smokeless tobacco is established as an independent risk factor for oral cancer; an increased risk of oral cancer has been identified with use of numerous tobacco products, including gutka, supari (areca nut) and betel quid (*Piper betle*), with an odds ratio between 5.1 and 11.4 (44).

Alcohol-related carcinogenesis. Ethanol serves as a risk factor for the development of oral cancer through its local and systemic effects. By its local effects, alcohol may increase the permeability of the mucosa by dissolving the lipid component of the epithelium; it may provide indirect cellular damage through acetaldehyde; it may amplify the toxicity of other carcinogenic agents; and it may cause hyposalivation, leading to a higher exposition to locally acting carcinogens.

As systemic effects, ethanol acts to reduce first pass hepatic metabolism of toxic substances; it may lead to immunosuppression; and it may be associated with malnutrition, which can enhance the risk of developing cancer (45).

Patients who stop drinking exhibit a decreased risk of developing OSCC and OPSCC over time; however, for oropharyngeal cancer, the risk does not return to baseline, remaining higher than in those without a history of alcohol consumption. Otherwise, for oral cancer, the risk in former

drinkers after more than 20 years is reportedly lower than in the 'never drinker' population (43).

EBV-related carcinogenesis. Epstein-Barr virus (EBV) was identified in 1964 by Epstein *et al* (46), in a Burkitt lymphoma-derived cell line. It is a herpesvirus (HHV-4), and therefore its genome is composed of DNA (47). EBV is the etiologic agent of mononucleosis, also known as the kissing disease for its transmission path; this is a self-limiting disease, after which the virus may remain latent, with infected individuals potentially becoming lifelong carriers of the virus (48). Worldwide, more than 90% of adults infected with EBV exhibit no sign of the infection (47).

EBV is established as a causative factor in numerous cancers, including Burkitt lymphoma, Hodgkin lymphoma, immunosuppression-related non-Hodgkin lymphoma and nasopharyngeal carcinoma (47,48). EBV-persistent infection has been associated with oral cancers, even though there are few epidemiological data available (49).

3. Epigenetics

Epigenetics refers to changes in DNA expression not attributable to alterations in DNA sequence. The main events responsible are DNA methylation, histone modification and post-transcriptional gene downregulation by microRNAs (miRNA/miRs). These alterations can persist for a cell lifetime and be inherited by subsequent generations.

The results of epigenetics vary from increased gene expression to complete silencing, depending on the interference of these alterations to activators and suppressors of specific promoters in chromatin. Therefore, epigenetic changes can induce overexpression of oncogenes as much as silencing of tumor suppressor genes (50).

DNA methylation is the most common epigenetic event (51). It is performed by a series of enzymes known as DNA methyltransferases (DNMTs). These enzymes catalyze the covalent addition of a methyl group to the carbon-5 position of cytosine bases that are located 5' to a guanosine base in a CpG dinucleotide (52).

Hypermethylation has been identified in the promoters of tumor suppressor genes in numerous cancers; this may lead to the reduction of their expression or to their complete silencing (49). Conversely, hypo/demethylation of the promoter of proto-oncogenes may lead to increased expression of these genes (50). An imbalance in tumor suppressor gene methylation status has often been reported following exposure to tobacco-derived carcinogens and other environmental compounds including cadmium, arsenic and nickel (52).

Many genes present an altered DNA methylation profile in OSCC and OPSCC (53). The galanin (GAL) gene maps onto chromosome 11q13.2, and is a neuropeptide widely distributed in the central and peripheral nervous system (54). It has the function of regulating anterior pituitary hormones secretion and acts as neurotransmitter (54). GALR1 and GALR2 are two of the three receptors for galanin; their genes are located on chromosome 18q23 and 17q25.1, respectively. These receptors are members of the G protein-coupled receptor (GPCR) superfamily. Galanin and its receptors regulate growth via the inhibition of extracellular signal-regulated kinase (ERK) 1/2, upregulation of the cyclin-dependent kinase inhibitors p27 and p57, and decreased expression of cyclin D1, which can altogether limit cell proliferation (55). Additionally, GALR2 can induce caspase-3 dependent apoptosis (55-57). Hypermethylation of these genes leads to a lack of their expression with a consequent lack of their tumor suppressor activity (57). A previous study identified 25 out of 48 (52%) OSCC cases to have hypermethylation of at least one of the three genes; furthermore in OPSCC, 13 out of 20 cases (65%) were determined to have hypermethylation of at least one of the genes (58).

Calprotectin S100A8/9 is a heterodimer of the two calcium-binding proteins S100A8 and S100A9. S100A8/9 is located both within epithelial cells and in the extracellular space (59). In normal epithelial cells, S100A8/9 works to arrest the cell cycle by activating the G2/M DNA damage checkpoint (59); a function that has been evidenced in the TR146 cell line (an oral cancer-derived cell line) (60). In HNSCC, it has been identified that S100A8/9 level was on average 10-fold lower compared with in normal adjacent tissues (60). Additionally, S100A8/9 in the extracellular space is released from polymorphonuclear, leukocytes and macrophages and works by signaling through the receptor for advanced glycation end products and toll-like receptor (TLR) 4 (61).

Notably, it has been reported that S100A9 promoter hypermethylation correlates with reduced expression of S100A9. S100A8 expression, conversely, does not correlate with its promoter methylation (60).

4. Saliva-detectable hypermethylation

Saliva is an emerging diagnostic medium for oral and oropharyngeal cancers. It represents an effective diagnostic alternative since it is produced and collected close to the sites of malignancy; this provides the possibility to analyze tumor-specific biomolecules with minimal interferences (61). Additionally, saliva allows analysis of regions including the tonsillar crypts, which are otherwise difficult to sample (62). Ultimately, saliva sampling is easy to perform (63,64).

Several hypermethylated genes have been detected via saliva sampling (Table I). Insulin-like growth factor (IGF)-binding protein 7 (IGFBP-7) is a protein involved in the regulation of free IGF concentration in tissues (65). It is also involved in tumor suppressor activity and in epithelial-mesenchymal transition (EMT) (66,67). In a previous cohort of 47 oral tongue cancer cases, IGFBP-7 promoter was reportedly hypermethylated, and consequently, IGFBP-7 marginally expressed. Furthermore, hypermethylation in these cases was documented to correlate with invasive depth, locoregional recurrence and poor prognosis (67).

Krüppel-like factor 4 (KLF4) is a is a zinc-finger transcription factor encoded by a gene located on chromosome 9q31.2. It is involved in epidermal barrier function (68), cell proliferation, differentiation and apoptosis (69) and stem cell renewal (70). The activity and expression of KLF4 are often associated with mutation in various cancers (71-74). Li *et al* (75), identified that KLF4 was downregulated in OSCC, and its downregulation correlated with cancer differentiation i.e., the poorer the cancer differentiation, the lower the

Table I. List of genes found hypermethylated in saliva samples, their reported function and the tumor type where modifications were present.

Gene	Gene product	Function	Tumor site (OSCC/OPSCC)	(Refs.)
CCNA1	Cyclin A1	Cell cycle regulation	OSCC and OPSCC	(62,99)
DAPK1	Death-associated protein kinase 1	Apoptosis and autophagy	OSCC and OPSCC	(62,63,99)
DCC	Deleted in colorectal carcinoma	Netrin 1 receptor	OSCC	(62,100)
EDNRB	Endothelin receptor type B	G protein-coupled receptor	OSCC	(62,100)
ERCC1	Excision repair cross-complementation group 1	Repair of DNA damage induced by ultraviolet light or cisplatin	OSCC and OPSCC	(62,101)
HOXA9	Homeobox protein Hox-A9	Gene expression, morphogenesis and differentiation	OSCC	(62,101)
KIF1A	Kinesin family member 1A	Membranous organelle transportation along axonal microtubules	OSCC	(62,102,103)
MED15	Mediator of RNA polymerase II transcription subunit 15	Transcriptional coactivator in RNA polymerase II transcription	OSCC	(62,104)
MINT31	Spen family transcriptional repressor family transcriptional repressor	Calcium channel regulator	OSCC	(62,99,105)
NID2	Nidogen-2	Cell adhesion	OSCC	(62,102)
PAXI	Paxillin	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix	OSCC	(62,106)
p16INK2A	p16 (also known as cyclin-dependent kinase inhibitor 2A)	Tumor suppressor gene	OSCC	(62,63,99)
RASSF1α	Ras association domain family member 1 α	Growth inhibition along the RAS-activated signaling pathway	OSCC	(62,63)
TIMP3	Metalloproteinase inhibitor 3	Inhibitor of the matrix metalloproteinases	OSCC and OPSCC	(62,99,105,107)

OSCC, oral squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma.

expression of KLF. The downregulation of KLF4 in OSCC has been associated with promoter hypermethylation (75).

Somatostatin (SST) and somatostatin SST receptor type 1 (SSTR1) are encoded by genes located on 3q27.3 and 14q21.1, respectively. SST is a peptide hormone that exists in two forms (14 amino acids and 28 amino acids), both encoded by the same gene. SST has several functions: It inhibits growth hormone (GH) secretion; it works as an immunomodulator to cause an inhibition of tumor necrosis factor-α, interferon-γ, corticotropin releasing hormone and substance P secretion; and it binds μ-opioid receptor (76). Additionally, SST interacts with SSTR1 to cause an antiproliferative effect. In fact, the activity of SSTR1 may lead to the activation of SHP-2 (also known as tyrosine-protein phosphatase non-receptor type 11); SHP-2 dephosphorylates the proto-oncogene tyrosine-protein kinase c-src on Tyr529 (inhibitory site) and makes possible phosphorylation on Tyr418 (stimulatory site). Once activated, c-src may also phosphorylate Raf-1,the function of which is to activate (through phosphorylation) mitogen activated-protein kinase signaling, which can eventually lead to cell cycle inhibition through p21/Cip1. Besides these direct effects, SST and its receptors may inhibit proliferation through indirect effects. In fact, the activation of the SST pathway may cause a reduction of IGF-1 synthesis and inhibition of angiogenesis (77).

SSTR1 is one of the five receptors for SST, and an inhibitory GPCR (78). Misawa *et al* (79), have demonstrated SST

Table II. List of miRNAs with altered expression, on saliva specimen testing, in oral and/or oropharyngeal cancers.

Name	Status	Tumor type	(Refs.)
miR-9	Overexpressed	HPV-negative HNSCC	(62)
miR-24	Overexpressed	OSCC (vs. healthy controls)	(62,108)
miR-27b	Overexpressed	OSCC (vs. healthy controls) OSCC in remission	(62,108)
miR-31	Overexpressed	HPV-related OSCC and OPSCC (vs. healthy controls and leukoplakia)	(62,109,110)
miR-134	Overexpressed	HNSCC	(62,111)
miR-191	Overexpressed	HNSCC	(62,111)
miR-125a	Underexpressed	OSCC	(62,112)
miR-136	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-147	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-148a	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-200a	Underexpressed	OSCC (vs. control samples)	(62,112,113)
miR-222	Underexpressed	OSCC and OPSCC	(62,109)
miR-323-5p	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-503	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-622	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-646	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-668	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-877	Underexpressed	OSCC (vs. healthy controls)	(62,108)
miR-1250	Underexpressed	OSCC (vs. healthy controls)	(62,108)

miRNA/miR, microRNA; OSCC, oral squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus.

and SSTR1 promoter hypermethylation in OSCC, OPSCC primary tumor samples and in UM-SCC cell lines; these findings suggest that SST and SSTR1 may be considered as important molecules in the tumorigenesis and progression of OSCC and OPSCC.

Other genes observed to be hypermethylated in saliva samples in oral/oropharyngeal cancers are listed in Table I.

5. miRNA

miRNAs represent a category of non-coding RNA molecules, each made of ~22 nucleotides. These molecules are involved in the control of gene expression by interacting with mRNA (80).

miR-143 is a miRNA capable of interacting with the 3' untranslated region of hexokinase 2 (HK2) mRNA (81). Elevated concentrations of HK2 have been observed in numerous cancers, including oral and oropharyngeal cancers; in particular, it has been determined in OSCC-derived cell lines that there was a lower concentration of miR-143, compared with in normal keratinocytes and, consequently, higher concentrations of HK2, since miR-143 inhibits HK2 synthesis (81).

Saliva-detectable miRNA. Saliva may be sampled and analyzed for the identification of miRNAs. Several miRNAs have been identified to be overexpressed or underexpressed in patients with oral and/or oropharyngeal cancer. For instance, miR-491-5p is a miRNA that has been implicated in a number of important processes, including migration, invasion and consequently metastasis. The function of miR-491-5p is to target GPCR kinase-interacting protein 1 (GIT1), leading to a downregulation of its expression. A well-established function of GIT1 is to initiate cell motility; this is achieved through interactions with members of the focal adhesion kinase and ERK1/2 pathways.

Huang *et al* (82), reported that miR-491-5p was downregulated in highly aggressive OSCC cell lines (SAS-I5, OECM-1-I8, SCC25-I6, OC-3-I5- and OC-3-I5-lung-IV2) and in 29 out of 33 OSCC samples compared with in normal tissue. They also demonstrated that cells with low miR-491-5p expression exhibited enhanced capability to migrate and metastasize, and furthermore, that miR-491-5 low expression was correlated with poor prognosis (82).

Examples of miRNAs observed to be over- or underexpressed in OSCC/OPSCC are listed in Table II.

6. Therapeutic implications

To date, there are few chemotherapeutic options for oral and oropharyngeal cancers. Platinum-based antineoplastics are the most frequently used; however, their efficacy is not satisfactory (83,84).

The biology of epigenetics can be used for therapeutic purposes; well known among such applications is in the context of viral mimicry. Viral mimicry refers to a characteristic behavior that certain cancer cells acquire when treated with demethylating agents; these treatments can lead to an effect that mimics a viral infection (85). The exact mechanism of action is not fully understood, nor is the reasoning behind

why certain cells are more susceptible than others, or why in certain cases the efficacy of demethylating agents decreases over time (85,86).

Viral mimicry is based on a series of immune effects on tumor cells. Demethylating agents may stimulate viral defense and type I interferon signaling; this effect can be achieved through RNA demethylation (85). In fact, demethylated RNA is known to stimulate an interferon response through TLR3 activation; a similar response to DNA demethylation is suggested to occur by a similar effect obtained using demethylating agents which target only DNA (85).

Another immune effect seen with demethylating agents involves double-stranded RNA (dsRNA). In fact, these drugs have been associated with an increase of cytosolic levels of dsRNA; dsRNA molecules are used by epithelial and other cells as a defense mechanism towards viral infection, which in turn may lead to a type I interferon response and apoptosis (85). Viral mimicry has been observed and studied in various cancers, including colon (86) and breast (87) cancers.

Another notable mechanism of action involves endogenous retroviral genes. These genes comprise ~8% of the human genome and are normally silenced through hypermethylation in somatic cells (82). With demethylating drugs these genes are unlocked, and their RNA levels increase in the cell cytoplasm. This may then promote the activation of a viral defense response through RNA sensors (85).

Additionally, demethylating agents may cause toxicity in cancer cells through non-immune mechanisms of action. One of the most plausible and studied mechanisms of action is based on the evidence that cancerous cells share an overall hypermethylation of tumor-suppressor gene promoters; with demethylating agents, these genes are 'unlocked' and therefore can provide a tumor-suppressor function (86).

Gene-body methylation may also be important. It affects transcription in a directly proportional way, in that the more a gene-body is methylated, the more it is expressed (86,88). However, this mechanism is poorly understood (86,88).

Another theory is based on the evidence that global demethylation may lead to direct toxicity in cancerous cells, causing their death or interrupting their proliferation; normal cells instead tend to survive against these drugs (86,89). However, the global DNA methylation state cannot predict response to therapy, and thus it is probably not the only mechanism involved (86).

Another mechanism of action describes the cytotoxicity of demethylating agents as the main cause of their efficacy in cancer therapy; specifically, they may trap DNMTs onto DNA, resulting in bulky adducts that can inhibit DNA synthesis and eventually cause cell death (86).

It is likely that all these theories have some involvement, though it is unknown which are more important than others.

5-Azacytidine (5-aza-CR) and 5-aza-2'-deoxycytidine are the main demethylating drugs. These are nucleoside analogs of cytosine and can inhibit DNA methylation by being incorporated in a DNA molecule at the position of cytosine during DNA replication (90). When a DNA methyltransferase interacts with a DNA molecule modified in this way, it ends adhere to the DNA, resulting in a long-lasting demethylation.

miRNA based therapy can be expected to be a critical topic in upcoming research. As discussed, miRNAs have a crucial role in regulating gene expression for both tumor suppressor genes and oncogenes. For therapeutic purposes, the aim is to inhibit the miRNAs that are overexpressed and to replace those that are insufficient.

Use of miRNA mimics is one such strategy to replace underexpressed miRNAs; these molecules must be modified to achieve optimal pharmacokinetics and pharmacodynamics, for example through conjugation with a sugar molecule or utilizing liposomes (91).

Conversely, treatment with miRNA inhibitors is the strategy for lowering the concentration of overexpressed miRNAs. These molecules are capable of binding specific miRNAs, preventing their binding with target mRNA (91).

7. Conclusions

Among the most considerable problems with treating OSCC and OPSCC is the tendency of these cancers to become chemo-radioresistant. However, this is among the issues that can be bypassed through epigenetic-based therapy; in fact, it is established that certain demethylase inhibitors can induce a chemo-radiotherapy resensitizing effect in various cancer types (92-94). HNSCC cell lines (including SQD9, SCC61, Cal27, SC179, SC263, JH011) have been identified to have an increased response to radiation therapy following exposure to demethylating agents in vitro (95,96), suggesting that these drugs may be used efficiently in combination with other drugs and radiation to achieve an improved clinical outcome.

Another issue that may be, at least in part, bypassed with demethylating drugs is radiation-related toxicity; radiation therapy is associated with numerous side effects caused by direct effect of the radiation on healthy tissues (97,98). In oral and oropharyngeal cancers, the most frequent of such effects are mucositis, dysphagia, trismus, dental problems, xerostomia and osteoradionecrosis (97,98). As discussed, demethylating drugs may enhance the radiation sensibility of cancer cells; this would make possible the use of lower doses of radiation to achieve the same therapeutic benefits while potentially minimizing the side effects.

In conclusion, oral and oropharyngeal cancers represent a major health issue, both regarding their epidemiological characteristics and poor clinical outcome. There have been several advances in our understanding of these malignancies, some of which have been used for prevention purposes (for instance by acknowledging the risk factors), and others have had an impact on therapeutic approaches.

Epigenetics influences all phases of cancer development and progression. The understanding of its functioning comprises an important aim of recent research due to the possible therapeutic implications. This is particularly important in oral and oropharyngeal cancer, since the relevant therapeutic approaches and clinical outcomes are still based almost exclusively on classical chemotherapy. This is probably a main reason for the lack of improved survival outcome over time, or at least for the lower extent of improvement than would be expected. In the short term, it is probable that epigenetics will be used as a basis for therapeutic purposes.

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Competing interests

All authors declare no competing interests.

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