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REVIEW ARTICLE

DNA methylation in oral squamous cell carcinoma: molecular mechanisms and clinical implications

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DNA methylation is an important regulator of gene transcription, and its role in carcinogenesis has been a topic of considerable interest in the last few years. Of the all epigenetic modifications, methylation, which represses transcription of the promoter region of tumor suppressor genes leading to gene silencing, has been most extensively studied. Oral squamous cell carcinoma (OSCC) has long been known to be the endpoint of many genetic changes, not only genomic mutations but also abnormal epigenetic modifications, as such, promoter methylation, contribute to development of this tumors. Recent studies have shown that promoter methylation of tumor suppressor genes is an important factor in carcinogenesis of OSCC. Some of the main genes that frequently showed promoter methylation in OSCC are those that participate in diverse processes such as regulation of the cell cycle, DNA repair, proliferation, and apoptosis. The aim of this review is to assess the current state of knowledge regarding promoter methylation of diverse genes in OSCC.

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Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy in the world today; approximately 405 000 cases of OSCC are diagnosed each year, with a rising incidence in many countries (Marsh *et al*, 2011). Accounting for between 90% and 95% of all malignant lesions of the mouth, OSCC has become practically synonymous with oral cancer.

Early detection of OSCC is important to reduce mortality rates and to help provide successful cancer treatment. The etiology of OSCC is multifactorial and involves intrinsic and extrinsic factors. Although it is well known that tobacco and alcohol are the two primary environmental risk factors associated with the development of OSCC (Hashibe *et al*, 2009), it is now recognized that HPV infection plays an important role in the pathogenesis of oral cancer, although it is higher for the oropharyngeal subset (Dayyani *et al*, 2010; Machado *et al*, 2010).

Methylation is a common epigenetic mechanism that leads to gene silencing in tumors and could be a useful biomarker in OSCC; thus, the promoter methylation of many tumor suppressor genes has been reported (Ha and Califano, 2006; Shaw, 2006; Perez-Sayans *et al*, 2009; Diez-Perez *et al*, 2011).

The aim of this review is to analyze the current state of DNA methylation in OSCC as well as to emphasize the important role of epigenetic biomarker usage in the prognosis, diagnosis, and therapy associated with OSCC.

DNA methylation

DNA methylation is an epigenetic modification that is significant in controlling gene activity as well as in cell structure (Esteller, 2008). DNA methylation usually takes place at the 5′ position of the cytosine ring within CpG nucleotides, and its consequence is the silencing of genes and noncoding genomic regions. The CpG dinucleotides are found in 1 per 80 dinucleotides in 98% of the human genome; when they are grouped in small fragments of DNA, they are called CpG islands, which cover from 1% to 12% of the total human genome; CpG islands are located throughout one of the DNA chains, usually placed near a promoter gene or in regions of large repetitive sequences (e.g., centromeric repeats, retrotransposon elements, rDNA, etc.) (Kargul and Laurent, 2009; Sharma *et al*, 2010). Although, in the latter case, most of the CpGs are methylated to prevent chromosome instability, the majority of CpG islands remain unmodified during development and in differentiated tissues (Suzuki and Bird, 2008). Recent findings also suggest that extensive DNA methylation

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changes caused by differentiation take place at CpG island ''shores'', regions of comparatively low CpG density close to CpG islands (Meissner *et al*, 2008; Doi *et al*, 2009).

The enzymes directly responsible for CpG island hypermethylation of tumor-suppressor genes are known as DNA methyltransferases (DNMTs), and there are three main DNMTs: DNMT1, which maintains the existing methylation patterns following DNA replication, and DNMT3A and DNMT3B, *de novo* enzymes that target previously unmethylated CpGs[5] (Rodriguez-Paredes and Esteller, 2011). The DNA methylation code has to be read by cell; the information stored by hypermethylation CpG islands is in part interpreted by methyl-CpG binding proteins (MBDs). MBDs are important ''translators'' between DNA methylation and histone-modifier genes that establish a transcriptionally inactive chromatin environment. This family of proteins consist of five well-characterized members (MeCP2, MBD1, MBD2, MBD3, and MBD4) (Ballestar and Esteller, 2005). MBDs proteins are associated with hypermethylated CpG island promoters of tumor-suppressor genes and their transcriptional silencing (Ballestar *et al*, 2003; Lopez-Serra *et al*, 2006). The finding that MeCP2 represses transcription of methylated DNA through the recruitment of a histone deacetylase-containing complex established for the first time a mechanistic connection between DNA methylation and transcriptional repression by the modification of chromatin (Jones *et al*, 1998).

Methylation is an important mechanism in transcriptional regulation and an actual correlation has been observed between the density of methylated cytosine residues and transcriptional activity (Jones and Baylin, 2007; Kargul and Laurent, 2009). Epigenetic changes in cancer have traditionally been evaluated by measuring the status of the CpG island cytosine methylation of a particular gene; the molecular techniques used to detect methylation have evolved from the Southern blot to more sensitive quantitative-PCR techniques. To date, diverse techniques used at present to detect methylation provide good sensitivity, specificity, and speed, as shown in Table 1.

Impact of diet, lifestyle, and environmental factors in DNA methylation

Dietary, lifestyle, and other environmental factors induce epigenetics alterations that may have important consequences for cancer development. The concept that carcinogens and lifestyle factors contribute to tumorigenesis through epigenetic mechanisms is critical and holds great promise in disease prevention and treatment (Marsit *et al*, 2009). Existing evidence supports the

Table 1 Molecular techniques for detecting DNA methylation

Method	*Description*	*Limitations*
Nearest neighbor analysis (Gruenbaum *et al*, 1981)	Residue detection 3′of methylated cytosines in the whole genome	No connection to genes or methylated positions in the DNA
Restriction digestion–Southern blot (SB) (Singer-Sam *et al*, 1990)	Methylation-sensitive and-insensitive restriction enzyme digestion and SB hybridization	For specific sites only, requires abundant DNA, relatively low sensitivity
Methylated CpG island amplification (Toyota *et al*, 1999)	Methylation-sensitive enzyme cutting and subtractive hybridization of normal and tumor DNA samples	Limited specificity owing to partial digestion, ligation or hybridization
Genomic bisulfite sequencing (BS) (Frommer *et al*, 1992)	Sodium bisulfate conversion of cytosine (but not methylcytosine) to uracil, PCR amplification and sequencing	Complicated procedure limiting efficiency
Combined bisulfite restriction analysis (Xiong & Laird, 1997)	As in BS but direct sequencing replaced by restriction digestion of sites potentially affected by bisulfite	For specific sites only
Methylation-specific PCR (MSP) (Herman *et al,* 1996)	As in BS but using PCR amplification with primers differentiating between cytosine and methylcytosine	Mainly for surveying CpG-rich regions of DNA (CpG island)
Methylation-sensitive single nucleotide primer extension (Gonzalgo & Jones, 1997)	Sodium bisulfate treatment with primer extension reaction involving radioactive labeled cytosine or thymidine	Slow in large scales, only few cytosines can be analyzed simultaneously
MSP with efficient detection (MethyLight) (Eads *et al*, 2000)	Sodium bisulfate treatment, MSP primer reaction, real-time fluorescence analysis	Mainly for surveying CpG-rich regions of DNA (CpG island)
Restriction landmark genomic scanning (Hatada *et al*, 1991)	Methylation-sensitive and-insensitive restriction digestions, isotope labeling, 2-D electrophoresis	Requires appropriate enzymes and relatively abundant DNA
Differential methylation hybridization (Huang *et al*, 1999)	Methylation-sensitive enzyme cutting, hybridization of PCR amplified and labeled normal and tumor DNA on array of fragments from CpG island genomic library	Requires appropriate enzymes and relatively abundant DNA
Methylation-specific oligonucleotide microarray method (MSO) (Gitan *et al*, 2002)	Sodium bisulfite treatment, PCR amplification, hybridization on glass slide array of oligonucleotides that discriminate between converted and unconverted CpG	Can miss sparse methylation
Microarray-based DNA methylation analysis (Adorjan *et al*, 2002)	As MSO but using PCR amplification with primers not overlapping the CpG dinucleotides of the target genes	Limited choice of suitable PCR primers

notion that all recognized epigenetic marks, such as DNA methylation, are influenced by environmental exposure, including diet, tobacco, alcohol, physical activity, stress, environmental carcinogens, genetic factors, and infectious agents, which play important roles in the etiology of cancer (Chen and Xu, 2010; Mathers *et al*, 2010; Ross, 2010).

Folate, found in fresh fruits and vegetables, is essential in the conversion of methionine to S-adenosylmethionine (SAM), the principle methyl donor for methylation (Ross, 2010). Alcohol intake has also been reported to impair folate absorption, increase folate excretion, and interfere with one carbon metabolism (Mason and Choi, 2005). Researchers recently have evaluated whether diet and multivitamin use influences the prevalence of gene promoter methylation in cells exfoliated from the aerodigestive tract of current and former smokers (Stidley *et al*, 2010). In this study, the promoter methylation of eight genes was analyzed; methylation status was categorized as low (fewer than two genes methylated) or high (two or more genes methylated); significant protection against methylation was observed for consumers of leafy green vegetables and folate as well as for current users of multivitamins. These findings support the concept that novel interventions to prevent cancer could be explored based on the ability of diet and dietary supplements to affect reprogramming of the epigenome (Stidley *et al*, 2010).

The folates function in cells as their reduced form conjugated with a polyglutamate chain; they have a single type of function in that they can accept so-called C_1 units from various donors and pass them on in various biosynthetic reactions (Scott and Weir, 1994; Scott, 1999). Thus, in cells folates will be a mixture of polyglutamyl tetrahydrofolates and various C_1 forms of tetrahydrofolate (e.g., 10-formyl-, 5, 10 methylene-and 5-methyltetrahydrofolate), depending on which C group is attached to them. Alternatively, the form of folate (5,10-methylenetetrahydrofolate) used for thymidylate synthase can be channeled up to the ''methylation cycle''. This cycle performs important function; it ensures that the cell always has an adequate supply of SAM, which is an activated form of methionine acting as a methyl donor to a whole range of methyltransferases. Other methyltransferases down-regulate DNA and suppress cell division, methylate 3,4-dihydroxyphenylalanine and lipids, etc. (Scott, 1999).

On the other hand, the relationship among tobacco, alcohol, HPV infection, and methylation in OSCC is not clear. Tobacco and alcohol use are the most important known risk factors for the development of OSCC, suggesting that the exposure to these risk factors may increase the predisposition for genetic and epigenetic alterations such as DNA methylation. Guerrero-Preston *et al* (2009) reported that global DNA methylation index was shown to vary for oral cancer cases with different etiologies. In this study, predictive simulations were performed to explore associations between etiological factors and global DNA methylation in OSCC samples. The global methylation index was found to be 4.28 (95% CI, 4.1–4.4) in an oral cancer series, without statistically significant differences between the global DNA methylation levels of patients who had smoking ($P = 0.21$), drinking ($P = 0.31$), and HPV insertion ($P = 0.34$) compared with patients who did not. According to predictive simulations, an inverse association between smoking and DNA methylation was observed; as the probability of smoking increases, the probability of DNA methylation decreases, and on the other hand, no associations between the probability of DNA methylation and drinking or DNA methylation and HPV insertion also were observed. Finally, no difference in global DNA methylation levels between cases with different etiologies was observed. Global genomic DNA hypomethylation may precede and subsequently coexist with gene-specific promoter hypermethylation and hypomethylation in cancer (Guerrero-Preston *et al*, 2009).

Nevertheless, Smith *et al* (2007) reported in squamous cell head and neck cancer (HNC) samples that about 67% of tumors showed a degree of global hypomethylation that exceeded the measure of any normal mucosal specimen; these results suggest that, despite promoter hypermethylation of individual tumor suppressor genes, HNC are global hypomethylated. In relation with smoking status and global status of methylation in tumors, tobacco exposure may be causing genome-wide damage apparent in this epigenetic assay; finally, global methylation mean levels were reduced in advanced clinical stage (IV/45.7), compared with the others clinical stages (I/48.4, II/47.0, III/48.8), suggesting this epigenetic change worsens as tumorigenesis progresses (Smith *et al*, 2007).

Moreover, according to a recent study (González-Ramírez *et al*, 2011), the promoter methylation of the hMLH1 gene was not associated with alcohol and tobacco consumption in OSCC, in agreement with others reported date (Taioli *et al*, 2009). Nevertheless, Sengupta *et al* (2007) found that a significant proportion of smokers exhibited hMLH1 methylation compared with non-smokers (35%) ($P = 0.001$) in head and neck carcinoma and oral leucoplakia samples (Sengupta *et al*, 2007). Additionally, de Freitas Cordeiro-Silva *et al* (2011) analyzed the methylation status of cancer-related genes by methylation-specific PCR (MSP) in normal oral exfoliated cells from patients with OSCC and their probable association with tobacco and alcohol consumption; the results showed no association between methylation and smoking/drinking habits (de Freitas Cordeiro-Silva *et al*, 2011).

On other hand, von Zeidler *et al* (2004) evaluated the methylation status of the $p16^{INK4A}$ gene in potentially malignant oral lesions with DNA samples from normal mucosa and the posterior tongue border from 258 smokers without oral cancer. Using methylation-sensitive restriction enzymes and PCR amplification, MSP, or direct DNA sequence of bisulfate modified DNA, hypermethylation was detected in 9.7% of the cases analyzed; these findings confirmed that methylation in tumor suppressor genes is an early event that might confer cell growth advantages contributing to the tumorigenic process. Thus, the detection of an abnormal

$p16^{INK4A}$ methylation pattern may be a valuable tool for early oral cancer detection in people with risk factors for the development of oral cancer (von Zeidler *et al*, 2004).

In relation to infectious agents, HPV was detected in about 20% of all HNC; while there is strong evidence for a causal etiological role in the case of tonsillar carcinomas, there is no conclusive evidence of a causal association with malignant lesions of the oral cavity (Machado *et al*, 2010). A previous study (Balderas-Loaeza *et al*, 2007) reported hypermethylation of important segments of the viral DNA in 10 of 12 HPV-16-positive oral carcinomas from Mexican patients. These data indicate epigenetic changes of HPV-16 in oral carcinomas similar to those of other carcinomas related to HPV, suggesting carcinogenic processes under the influence of HPV-16 in most, if not all, of these oral malignant lesions.

Silencing of genes by promoter methylation in OSCC

Several studies have shown that promoter methylation of many genes is an important factor in the carcinogenesis of OSCC. As shown in Table 2, many genes have been tested for methylation in OSCC tissue, and it is important to note numerous studies in head and neck cancer, including the subset of the oral cavity. These tumor-suppressor genes all have a mechanistic basis for their role in carcinogenesis and are generally implicated in other tumor types (Ha and Califano, 2006). Some of the main genes that frequently showed promoter methylation in OSCC are those that participate in the different cellular pathways such as: regulation of the cell cycle, such as the $p16^{INK4A}$ gene, an inhibitor of kinase dependent on the cyclin, located in the 9p21 chromosome; this gene was first identified as a putative tumor-suppressor gene. The $p16^{INK4A}$ gene has been reported as methylated in OSCC at frequencies of 23–67% (Viswanathan *et al*, 2003; Kulkarni and Saranath, 2004). The alterations in this gene affect cell cycle regulation, specifically when suppressing the G1 phase. Other participant genes in cell cycle regulation are $p14^{ARF}$ and $p15^{INK4B}$, having reported methylated rates ranging from 14% to 43% (Ogi *et al*, 2002; Ishida *et al*, 2005) for $p14^{ARF}$ and from 9% to 23% for $p15^{INK4B}$ (Ogi *et al*, 2002; Viswanathan *et al*, 2003). Existing date suggest that co-methylation of $p16^{INK4A}$ and $p14^{ARF}$ is associated with tumor progression. Ishida *et al* (2005) detected correlating of $p16^{INK4A}/p14^{ARF}$ co-methylation with lymph node metastasis and tumor clinical stage. Hypermethylation of $p14^{ARF}$ alone also appeared to be significantly associated with clinical stage (Ishida *et al*, 2005).

The genes involved in DNA repair are also affected by methylation; for example, the genes of the system of

Table 2 Promoter methylation in oral squamous cell carcinoma (OSCC) and head and neck carcinoma

References	*Tissue*	n	$p14^{ARF}$ *(%)*	$p15^{INK4B}$ *(%)*	$p16^{INK4A}$ *(%)*	*E-cadherin (%)*	*DAPK (%)*	*MGMT (%)*	*MLH1 (%)*
Nakahara *et al* (2001)	OSCC	32	NT	NT	50	NT	NT	NT	NT
Chang *et al* (2002)	OSCC	70	NT	NT	NT	64	NT	NT	NT
Huang *et al* (2002)	OSCC	48	NT	NT	42	NT	NT	NT	NT
Ogi *et al* (2002)	OSCC	96	14	9	29	NT	7	NT	0
Yeh *et al* (2002)	OSCC	48	NT	NT	NT	85	NT	NT	NT
Viswanathan *et al* (2003)	OSCC	99	NT	23	23	35	NT	41	8
Kulkarni and Saranath (2004)	OSCC	60	NT	NT	67	NT	68	52	NT
Ishida *et al* (2005)	OSCC	49	20	NT	35	NT	NT	12	NT
Tran *et al* (2005)	OSCC	27	NT	NT	63	NT	NT	NT	NT
Kato *et al* (2006)	OSCC	55	NT	NT	51	NT	NT	56	NT
Czerninski *et al* (2009)	OSCC	28	NT	NT	NT	NT	NT	NT	17
Ohta *et al* (2009)	OSCC	44	NT	NT	64	NT	NT	NT	NT
Supic *et al* (2009)	OSCC	77	NT	NT	58	43	37	34	NT
Kordi-Tamandani *et al* (2010)	OSCC	76	NT	NT	NT	62	NT	74	NT
González-Ramírez *et al* (2011)	OSCC	50	NT	NT	NT	NT	NT	NT	76
Sanchez-Cespedes *et al* (2000)	HNC	95	NT	NT	27	NT	18	33	NT
Rosas *et al* (2001)	HNC	30	NT	NT	47	NT	33	23	NT
Hasegawa *et al* (2002)	HNC	80	NT	NT	32	36	24	NT	NT
Liu *et al* (2002)	HNC	62	NT	NT	NT	NT	NT	NT	92
Liu *et al* (2003)	HNC	78	NT	NT	NT	NT	NT	NT	50
Maruya *et al* (2004)	HNC	32	20	NT	33	2	25	37	NT
Puri *et al* (2005)	HNC	62	NT	NT	36	NT	NT	30	23
Demokan *et al* (2006)	HNC	116	NT	NT	NT	NT	NT	NT	47
Calmon *et al* (2007)	HNC	43	30	NT	63	88	81	NT	NT
Righini *et al* (2007)	HNC	90	11	18	29	36	27	29	2
Sengupta *et al* (2007)	HNC	99	NT	23	23	35	NT	41	8
De Schutter *et al* (2009)	HNC	46	NT	NT	5	13	11	42	NT
Zuo *et al* (2009)	HNC	120	NT	NT	NT	NT	NT	NT	32
Tawfik *et al* (2011)	HNC	49	NT	NT	NT	NT	NT	NT	87

NT = no tested, HNC included OSCC samples.

DNA mismatch reparation, such as hMLH1, that contribute to the maintenance of genetic sequence, minimizing cell death, mutation rates, replication errors, DNA damage persistence, and genomic instability. The hMLH1 gene is located in the 3p21 chromosome and is expressed in constantly renewed cells such as the epithelial cells of the oral mucosa and the gastrointestinal tract. This gene has reported methylation rates ranging from 8% to 76% in OSCC (Viswanathan *et al*, 2003; González-Ramírez *et al*, 2011). The silencing of this gene through methylation avoids the elimination of base–base likings, facilitating malignant transformations by genetic mutation accumulation. Recently, González-Ramírez *et al* (2011), in a case–control study among Mexican people with 50 patients with OSCC and 200 control samples of healthy individuals, found that promoter methylation of hMLH1 was detected in 38 (76%) patients with OSCC but in none of the control samples; additionally, the study showed a high frequency of methylation cases with negative expression for the hMLH1 protein corresponding to an early clinical stage. These results suggest that promoter methylation of hMLH1 is an early event in oral cancer (González-Ramírez *et al*, 2011).

Another important DNA repair gene is the MGMT gene (O^6-methylguanine-DNA methyltransferase), located at the 9p21 chromosome. MGMT is a detoxifying agent of DNA adducts. It is important in preventing alkylation and, thus, could be predictive of chemosensibility (Ha and Califano, 2006). The importance of the MGMT gene lies in the repairing of the genome when it suffers damage from by alkylation because it can eliminate the alkyl groups found in position O^6 of guanine, which safeguards genomic stability (Kato *et al*, 2006). This gene has a reported methylation rate of 26–74% in OSCC cases (Ishida *et al*, 2005; Kordi-Tamandani *et al*, 2010). Kato *et al* (2006) described that MGMT promoter hypermethylation and loss of their protein expression can be used as reliable and prognostic predictors for tumor recurrence and patient survival in HNC (Kato *et al*, 2006). Moreover, Kordi-Tamandani *et al* (2010) recently showed that MGMT methylation may be considered as a potential molecular marker for the poor survival in advanced OSCC (Kordi-Tamandani *et al*, 2010).

The role of MGMT in the resistance to alkylating chemotherapy is associated with the silencing of the MGMT gene by promoter methylation that results in decreased MGMT expression in tumor cells (Qian and Brent, 1997). This is then followed by the removal of the methyl group from the O^6 position of guanine, thereby restoring the nucleotide to its native form without causing any DNA strand breaks. On transfer of the alkyl group to an internal cysteine residue in the active site of MGMT (Hegi *et al*, 2008). Although the O^6 position of guanine is not the most common target of alkylating agents, the resulting promutagenic lesions act as an important trigger for cytotoxicity and apoptosis (Ochs and Kaina, 2000).

A positive mediator in apoptosis induction is the protein produced by the DAPK gene, which, in OSCC cases, has been found to be methylated at rates ranging from 7% to 68% (Ogi *et al*, 2002; Kulkarni and Saranath, 2004). Interestingly, for head and neck cancer, a correlation between the methylation of DAPK and the presence of metastatic lymph nodes has been reported (Sanchez-Cespedes *et al*, 2000).

The promoter methylation patterns of the MGMT, $p16^{INK4A}$, and DAPK genes have been used as a molecule marker in neoplastic cells and in other human fluids. A previous research study reported that in 50 patients with head and neck cancer, including OSCC, there was 55% aberrant methylation in the promoter of the MGMT, $p16^{INK4A}$, and DAPK genes; the same methylation pattern was detected in the corresponding serum DNA (42%) of the cases (Sanchez-Cespedes *et al*, 2000). The epigenetic silencing of the MGMT, $p16^{INK4A}$, and DAPK genes imply alterations in the DNA reparation process, the cell cycle, and the processes related to metastasis, respectively (Ha and Califano, 2006). Table 3 shows a selected list of genes frequently methylated in OSCC, with their principal characteristics.

Epigenetic therapy in OSCC

The reversible nature of the profound epigenetic changes that occur in cancer has led to the possibility of epigenetic therapy as a treatment option. The aim of epigenetic therapy is to reverse the causal epigenetic

Table 3 Selected genes that undergo methylated in oral squamous cell carcinoma

Gene	*Function*	*Location*	*Consequences*
$p14^{ARF}$	MDM2 inhibitor	9p21	Degradation of p53
$p15^{INK4B}$	Cyclin-dependent kinase inhibitor	9p21	Entrance in cell cycle
$p16^{INK4A}$	Cyclin-dependent kinase inhibitor	9p21	Entrance in cell cycle
MLH1 Mut L homologue 1	DNA mismatch repair	3p21.3	Frameshift mutations
MGMT O-6 methylguanine-DNA methyltransferase	DNA repair of O6-alkyl-guanine	10q26	Mutations, chemosensitivity
CDH1 E-cadherin	Increasing proliferation, invasion	16q24	Dissemination
DAPK Death-associated protein kinase	Pro-apoptotic	9q34.1	Resistance to apoptosis

aberrations that occur in cancer, leading to the restoration of a ''normal epigenome''. Many epigenetic drugs have been discovered in the recent past that can effectively reverse DNA methylation that occurs in cancer (Esteller, 2008; Sharma *et al*, 2010).

DNA methylation inhibitors were the first epigenetic drugs proposed for use as cancer therapeutics; this drug-induced reduction of DNA methylation causes growth inhibition in cancer cells by activating tumor suppressor genes that are aberrantly silenced in cancer (Yoo and Jones, 2006). Demethylating therapy has been used in solid tumors and in some other types of hematological malignancies, such as myelodysplastic syndrome and leukemia; in both cases, the 5-Aza-CR (azacitidine) and 5-aza-CdR (decitabine) have been FDA approved for use in treatment (Rudek *et al*, 2005; Mack, 2006; Plimack *et al*, 2007).

The information about demethylation induction en OSCC is scarce; nevertheless, recently, Brieger *et al* (2010) analyzed the methylation status and expression of hic1, a potential tumor suppressor gene frequently hypermethylated in several HNC, including OSCC. In this study, 21 of 22 analyzed primary tumor samples methylated, as well as in the three analyzed cell lines, suggesting the inactivation of this gene. In this study, the three cell lines were treated with the demethylating agent 5-azacytidine (5-Aza) for 72 h; methylation analysis of the promoter confirmed the demethylating activity of the treatment. The hic1-expression was restored after demethylation treatment in the previously methylated cell lines at both the mRNA and protein level and was accompanied by a significant decrease in proliferative activity and clonogenic survival (Brieger *et al*, 2010). Reactivation of silenced tumor suppressor gene by pharmacologic unmasking might therefore become an option in OSCC treatment.

Conclusions

Oral carcinogenesis is a multifactorial process that can alter the functions of oncogenes, tumor suppressor genes, and other related molecules. The determination of promoter methylation status may be a useful molecular target for identifying tumor cells in patients at risk of OSCC and has shown promise in detecting oral cancer from tissue, saliva, and serum samples and in real time analysis of margins during surgery. The creation of methylation gene panels could be useful for OSCC screening to assist in early detection, monitoring, and treatment.

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Author contributions

The categories for author contributions are as follows: I González Ramírez was responsible for conception, intellectual design, preparation, editing and review of the manuscript; C García Cuellar was responsible for conception, intellectual design, editing and review of the manuscript; Y Sánchez Pérez and M Granados García were responsible for manuscript concepts review.

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