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REVIEW

Role of DNA methylation in head and neck cancer

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Abstract Head and neck cancer (HNC) is a heterogenous and complex entity including diverse anatomical sites and a variety of tumor types displaying unique characteristics and different etilogies. Both environmental and genetic factors play a role in the development of the disease, but the underlying mechanism is still far from clear. Previous studies suggest that alterations in the genes acting in cellular signal pathways may contribute to head and neck carcinogenesis. In cancer, DNA methylation patterns display specific aberrations even in the early and precancerous stages and may confer susceptibility to further genetic or epigenetic changes. Silencing of the genes by hypermethylation or induction of oncogenes by promoter hypomethylation are frequent mechanisms in different types of cancer and achieve increasing diagnostic and therapeutic importance since the changes are reversible. Therefore, methylation analysis may provide promising clinical applications, including the development of new biomarkers and prediction of the therapeutic response or prognosis. In this review, we aimed to analyze the available information indicating a role for the epigenetic changes in HNC.

Keywords Methylation · Epigenetics · Head and neck cancer

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Introduction

Head and neck cancer (HNC) is a highly heterogenous group of malignant diseases and the sixth most frequently observed cancer type in developing countries (Crowe et al. 2002; Ohshima et al. 2005). It reveals different combinations of various sites and disease types which result from sequential genetic changes in multiple intracellular pathways and inherent viral infections. HNC displays serial dysplastic alterations before acquiring invasive characteristics. Tobacco and/or alcohol use are the main etiological factors and play an important role in oral cavity, pharynx, and larynx cancer (Ohshima et al. 2005). Acute laryngitis which can be caused by chronic irritation, inhalation of irritants, viral infections, or allergic reactions may also increase the risk of cancer development (Kumar et al. 2000). The prognosis of the disease varies according to tumor size, local invasion, histology, and grade as well as ethnic origin. These diverse varieties reflect the versatile pathogenesis of the disease. Identification of novel therapeutic targets and new and specific biomarkers for the early detection of HNC could greatly increase the survival rate and might also help as prognostic indicators.

Head and neck carcinogenesis is a multistep and multifactorial complex mechanism containing a variety of genetic and epigenetic abnormalities in DNA repair, signal transduction, apoptosis, angiogenesis, proliferation, differentiation, and cell cycle regulation (Scully et al. 2000). In recent years, the role of epigenetic alterations have been increasingly recognized. Changes in the methylation patterns are one of the most frequent events in human tumors (Jones

and Baylin 2002). Two types of methylation changes are usually observed : Hypermethylation of the CpG islands and global hypomethylation in a variety of tumors. DNA hypomethylation has been associated with the activation of oncogenes and chromosomal instability leading to over-expression of the oncogenes, while DNA hypermethylation is associated with the repression of tumor suppressor genes (TSG) and genomic instability. DNA methylation also plays an important role in tumor initiation and progression (Jones and Baylin 2002; Momparler and Bovenzi 2000). Thus, gene silencing by hypermethylation is an important mechanism that has great promise for therapy and for the discovery of new biomarkers. Hovewer, the information on the frequency and specificity of methylation changes is still insufficient. It presents a challenge to identify crucial genes that are susceptible to methylation-induced silencing and are directly associated with the development of HNC. Since HNC is a heterogenous disease, the methylation status may vary according to clinical characteristics and environmental and genetic factors such as anatomic involvement of tumor (Dikshit et al. 2007; Azarschab et al. 2003), human papilloma virus (HPV) positivity (Bennett et al. 2010; Richards et al. 2009), smoking (Sharma et al. 2010), microsatellite instability (MSI) (Zuo et al. 2009; Demokan et al. 2006; Sengupta et al. 2007; Liu et al. 2003a, b, 2002), and geographic location (Li et al. 2003a, b; Ayadi et al. 2008). Therefore, analysis of the epigenetic changes in genes playing important roles in these vital molecular mechanisms is crucial in order to understand the molecular biology of head and neck carcinogenesis.

Genetic evidence indicates that the majority of squamous HNC originate from hyperplastic fields characterized by higher numbers of proliferating cells and clonal selection (Rubin 2011; Tabor et al. 2002). Almost two thirds of the tumor recurrences following resection occur in these regions (Tabor et al. 2004; Ha and Califano 2003). Clonal selection, divergence, and expression are the basic characteristics of the field development. Molecular evidence including cells harboring mutations of the TSG and recent data on *p53* mutations support the field cancerization theory. The presence of cells with *p53* gene mutations in these fields have also been shown in HNC (Tabor et al. 2001). The genetic changes characterizing these regions can be used to identify individuals at risk of developing cancer (Dakubo et al. 2007). Furthermore, methylation in noncancerous tissues is now considered as a marker for potential cancer risk and previous exposure to certain carcinogenic factors (Nakajima et al. 2008). Knowledge of the methylation status in these regions may enable intervention by using demethylating agents as chemopreventive means (Dakubo et al. 2007). An epigenetic field effect has been first described by increased aberrant methylation in normal tissue from patients with liver cancer (Kondo et al. 2000). Later studies have unequivocally shown the presence of an epigenetic field for cancerization (Ushijima 2007). Recently, the presence of an epigenetic field for cancerization has also been shown in colon (Shen et al. 2005), breast (Yan et al. 2006), and renal (Arai et al. 2006) cancers.

Recent advances in technology make it possible to analyze DNA methylation by highly sensitive and quantitative methods. For DNA methylation analysis, a variety of different methodologies have been used, almost all of which rely on three different approaches for treating DNA: the action of methylation-specific endonucleases, bisulfite modification of unmethylated cytosines, or immunoprecipitation (IP) of the methylated DNA fragments. Combination of these initial steps with different detection techniques for analysis have resulted in various analytical methods to investigate candidate genes or to study global DNA methylation. The main methodologies used for methylation analysis are summarized in Table 1. Several techniques initially confined to smaller regions of the genome have now been extended to perform analysis of the whole genome. New high-throughput methodologies provide information to characterize methylated sequences at single-base resolution on a genome-wide scale.

Recent studies have shown that hypermethylation of some TSG may be a valuable biomarker (Kim et al. 2006; Demokan et al. 2010; Kaur et al. 2010; Pattani et al. 2010) in different cancer types. Furthermore, results of clinical

Table 1 The main methodologies used in DNA methylation analysis

Sequence-specific methylation
Quantitative methylation-specific PCR (QMSP)
Methylation-sensitive restriction enzyme analysis
Sensitive restriction-multiplex PCR
Combined bisulfite restriction analysis (COBRA)
Bisulfite sequencing
Pyrosequencing
Multiplex ligation-dependent probe amplification (MLPA)
Mass array analysis
Global methylation
Restriction landmark genomic scanning (RGLS)
Arbitrarily primed methylation-sensitive PCR (AP-MSP)
Methylated CpG island recovery assay (MIRA)
Differential methylation hybridization (DMH)
Analysis of intermethylated sites (AIMS)
Methylated DNA immunoprecipitation (MeDIP)
Whole-genome shotgun bisulfite sequencing (WGSBS)

studies using DNA methyltransferase and histone deacetylase inhibitors indicate the potential of epigenetic therapeutics in clinical oncology (Ren et al. 2011; Wagner et al. 2010). The epigenetic changes may help to predict the prognosis and identify individuals who may benefit from the therapy with demethylating agents (Issa et al. 2004).

In this review, we aimed to summarize the present state of knowledge in head and neck carcinogenesis by analyzing the studies investigating the relationship between methylation and HNC. The publications in the literature were retrieved by literature and keyword search using the ISI, PubMed, and Scopus databases to identify the studies relevant to this review. Citations in these publications were also evaluated for their relevance.

Data from analysis of tumor suppressor gene panels

In recent years, most studies have focused on the analysis of promoter methylation of TSG panels playing a role in vital cellular mechanisms via the candidate gene strategy approach (Table 2). In a study among the North American population (Demokan et al. 2010), *KIF1A* and *EDNRB* genes were highly methylated (97% and 98%, respectively) in the primary tumor tissue and salivary rinse samples from patients with squamous cell carcinoma, while normal salivary and normal mucosal samples were minimally methylated. A significant association between *KIF1A* methylation and tumor site was reported. Kaur et al. (2010) found high methylation levels of the *KIF1A*, *EDNRB*, *DCC*, and *p16* genes in Indian patients with oral squamous cell carcinoma (OSCC) and Pattani et al. (2010) have shown that, in salivary samples from patients with premalignant or malignant oral cavity lesions, promoter hypermethylation of the *KIF1A* and *EDNRB* genes was associated with malignancy. The endothelin receptor type B (*EDNRB*) is a G protein-coupled receptor which activates a phosphatidylinositol–calcium second messenger system (Smollich and Wülfing 2008). The kinesin family member 1A (*KIF1A*) gene encodes a protein that is a microtubule-dependent molecular motor involved in important intracellular functions such as organelle transport and cell division (Okada et al. 1995).

We have evaluated the epigenetic changes specific to head and neck squamous cell carcinoma (HNSCC) by investigating promoter hypermethylation of a panel of 24 TSG via candidate gene approach in a recent study (Yalniz et al. 2011). *CHFR*, *RARβ*, *DAPK1*, and *RASFF1A* genes were found to be the most frequently methylated genes in HNC tumor tissue by methylation-specific multiplex ligation-dependent probe amplification. A further collaborative study analyzing a panel of 22 genes confirmed these findings and found that the *RARβ*, *APC*, and *CHFR* genes were frequently hypermethylated in HNC (Chen et al. 2007).

In another study, six genes were analyzed in patients with HNSCC treated by radiotherapy (de Schutter et al. 2009). The *MGMT* and *TIMP3* genes displayed higher methylation rates. Promoter hypermethylation of the *TIMP3* and *CDH1* genes were significantly associated with better locoregional control (LRC) and overall survival (OS) or disease-free survival (DFS). *TIMP3* methylation highly correlated with *DAPK* methylation, indicating a very strong functional association between these two genes (Nayak et al. 2007). In other gene panel studies, methylation of the *TIMP3*, *CDH1*, *p16*, *MGMT*, *DAPK*, and *RASSF1* genes were observed in HNSCC tumors and paired saliva samples (Righini et al. 2007; Hasegawa et al. 2002; Rosas et al. 2001; Sanchez-Cespedes et al. 2000). A study analyzing promoter methylation of 15 candidate genes (Steinmann et al. 2009) in tumors and matched normal tissue from patients with HNSCC has shown that methylation of the *p16*, *MGMT*, *DAPK*, *RARβ*, *hMLH1*, *CDH1*, *RASSF2*, *RASSF5*, and *MST1* genes was significantly more frequent in the tumors than the normal tissue (Steinmann et al. 2009). The *hMLH1* (Puri et al. 2005), *RARβ* (Maruya et al. 2004), *p16*, and *MGMT* (Puri et al. 2005; Maruya et al. 2004) genes have also been found to be frequently methylated in HNSCC. The higher increased methylation of the TSG is usually associated with advanced tumor stages and undifferentiated HNSCC. This has been primarily shown for the *p16* and *RASSF5* genes (Steinmann et al. 2009). In the same study, *RASSF4* gene methylation was more frequent in patients with advanced tumor stage and recurrent HNSCC than patients without relapse. Higher methylation of the *p16*, *RARβ*, and *RASSF1* genes was also reported in a study in which matched normal samples were not available (Okami et al. 2005).

In a study investigating three TSG functioning in carcinogen metabolism (*CYP1A1*, *CYP2A13*, and *GSTM1*), the genes were found to be moderately (27.4–58.1%) methylated in the tumors, while methylation levels in normal tissue were much lower. A significant correlation was reported between smoking and the methylation status of the *CYP1A1* and *CYP2A13* genes (Sharma et al. 2010).

Hypermethylation of the *hMLH1* gene, which plays an important role in DNA mismatch repair, has been found to be significantly associated with decreased *hMLH1* protein expression, MSI, and decreased cause-specific survival for HNSCC patients in various studies (Zuo et al. 2009; Demokan et al. 2006; Sengupta et al. 2007; Liu et al. 2003a, b, 2002). Methylation of both *hMLH1* and *hMSH2*

Table 2 Candidate genes frequently methylated in HNC

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
APC	Adenomatous polyposis coli	Tumor suppression	HNSCC (Chen et al. 2007), SGC (Durr et al. 2010; Lee et al. 2010), NPC (Loyo et al. 2011)	MS-MLPA (Chen et al. 2007) QMSP (Durr et al. 2010; Loyo et al. 2011), MSP (Lee et al. 2010)	(HG,St,H) (Lee et al. 2010)	T (Chen et al. 2007; Durr et al. 2010; Lee et al. 2008; Loyo et al. 2011), MN (Durr et al. 2010; Lee et al. 2010)	21–34
BLU	MYND-type containing 10	Tumor suppression	NPC (Ayadi et al. 2008; Liu et al. 2003b; Loyo et al. 2011; Qiu et al. 2004)	QMSP (Loyo et al. 2011), MSP (Ayadi et al. 2008; Liu et al. 2003b; Qiu et al. 2004)	H (Ayadi et al. 2008)	T (Ayadi et al. 2008; Liu et al. 2003b; Loyo et al. 2011; Qiu et al. 2004), NE (Liu et al. 2003b; Qiu et al. 2004)	34–74
CCNA1	Cyclin A1	Cell cycle	OSCC (Shaw et al. 2006) HNC (Tan et al. 2008; Tokumaru et al. 2004), NPC (Yanatatsaneejit et al. 2008)	PS (Shaw et al. 2006), BS (Tokumaru et al. 2004), QMSP (Tan et al. 2008) MSP (Yanatatsaneejit et al. 2008), COBRA (Yanatatsaneejit et al. 2008)	HG (Shaw et al. 2006)	T (Shaw et al. 2006; Tan et al. 2008; Tokumaru et al. 2004; Yanatatsaneejit et al. 2008), NE (Tokumaru et al. 2008; Yanatatsaneejit et al. 2008) MN (Shaw et al. 2006), SM (Tan et al. 2008), L (Yanatatsaneejit et al. 2008)	45–53
CDH1	E-cadherin	Cell adhesion	HNSCC (De Schutter et al. 2009; Hasegawa et al. 2002; Righini et al. 2007; Steinmann et al. 2009), HPC (Dikshit et al. 2007) SGC (Zhang et al. 2007a), OSCC (Chang et al. 2002; De Moraes et al. 2008; Maeda et al. 2007a; Viswanathan et al. 2003; Yeh et al. 2002) LC (Azarschab et al. 2003; Dikshit et al. 2007) NPC (Ayadi et al. 2008; Chang et al. 2003b; Kao et al. 2002; Krishna et al. 2005;	MSP (Ayadi et al. 2008; Azarschab et al. 2003; Chang et al. 2003b; Chang et al. 2002; De Moraes et al. 2008; De Schutter et al. 2009; Dikshit et al. 2007; Hasegawa et al. 2002; Krishna et al. 2005; Li et al. 2003a, b) MSP (Maeda et al. 2007a; Niemhom et al. 2008; Righini et al. 2007; Steinmann et al. 2009; Tsao et al. 2003; Wong et al. 2003b) MSP (Yeh et al. 2002;	(B-LRC, B-S) (De Schutter et al. 2009) (B-DFS, B-DSS, B-O) (De Schutter et al. 2009), (TU,St,TS) (Hasegawa et al. 2002) MET (Azarschab et al. 2003; Li et al. 2003a), (PD,PI) (Zhang et al. 2007a) LNM (Ayadi et al. 2008; De Moraes et al. 2008; Li et al. 2003b), I (Li et al. 2003a) EBV (Kao et al. 2002;	M-SA (Hasegawa et al. 2002; Righini et al. 2007), NE (Chang et al. 2003b; Niemhom et al. 2008; Tsao et al. 2003) MN (Righini et al. 2007; Steinmann et al. 2009; Viswanathan et al. 2003; Yeh et al. 2002) SA-H (Righini et al. 2007) LN (Chang et al. 2002; Li et al. 2003a; Li et al. 2003b) T (Ayadi et al. 2008; Azarschab et al. 2003; Chang et al. 2002, 2003b;	23–85

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
			Li et al. 2003a, b; Niemhom et al. 2008; Tsao et al. 2003; Wong et al. 2003b)	Zhang et al. 2007a), SRM-PCR (Viswanathan et al. 2003) BS (Kao et al. 2002)	Krishna et al. 2005; Li et al. 2003a; Niemhom et al. 2008; Tsao et al. 2003) St (Niemhom et al. 2008)	De Moraes et al. 2008; De Schutter et al. 2009; Dikshit et al. 2007; Hasegawa et al. 2002; Kao et al. 2002; Krishna et al. 2005; Li et al. 2003a) T (Li et al. 2003b; Maeda et al. 2007a; Niemhom et al. 2008; Righini et al. 2007; Steinmann et al. 2009; Tsao et al. 2003; Viswanathan et al. 2003) T (Wong et al. 2003b; Yeh et al. 2002; Zhang et al 2007a)	
CHFR	Checkpoint with forkhead and ring finger domains	Early G2/M checkpoint	HNSCC (Chen et al. 2007; Yalniz et al. 2011) OSCC (Baba et al. 2009; Toyota et al. 2003), NPC (Cheung et al. 2005)	MS-MLPA (Chen et al. 2007; Yalniz et al. 2011) MSP (Baba et al. 2009; Cheung et al. 2005), MSRE (Toyota et al. 2003)	n.a.	T (Baba et al. 2009; Chen et al. 2007; Cheung et al. 2005; Toyota et al. 2003; Yalniz et al. 2011), MN (Baba et al. 2009; Toyota et al. 2003) NE (Baba et al. 2009; Cheung et al. 2005; Toyota et al. 2003)	19–61
DAPK1	Death-associated protein kinase 1	Apoptosis	HNSCC (Chen et al. 2007; Hasegawa et al. 2002; Nayak et al. 2007; Righini et al. 2007; Rosas et al. 2001; Sanchez-Cespedes et al. 2000; Steinmann et al. 2009) OSCC (Ogi et al. 2002), SGC (Li et al. 2005), LC (Zhang and Kong 2004) LC-HPC (Dikshit et al. 2007), OPC (Calmon et al. 2007) NPC (Chang et al. 2003b; Fendri et al. 2009; Kong et al. 2006; Kwong et al. 2002; Wong et al. 2002)	MS-MLPA (Chen et al. 2007) QMSP (Nayak et al. 2007), MSRE (Ogi et al. 2002) MSP (Calmon et al. 2007; Chang et al. 2003b; Dikshit et al. 2007; Fendri et al. 2009; Hasegawa et al. 2002; Kong et al. 2006; Kwong et al. 2002; Li et al. 2005) MSP (Righini et al. 2007; Rosas et al. 2001; Sanchez-Cespedes et al. 2000; Steinmann et al. 2009; Wong et al. 2002; Zhang and Kong 2004),	(St,TS,TU) (Hasegawa et al. 2002) LNM (Calmon et al. 2007; Fendri et al. 2009; Hasegawa et al. 2002; Sanchez-Cespedes et al. 2000) St (Sanchez-Cespedes et al. 2000)	M-SA (Chang et al. 2003b; Hasegawa et al. 2002; Righini et al. 2007; Rosas et al. 2001) NE (Chang et al. 2003b; Kwong et al. 2002; Wong et al. 2002), M-SE (Sanchez-Cespedes et al. 2000) SA-H (Righini et al. 2007), CINT (Kong et al. 2006) MN (Fendri et al. 2009; Righini et al. 2007; Steinmann et al. 2009; Zhang and Kong 2004), (L) (Wong et al. 2002) T (Calmon et al. 2007; Chang et al. 2003b;	7–77

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
						Chen et al. 2007; Dikshit et al. 2007; Fendri et al. 2009; Hasegawa et al. 2002; Kong et al. 2006; Kwong et al. 2002; Li et al. 2005; Nayak et al. 2007; Ogi et al. 2002) T (Righini et al. 2007; Rosas et al. 2001; Sanchez-Cespedes et al. 2000; Steinmann et al. 2009; Wong et al. 2002; Zhang and Kong 2004)	
DCC	Deleted in colorectal cancer	Tumor suppression	OSCC (Kaur et al. 2010; Ogi et al. 2002), HNSCC (Carvalho et al. 2006; Tan et al. 2008), NPC (Loyo et al. 2011)	QMSP (Carvalho et al. 2006; Kaur et al. 2010; Loyo et al. 2011; Tan et al. 2008), BS (Ogi et al. 2002)	(I, P-S) (Ogi et al. 2002)	T (Carvalho et al. 2006; Kaur et al. 2010; Loyo et al. 2011; Ogi et al. 2002; Tan et al. 2008) SA-H (Carvalho et al. 2006; Kaur et al. 2010) SE-H (Kaur et al. 2010), NE (Kaur et al. 2010), SM (Carvalho et al. 2006; Tan et al. 2008)	50–75
DLC1	Deleted in liver cancer 1	Tumor suppression	NPC (Loyo et al. 2011; Seng et al. 2007)	QMSP (Loyo et al. 2011; Seng et al. 2007)	n.a.	T (Loyo et al. 2011; Seng et al. 2007), (MN,NE, SM) (Seng et al. 2007)	43–89
DLEC1	Deleted in lung and esophageal cancer	Tumor suppression	NPC (Ayadi et al. 2008; Loyo et al. 2011)	QMSP (Loyo et al. 2011), MSP (Ayadi et al. 2008)	H (Ayadi et al. 2008)	T (Ayadi et al. 2008; Loyo et al. 2011)	60–86
EDNRB	Endothelin receptor type B	Signaling mechanism	HNSCC (Demokan et al. 2010), OSCC (Kaur et al. 2010; Pattani et al. 2010), PM-OCL (Pattani et al. 2010), NPC (Lo et al. 2002; Zhou et al. 2007)	QMSP (Demokan et al. 2010; Kaur et al. 2010; Pattani et al. 2010), MSP (Lo et al. 2002; Zhou et al. 2007)	H (Pattani et al. 2010)	T (Demokan et al. 2010; Kaur et al. 2010; Lo et al. 2002; Pattani et al. 2010; Zhou et al. 2007) SA-H (Demokan et al. 2010; Kaur et al. 2010; Pattani et al. 2010), SE-H (Kaur et al. 2010) NE (Kaur et al. 2010; Lo et al. 2002)	60–97

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
HIN-1	High-in-normal 1	Tumor suppression	OSCC (Huang et al. 2009), NPC (Wong et al. 2003a)	PCR-DHPLC (Huang et al. 2009), MSP (Wong et al. 2003a)	P-DFS (Huang et al. 2009)	T (Huang et al. 2009; Wong et al. 2003a), (NE, SA-H, L) (Wong et al. 2003a)	7.3–77
hMLH1	mutL homolog 1	DNA repair	HNSCC (Demokan et al. 2006; Liu et al. 2002; Liu et al. 2003a; Puri et al. 2005; Sengupta et al. 2007; Steinmann et al. 2009; Zuo et al. 2009) PM-OCL (Sengupta et al. 2007) OSCC (Czerninski et al. 2009), NPC (Tan et al. 2006; Wong et al. 2003b)	MSP (Czerninski et al. 2009; Liu et al. 2003a; Puri et al. 2005; Steinmann et al. 2009; Tan et al. 2006) MSP (Wong et al. 2003b; Zuo et al. 2009) , MSRE (Demokan et al. 2006; Liu et al. 2003a; Sengupta et al. 2007)	H-MSI (Demokan et al. 2006; Sengupta et al. 2007; Zuo et al. 2009) P-S (Zuo et al. 2009)	MN (Sengupta et al. 2007; Steinmann et al. 2009; Zuo et al. 2009) NE (Czerninski et al. 2009) , T (Czerninski et al. 2009; Demokan et al. 2006; Liu et al. 2002, 2003a) T (Puri et al. 2005; Sengupta et al. 2007; Steinmann et al. 2009; Tan et al. 2006; Wong et al. 2003b; Zuo et al. 2009)	32.5–50
hMSH2	mutS homolog 2	DNA repair	HNSCC (Demokan et al. 2006; Sengupta et al. 2007), PM-OCL (Sengupta et al. 2007) OSCC (Czerninski et al. 2009)	MSP (Czerninski et al. 2009), MSRE (Demokan et al. 2006; Sengupta et al. 2007)	n.a.	MN (Sengupta et al. 2007) , T (Czerninski et al. 2009; Demokan et al. 2010; Sengupta et al. 2007) ,NE (Czerninski et al. 2009)	30–50
KIF1A	Kinesin family member 1A	Axonal transport of synaptic vesicles and cell division	HNSCC (Demokan et al. 2010; Hoque et al. 2008), OSCC (Kaur et al. 2010; Pattani et al. 2010), PM-OCL (Pattani et al. 2010), NPC (Loyo et al. 2011)	QMSP (Demokan et al. 2010; Hoque et al. 2008; Kaur et al. 2010; Loyo et al. 2011; Pattani et al. 2010)	ANS (Demokan et al. 2010), H (Pattani et al. 2010)	T (Demokan et al. 2010; Hoque et al. 2008; Kaur et al. 2010; Loyo et al. 2011; Pattani et al. 2010) SA-H (Demokan et al. 2010; Kaur et al. 2010; Pattani et al. 2010), SE-H (Kaur et al. 2010) NE (Hoque et al. 2008; Kaur et al. 2010)	35–98
MGMT	O(6)-methylguanine-DNA methyltransferase	DNA repair	HNSCC (De Schutter et al. 2009; Hasegawa et al. 2002; Maruya et al. 2004; Puri et al. 2005; Righini et al. 2007; Rosas et al. 2001) HNSCC (Sanchez-Cespedes et al. 2000;	MSP (De Schutter et al. 2009; Dikshit et al. 2007; Hasegawa et al. 2002; Kato et al. 2006; Maruya et al. 2004; Puri et al. 2005) MSP (Righini et al. 2007;	(St, TS,TU) (Hasegawa et al. 2002), AU (Puri et al. 2005) (P-S, P-DFS) (Taioli et al. 2009) (I-RC,P-P) (Zuo et al. 2004)	M-SA (Hasegawa et al. 2002; Righini et al. 2007; Rosas et al. 2001) , M-SE (Sanchez-Cespedes et al. 2000) SA-H (Righini et al. 2007)	6–56

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
			Steinmann et al. 2009; Zuo et al. 2004) OSCC (Dikshit et al. 2007; Kato et al. 2006; Taioli et al. 2009; Viswanathan et al. 2003) PM-HNL (Maruya et al. 2004) , LC (Zhang et al. 2004, 2006) PC (Dikshit et al. 2007; Taioli et al. 2009), NPC (Wong et al. 2003b)	Rosas et al. 2001; Sanchez-Cespedes et al. 2000; Steinmann et al. 2009; Taioli et al. 2009) MSP (Wong et al. 2003b; Zhang et al. 2004, 2006; Zuo et al. 2004) SRM-PCR (Viswanathan et al. 2003)		MN (Kato et al. 2006; Maruya et al. 2004; Righini et al. 2007; Steinmann et al. 2009; Viswanathan et al. 2003; Zhang et al. 2004, 2006) NE (Zhang et al. 2004, 2006) T (De Schutter et al. 2009; Dikshit et al. 2007; Hasegawa et al. 2002; Kato et al. 2006; Maruya et al. 2004; Puri et al. 2005; Righini et al. 2007; Rosas et al. 2001) T (Sanchez-Cespedes et al. 2000; Steinmann et al. 2009; Taioli et al. 2009; Viswanathan et al. 2003; Wong et al. 2003b; Zhang et al. 2004, 2006; Zuo et al. 2004)	
MINT1	Methylated in tumor-1	Synaptic vesicle exocytosis	OSCC (Ogi et al. 2002), SGC (Durr et al. 2010)	MSRE (Ogi et al. 2002), QMSP (Durr et al. 2010)	(ANS, P-P) (Ogi et al. 2002)	T (Durr et al. 2010; Ogi et al. 2002), MN (Durr et al. 2010)	23–48
MINT31	Methylated in tumor-31	Tumor suppression	OSCC (Ogi et al. 2002), HNSCC (Carvalho et al. 2008)	MSRE (Ogi et al. 2002), QMSP (Carvalho et al. 2008)	(St, P-P) (Ogi et al. 2002)	T (Carvalho et al. 2008; Ogi et al. 2002), NE (Carvalho et al. 2008)	15–64
p14	Alternative open reading frame (ARF) of INK4a locus	Tumor suppression	OSCC (Ishida et al. 2005; Kordi-Tamandani et al. 2010; Ogi et al. 2002; Sailasree et al. 2008; Shintani et al. 2001) HNSCC (Weber et al. 2002), PM-OCL (Takeshima et al. 2008) SGC (Nishimine et al. 2003)	MSRE (Ogi et al. 2002) MSP (Ishida et al. 2005; Kordi-Tamandani et al. 2010; Nishimine et al. 2003; Sailasree et al. 2008; Shintani et al. 2001; Takeshima et al. 2008; Weber et al. 2002)	G-P (Ogi et al. 2002; Sailasree et al. 2008), R-RC (Sailasree et al. 2008) (LNM,TS,St) (Ishida et al. 2005)	T (Ishida et al. 2005; Kordi-Tamandani et al. 2010; Nishimine et al. 2003; Ogi et al. 2002; Sailasree et al. 2008; Shintani et al. 2001; Takeshima et al. 2008; Weber et al. 2002) NE (Kordi-Tamandani et al. 2010; Takeshima et al. 2008)	14–34

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
p15	Cyclin-dependent kinase inhibitor 2B	Tumor suppression	OSCC (Ogi et al. 2002; Shintani et al. 2001; Viswanathan et al. 2003; Yeh et al. 2002), HNC (Chang et al. 2004) PM-OCL (Takeshima et al. 2008), NPC (Chang et al. 2003b; Wong et al. 2003b)	SRM-PCR (Viswanathan et al. 2003), MSRE (Ogi et al. 2002) QMSP (Chang et al. 2004) MSP (Chang et al. 2003b; Shintani et al. 2001; Takeshima et al. 2008; Wong et al. 2003b; Yeh et al. 2003)	ANS (Ogi et al. 2002), (TU, AU) (Chang et al. 2004)	MN (Viswanathan et al. 2003), M-SA (Chang et al. 2003b; Chang et al. 2004; Takeshima et al. 2008) NE (Chang et al. 2003b; Takeshima et al. 2008) T (Chang et al. 2003b; Chang et al. 2004; Ogi et al. 2002; Shintani et al. 2001; Takeshima et al. 2008; Viswanathan et al. 2003; Wong et al. 2003b; Yeh et al. 2003)	22–65
p16	Cyclin-dependent kinase inhibitor 2A (CDKN2A)	Tumor suppression	OSCC (El-Naggar et al. 1997; González et al. 1997; Hall et al. 2008; Huang et al. 2002; Ishida et al. 2005; Kato et al. 2006; Kaur et al. 2010; Miracca et al. 1999; Nakahara et al. 2006) OSCC (Nakahara et al. 2001; Ogi et al. 2002; Ohta et al. 2009; Reed et al. 1996; Riese et al. 1999; Ruesga et al. 2007; Sailasree et al. 2008) OSCC (Shaw et al. 2006; Shintani et al. 2001; Sinha et al. 2009; Tao et al. 1997; Viswanathan et al. 2003; Yakushiji et al. 2001; Yeh et al. 2003) HNSCC (Ai et al. 2003; Carvalho et al. 2008; Hasegawa et al. 2002; Koscielny et al. 2007; Maruya et al. 2004; Okami et al. 2005; Puri et al. 2005) HNSCC (Righini et al. 2007; Rosas et al. 2001; Sanchez-Cespedes	QMSP (Carvalho et al. 2008; Kaur et al. 2010; Tan et al. 2008) SRM-PCR (Viswanathan et al. 2003), PS (Hall et al. 2008; Shaw et al. 2006) SB (Reed et al. 1996), MSRE (El-Naggar et al. 1997; González et al. 1997; Miracca et al. 1999; Ogi et al. 2002; Tao et al. 1997) MSP (Agnese et al. 2006; Ai et al. 2003; Cao et al. 2009; Chang et al. 2003b; Dikshit et al. 2007; Hasegawa et al. 2002; Huang et al. 2002; Ishida et al. 2005; Kato et al. 2006; Kresty et al. 2002) MSP (Kwong et al. 2002; Li et al. 2005; Lo et al. 1996; Maruya et al. 2004; Nakahara et al. 2006; Nakahara et al. 2001; Nishimine et al.	(A-TU,TU) (Hasegawa et al. 2002) (St, LNM, TS) (Hasegawa et al. 2002; Ishida et al. 2005) ANS (Kresty et al. 2002), PD (Steinmann et al. 2009) (I-RC, P-P) (Sailasree et al. 2008)	T (Agnese et al. 2006; Ai et al. 2003; Cao et al. 2009; Carvalho et al. 2008; Chang et al. 2003b; Dikshit et al. 2007; El-Naggar et al. 1997; González et al. 1997; Guo et al. 2007; Ha and Califano 2003; Hall et al. 2008; Hasegawa et al. 2002; Huang et al. 2002; Ishida et al. 2005) T (Kato et al. 2006; Kaur et al. 2010; Koscielny et al. 2007; Kresty et al. 2002; Kwong et al. 2002; Li et al. 2005; Lo et al. 1996; Maruya et al. 2004; Miracca et al. 1999; Nakahara et al. 2006) T (Nakahara et al. 2001; Nishimine et al. 2003; Ogi et al. 2002; Ohta et al. 2009; Okami et al. 2005; Puri et al. 2005; Reed et al.	5–68

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
			et al. 2000; Steinmann et al. 2009) PM-HNL (Cao et al. 2009; Hall et al. 2008; Kresty et al. 2002; Maruya et al. 2004; Takeshima et al. 2008), SGC (Agnese et al. 2006; Guo et al. 2007; Li et al. 2002; Nishimine et al. 2003), LC (Agnese et al. 2006; Dikshit et al. 2007; Smigiel et al. 2004) HPC (Dikshit et al. 2007), NPC (Chang et al. 2003b; Kwong et al. 2002; Lo et al. 1996; Tan et al. 2006; Wong et al. 2003b)	2003) MSP (Ohta et al. 2009; Okami et al. 2005; Puri et al. 2005; Riese et al. 1999; Righini et al. 2007; Rosas et al. 2001; Rubin 2011; Ruesga et al. 2007; Sailasree et al. 2008; Sanchez-Cespedes et al. 2000) MSP (Shintani et al. 2001; Sinha et al. 2009; Smigiel et al. 2004; Steinmann et al. 2009; Takeshima et al. 2008; Tan et al. 2006) MSP (Weber et al. 2002; Wong et al. 2003b; Yakushiji et al. 2001; Yeh et al. 2003)		1996) T (Riese et al. 1999; Righini et al. 2007; Rosas et al. 2001; Rubin 2011; Ruesga et al. 2007; Sailasree et al. 2008; Sanchez-Cespedes et al. 2000; Shaw et al. 2006; Shintani et al. 2001; Sinha et al. 2009; Smigiel et al. 2004; Steinmann et al. 2009) T (Takeshima et al. 2008; Tan et al. 2006, 2008; Tao et al. 1997; Viswanathan et al. 2003; Weber et al. 2002; Wong et al. 2003b; Yakushiji et al. 2001; Yeh et al. 2003) SA-H (Carvalho et al. 2008; Kaur et al. 2010; Righini et al. 2007) SE-H (Kaur et al. 2010), SM (Sinha et al. 2009; Tan et al. 2008) M-SA (Carvalho et al. 2008; Hasegawa et al. 2002; Righini et al. 2007; Rosas et al. 2001) NE (Carvalho et al. 2008; Chang et al. 2003b; Guo et al. 2007; Kaur et al. 2010; Kwong et al. 2002; Lo et al. 1996; Takeshima et al. 2008) M-SE (Carvalho et al. 2008; Nakahara et al. 2006; Sanchez-Cespedes et al. 2000) MN (Huang et al. 2002; Kato et al. 2006; Kresty et al. 2002)	

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
						MN (Maruya et al. 2004; Miracca et al. 1999; Riese et al. 1999; Righini et al. 2007; Shaw et al. 2006; Steinmann et al. 2009; Viswanathan et al. 2003)	
PGP9.5 (UCHL1)	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	Processing of ubiquitin precursors and of ubiquitinated proteins	SGC (Durr et al. 2010), HNC (Tokumaru et al. 2004, 2008) OSCC (Tokumaru et al. 2008), NPC (Li et al. 2010; Loyo et al. 2011)	BS (Tokumaru et al. 2004), MSP (Li et al. 2010) QMSP (Durr et al. 2010; Loyo et al. 2011; Tokumaru et al. 2008)	n.a.	T (Durr et al. 2010; Li et al. 2010; Loyo et al. 2011; Tokumaru et al. 2004, 2008) MN (Durr et al. 2010) , NE (Tokumaru et al. 2004)	60–82
RARß	Retinoic acid receptor beta	Tumor suppression	HNSCC (Chen et al. 2007; Maruya et al. 2004; Okami et al. 2005; Steinmann et al. 2009; Yalniz et al. 2011; Youssef et al. 2004) PM-HNL (Maruya et al. 2004), PM-OCL (Youssef et al. 2004) SGC (Durr et al. 2010), NPC (Fendri et al. 2009; Kwong et al. 2002; Kwong et al. 2005b)	MS-MLPA (Chen et al. 2007; Yalniz et al. 2011) MSP (Fendri et al. 2009; Kwong et al. 2005b; Kwong et al. 2002; Maruya et al. 2004; Okami et al. 2005) MSP (Steinmann et al. 2009; Youssef et al. 2004), QMSP (Durr et al. 2010)	(St, H) (Fendri et al. 2009)	MN (Durr et al. 2010; Fendri et al. 2009; Maruya et al. 2004; Steinmann et al. 2009) NE (Kwong et al. 2002; Youssef et al. 2004) T (Chen et al. 2007; Durr et al. 2010; Fendri et al. 2009; Kwong et al. 2002, 2005b; Maruya et al. 2004; Okami et al. 2005; Steinmann et al. 2009; Yalniz et al. 2011; Youssef et al. 2004)	15–80
RASSF1A	Ras association (RalGDS/AF-6) domain family member 1A	Tumor suppression	HNSCC (Carvalho et al. 2008; Chen et al. 2007; Dong et al. 2003; Hasegawa et al. 2002; Okami et al. 2005; Righini et al. 2007; Steinmann et al. 2009), OSCC (Huang et al. 2009), SGC (Li et al. 2005), NPC (Chang et al. 2003b; Fendri et al. 2009; Kwong et al. 2002; Lo et al. 2001;	MS-MLPA (Chen et al. 2007), QMSP (Carvalho et al. 2008) PCR-DHPLC (Huang et al. 2009) MSP (Chang et al. 2003b; Dong et al. 2003; Fendri et al. 2009; Hasegawa et al. 2002; Kwong et al. 2002; Li et al. 2005) MSP (Lo et al. 2001; Okami et al. 2005) (Qiu et al. 2004; Righini et al. 2007; Steinmann et al. 2009),	HPV (Dong et al. 2003) (St,TS,TU) (Hasegawa et al. 2002) (P-DFS) (Huang et al. 2009) (HG,St,MET) (Li et al. 2005) (LNM, A) (Fendri et al. 2009), EBV (Zhou et al. 2005)	M-SA (Chang et al. 2003b; Hasegawa et al. 2002; Righini et al. 2007) MN (Righini et al. 2007; Steinmann et al. 2009; Zhou et al. 2005) , SA-H (Righini et al. 2007) NE (Carvalho et al. 2008; Chang et al. 2003b; Dong et al. 2003; Fendri et al. 2009; Kwong et al. 2005b; Lo et al. 2001; Qiu et al. 2004; Wang et al.	2.4–84

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
			Qiu et al. 2004; Tan et al. 2006; Wang et al. 2009; Wong et al. 2003b; Zhou et al. 2005)	MSP (Tan et al. 2006; Wang et al. 2009; Wong et al. 2003b; Zhou et al. 2005)		2009) T (Carvalho et al. 2008; Chang et al. 2003b; Chen et al. 2007; Dong et al. 2003; Fendri et al. 2009; Hasegawa et al. 2002; Huang et al. 2009; Kwong et al. 2002; Li et al. 2005; Lo et al. 2001) T (Okami et al. 2005; Qiu et al. 2004; Righini et al. 2007; Steinmann et al. 2009; Tan et al. 2006; Wang et al. 2009; Wong et al. 2003b; Zhou et al. 2005)	
RASSF2	Ras association (RalGDS/AF-6) domain family |member 2	Tumor suppression	HNSCC (Steinmann et al. 2009) OSCC (Huang et al. 2009; Imai et al. 2008), NPC (Zhang et al. 2007b)	COBRA (Imai et al. 2008; Steinmann et al. 2009) PCR-DHPLC (Huang et al. 2009), MSP (Zhang et al. 2007b)	P-DFS (Huang et al. 2009) , LNM (Zhang et al. 2007b)	T (Huang et al. 2009; Imai et al. 2008; Steinmann et al. 2009; Zhang et al. 2007b), MN (Imai et al. 2008; Steinmann et al. 2009) NE (Zhang et al. 2007b)	22–51
SFRP1	Secreted protein 1	Inhibition of Wnt signaling	SGC (Lee et al. 2010), OSCC (Sogabe et al. 2008)	MSP (Lee et al. 2010; Sogabe et al. 2008)	(HG,St,H) (Lee et al. 2010), G (Sogabe et al. 2008)	(T, MN) (Lee et al. 2010; Sogabe et al. 2008)	24–58.6
TIG1	Tazarotene-induced gene-1	Response to retinoic acid receptor	HNSCC (Tokumaru et al. 2005), NPC (Kwong et al. 2005a; Yanatatsaneejit et al. 2008)	MSP (Kwong et al. 2005a; Tokumaru et al. 2005) MSP-COBRA (Yanatatsaneejit et al. 2008)	n.a.	T (Kwong et al. 2005a; Tokumaru et al. 2005; Yanatatsaneejit et al. 2008), NE (Kwong et al. 2005a; Tokumaru et al. 2005; Yanatatsaneejit et al. 2008) L (Yanatatsaneejit et al. 2008)	51–91
TIMP3	Tissue inhibitor of metalloproteinases 3	Inhibition of angiogenesis and tumor growth	HNSCC (De Schutter et al. 2009; Nayak et al. 2007; Righini et al. 2007), SGC (Durr et al. 2010)	MSP (De Schutter et al. 2009; Righini et al. 2007), QMSP (Durr et al. 2010; Nayak et al. 2007)	(B-LRC, B-S) (De Schutter et al. 2009) (B-DFS, B-DSS, B-O) (De Schutter et al. 2009)	T (De Schutter et al. 2009; Durr et al. 2010; Nayak et al. 2007; Righini et al. 2007), MN (Durr et al. 2010; Righini et al. 2007)	40.5–71.8

Table 2 (continued)

Gene	Gene name	Gene function	Tumor type[a]	Method[b]	Clinical association[c]	Samples[d]	Methylation range (%)
						M-SA (Righini et al. 2007), SA-H (Righini et al. 2007)	
WIF1	Wnt inhibitory factor 1	Inhibition of Wnt signaling	NPC (Chan et al. 2007; Fendri et al. 2010)	MSP (Chan et al. 2007; Fendri et al. 2010)	(TS, LNM) (Chan et al. 2007; Fendri et al. 2010) (MET, A) (Chan et al. 2007; Fendri et al. 2010)	(T, NE) (Chan et al. 2007; Fendri et al. 2010)	85–89.7
14-3-3 sigma	Stratifin	Signaling mechanism	SGC (Gasco et al. 2002; Uchida et al. 2004), OSCC (Gasco et al. 2002) PM-OCL (Gasco et al. 2002), NPC (Yi et al. 2009)	MSP (Gasco et al. 2002; Uchida et al. 2004; Yi et al. 2009)	H (Uchida et al. 2004) (LNM, MET, S) (Yi et al. 2009)	T (Gasco et al. 2002; Uchida et al. 2004; Yi et al. 2009), MN (Gasco et al. 2002; Yi et al. 2009)	35–84

[a] Tumor types: *OSCC* oral squamous cell carcinoma, *PM-OCL* premalignant oral cavity lesions, *PM-HNL* premalignant head and neck lesions, *SGC* salivary gland carcinomas, *LC* larynx cancer, *HPC* hypopharyngeal cancer, *PC* pharyngeal cancer, *NPC* nasopharyngeal carcinoma

[b] Methods: *PCR* polymerase chain reaction, *MSP* methylation-specific PCR, *QMSP* quantitative methylation-specific PCR, *QRT-PCR* quantitative reverse transcriptase PCR, *MSRE* methylation-specific restriction enzyme analysis, *SRM-PCR* sensitive restriction-multiplex PCR, *MS-MLPA* methylation-specific multiplex ligation-dependent probe amplification, *COBRA* combined bisulfite restriction analysis, *PS* pyrosequencing, *SB* Southern blot, *BS* bisulfite sequencing, *PCR-DHPLC* PCR-denaturing high-performance liquid chromatography

[c] Clinical: *B-LRC* better local recurrence, *B/P-S* better/poor survival, *B/P-DFS* better/poor disease-free survival, *B/P-DSS* better/poor disease-specific survival, *B/P-O* better/poor outcome, *ANS* anatomic site, *I* invasion, *A-S* age of smoking, *St* stage, *LNM* lymph node metastasis, *TS* tumor size, *TU* tobacco usage, *I/R-RC* increased/reduced recurrence, *P/G-P* poor/good prognosis, *AU* alcohol usage, *H* histology, *HG* high grade, *MET* distant metastasis, *H-MSI* high microsatellite instability, *PD* poor differentiation, *PI* perineural invasion, *G* gender, *A* age

[d] Samples: *T* tumor, *MN* matched normal tissue, *NE* normal epithelium, *M-SA* matched salivary rinse, *M-SE* matched serum, *SA-H* salivary rinses from healthy people, *SM* surgical margin, *SE-H* serum from healthy people, *LN* lymph node, *L* lymphocytes, *CINT* chronic inflammatory nasopharyngeal tissues

genes may have an important role in oral carcinogenesis and have been associated with the susceptibility for oral malignancies (Czerninski et al. 2009).

Biomarkers predicting clinical response, tumor recurrence, or patient survival are not available for many cancer types, particularly for oral and pharyngeal cancer. Taioli et al. (2009) have studied the methylation of a panel of TSG in order to identify a possible correlation with survival and recurrence rates in patients with oral or pharyngeal cancer. *MGMT* promoter methylation was inversely associated with poor OS and DFS, indicating that *MGMT* promoter methylation may act as a possible prognostic biomarker for oral and pharyngeal cancer (Taioli et al. 2009). In oral epithelial dysplasia that transforms to OSCC, *p16* methylation may act as a candidate biomarker of malignant transformation, whereas methylation of the *MGMT*, *CYGB*, and *CCNA1* genes are not associated with malignancy (Hall et al. 2008). In oral carcinomas, methylation of the *p16* and *MGMT* genes are frequently observed as an early event (Kato et al. 2006). Promoter hypermethylation of the *p16*, *p15*, *hMLH1*, *MGMT*, and *CDH1* genes has been reported in OSCC (Viswanathan et al. 2003). The *Ras/PI3K/AKT* pathway is a major mechanism associated with radioresistance in OSCC. A study investigating four genes (*RASSF1A*, *RASSF2A*, *PTEN*, and *HIN-1*) in this pathway revealed that *RASSF1A* and *RASSF2A* methylation were more frequent in the tumors and significantly associated with poor DFS (Huang et al. 2009). Methylation of *CCNA1*, *CYGB*, and *p16* genes has also been correlated with the clinicopathological parameters in oral cancer (Shaw et al. 2006). In OSCC, different levels of methylation have been reported for the *p16*, *p15*, *p14*, *DCC*, *DAPK*, *MINT1*, *MINT2*, *MINT27*, and *MINT31* gene promoters and *DCC* methylation was associated with bone invasion of gingival tumors, invasiveness, and reduced survival (Ogi et al. 2002). Aberrant methylation of the *p14*, *p15*, and *p16* gene promoters has been reported in HNSCC (Weber et al. 2002), oral precancerous lesions (Takeshima et al. 2008), oral carcinomas (Sailasree et al. 2008; Yeh et al. 2003; Shintani et al. 2001), and salivary gland carcinomas (Nishimine et al. 2003). When normal tissue and benign and malignant salivary gland tumors were compared, significantly higher methylation of the *APC*, *RARβ*, *MINT1*, *PGP9.5*, and *TIMP3* genes were observed in salivary duct carcinoma (Durr et al. 2010). In adenoid cystic carcinomas of the salivary gland, *p16*, *RASSF1A*, and *DAPK* gene methylation is also a common event (Li et al. 2005).

In patients with laryngeal and hypopharyngeal cancers, promoter methylation of the *p16*, *MGMT*, *DAPK*, and *CDH1* genes are observed frequently (Dikshit et al. 2007; Azarschab et al. 2003). For tumors in the oropharynx, a statistically significant association between hypermethylation of the *DAPK1* gene and risk of lymph node metastases has been reported, and significant evidence indicate an association between hypermethylation of the *ADAM23* gene and advanced tumor stage in larynx cancer (Calmon et al. 2007).

In a study investigating the methylation status of candidate TSG in surgical margins as a predictor of local recurrence in HNSCC, it has been shown that analysis of the *CDKN2A*, *CCNA1*, and *DCC* genes in the surgical margins by quantitative methylation-specific PCR (QMSP) can correctly predict local recurrences in HNSCC (Tan et al. 2008). Using candidate gene and discovery approaches, 21 genes were investigated in HNSCC and normal tissue samples. *p16*, *MINT31*, and *RASSF1A* gene methylation were detected only in HNSCC but not in the controls (Carvalho et al. 2008).

Data from mRNA expression array studies

Recently, studies investigating novel methylated TSG specific to the tumor types have made use of mRNA expression arrays to analyze more than 40,000 genetic regions on a single platform via pharmacological unmasking and discovery approaches (Yamashita et al. 2002; Tokumaru et al. 2004).

After bisulfite sequencing, rapid subtractive hybridization, and microarray analysis in order to determine genes that are induced to reexpression by the demethylating agent 5-aza-2′-deoxycytidine in HNSCC cell lines, 35 out of 78 genes were selected and only 3 of these (*CRABP2*, *MX1*, and *SLC15A3*) were verified by QMSP (Calmon et al. 2009). After methylation-specific PCR (MSP) analysis, *CRABP2* and *MX1* genes were highly methylated in primary HNSCC compared with lymphocytes from a healthy cohort. In addition, lack of the CRABP2 protein was associated with poor survival rates, indicating that *CRABP2* expression may be a potential prognostic biomarker for patients with HNSCC.

In a study, *PGP9.5*, *CCNA1*, bone morphogenetic protein 2A (*BMP2A*), metallothionein 1G (*MT1G*), and neuromedin U (*NmU*) genes were highly methylated and *CCNA1* hypermethylation displayed an inverse correlation with *p53* mutations (Tokumaru et al. 2004).

Methylation of the Nischarin (*NISCH*), p21-activated protein kinase 3 (*PAK3*), *KIF1A*, and *OGDHL* genes has been reported in 8–52% of the patients with HNC, whereas no methylation was observed in the normal cohort (Hoque et al. 2008).

Global methylation

A recent study indicated the involvement of global DNA hypermethylation in the pathogenesis of HNSCC (Worsham

et al. 2010). Using DNA IP and Affymetrix whole-genome tiling arrays, 231 new and previously unreported genes out of 1,143 cancer genes on the array were identified via the whole-genome methylation approach.

In a study aiming to characterize early molecular changes in premalignant lesions of the oral cavity and the role of tobacco and alcohol consumption or HPV infections, the global methylation index was found to be 4.28 (95%CI, 4.1, 4.4) in the oral cancer case series. Methylation was inversely associated with tobacco use (Guerrero-Preston et al. 2009).

In cancer, abnormal demethylation leads to the loss of silencing in repetitive elements which are located on approximately 50% of the human genome, whereas in normal cells, these repetitive sequences are regulated by epigenetic silencing. DNA methylation alterations in HNC and adjacent nontumor tissues was investigated via a genome-wide microarray approach (Szpakowski et al. 2009). Of the more than 250,000 repetitive elements probed, between 5% and 8% displayed disease-related DNA methylation changes. Among the *SVA*, *HERV*, *LINE-1P*, *AluY*, and *MaLR* families, *LINE-1* (Richards et al. 2009; Subbalekha et al. 2009; Smith et al. 2007), *SINE* (*Alu*) (Richards et al. 2009) repetitive elements, and *LRE1* (Furniss et al. 2008; Hsiung et al. 2007) showed loss of DNA methylation in the tumors when compared to matched normal adjacent tissue. *LINE* and *LRE1* hypomethylation were more frequently observed in HPV-negative than in HPV-positive tumors (Richards et al. 2009).

The Runt-related transcription factor 3 (*RUNX3*) gene plays a role in the transforming growth factor-beta (TGF-β)-induced tumor suppression pathway. Although *RUNX3* has been considered as a TSG in some studies (Bae et al. 1995; Li et al. 2002), it has been shown that the expression level of *RUNX3* in HNSCC tissues are higher than that in normal oral epithelial tissues due to demethylation (Ginos et al. 2004; Salto-Tellez et al. 2006). Therefore, it has been suggested that *RUNX3* may have an oncogenic role in HNSCC and its expression may predict malignant behavior and the effect of chemotherapeutic drugs in HNSCC as a potential biomarker (Tsunematsu et al. 2009). In contrast to HNSCC, the *RUNX3* gene is underexpressed in OSCC due to promoter hypermethylation, indicating that *RUNX3* plays an important role in oral carcinogenesis and may be a useful diagnostic marker and a potential therapeutic target for OSCC (Gao et al. 2009).

A recent report has shown that the *SEPT9*, *SLC5A8*, *FUSSEL18*, *EBF3*, and *IRX1* genes which act in the *TGF-β* signaling pathway are commonly methylated and down-regulated in HNC (Bennett et al. 2009). In IP studies, all these candidate genes were observed to interact with the components of the *TGF-β* pathway (Bennett et al. 2008). It was reported that decreased mitotic activity and increased apoptosis rates were observed when the *SLC5A8*, *EBF3*, and *IRX1* genes are overexpressed.

Recently, HPV-infected HNSCC tumors have been shown to display higher levels of global DNA methylation. These tumors were universally methylated irrespective of the clinical factors and methylation of the *FUSSEL18*, *IRX1*, and *EBF3* genes were likely corralated with recurrences. Promoter methylation of the *FUSSEL18* and *SEPTIN9* genes was significantly associated with alcohol and tobacco consumption. A trend between HPV16 positivity and methylation of the *IRX1*, *EBF3*, *SLC5A8*, and *SEPT9* genes was noted and it has been suggested that this gene panel may be used for the selection of treatment modality (Bennett et al. 2010).

The Transketolase-like 1 (*TKTL1*) gene is a novel candidate oncogene, which is hypomethylated in human HNSCC tumor samples and contributes to HNSCC carcinogenesis via aerobic glycolysis and HIF1-α stabilization (Sun et al. 2010).

Studies of individual genes

Tumor suppressor genes

Significantly higher methylation of the *p14ARF* gene has been reported in OSCC compared to normal control tissues, implying that the methylation status of *p14ARF* may be an important determinant in the early diagnosis and treatment of OSCC (Kordi-Tamandani et al. 2010; Ishida et al. 2005).

Data on the methylation of the *p16* gene promoter is not consistent. The gene has been analyzed individually or in gene panels but with discordant results. In a study, *p16* methylation has been associated with malignant transformation of oral epithelial dysplasia and was considered a potential biomarker for the prediction of prognosis of mild or moderate oral epithelial dysplasia (Cao et al. 2009; Kresty et al. 2002). High methylation of the *p16* promoter region has also been reported in carcinomas of the tongue and methylation in the surgical margins were found to increase the risk of local recurrences 6.3-fold when compared with patients with negative margins (Sinha et al. 2009). Varying degrees of *p16* methylation has been reported in OSCC (Ohta et al. 2009; Ruesga et al. 2007; Nakahara et al. 2006; Huang et al. 2002; Yakushiji et al. 2001; Nakahara et al. 2001; Miracca et al. 1999; Riese et al. 1999; El-Naggar et al. 1997; Tao et al. 1997; González et al. 1997; Reed et al. 1996), in mucoepidermoid carcinoma (MEC) of the salivary glands (Guo et al. 2007; Agnese et al. 2006), in HNC (Yalniz et al. 2011; Koscielny et al. 2007; Ai et al. 2003), and in larynx cancer (Smigiel et al. 2004). Conversely, there is a also a report of low *p16* methylation in larynx cancer (Agnese et al. 2006).

DCC is a candidate TSG located on chromosome 18q21. Hypermethylation of *DCC* as a mechanism for inactivation

in HNSCC has been investigated (Carvalho et al. 2006). The *DCC* promoter was highly methylated in the tumors and there was a significant correlation between *DCC* promoter region hypermethylation and lack of *DCC* expression.

In a study investigating the methylation and expression levels of the *APC* and *SFRP* genes, both genes were highly methylated in MEC but not in adjacent normal tissue. There was significant correlation between methylation and low *SFRP1* expression. Methylation of the *SFRP1* gene was the main cause of decreased *SFRP1* expression. Beta-catenin expression was also associated with reduced *SFRP1* expression. In addition, both *SFRP1* and beta-catenin expression were associated with tumor grade and stage. Survival was particularly poor in patients with reduced *SFRP1* and cytoplasmic/nuclear beta-catenin expression. It has been suggested that detection of *SFRP1* expression and aberrant beta-catenin expression in the cell may be useful biomarkers of tumor progression and prognosis in patients with MEC (Lee et al. 2010).

Alterations in *TGF-β* signaling are common in HNSCC. Hypermethylation of TGF-β type I receptor (*TGFBR-I*) gene was evaluated via MSP and restriction enzyme-mediated PCR (MSRE). *TGFBR-I* expression was lost in 83% of the HNSCC tumors and was linked to DNA hypermethylation of the CpG-rich promoter region in 62% of the samples (Muñoz-Antonia et al. 2009).

NDRG2 is a candidate TSG involved in oral squamous cell cancers via the Akt signaling pathway. Reduced *NDRG2* mRNA levels, caused by promoter methylation, have been reported in most of the OSCC patients and in several cases of precancerous leukoplakia with dysplasia (Furuta et al. 2010).

Hypermethylation of the *FancB* gene has been observed in sporadic HNSCC tumors (Smith et al. 2010), while methylation of the *SYK* gene has been frequently observed in OSCC; the downregulation of *SYK* expression due to promoter methylation was associated with metastasis (Ogane et al. 2009). *MGMT* gene methylation is observed in laryngeal cancer (Zhang et al. 2006, 2004) and HNSCC (Zuo et al. 2004), but not in normal larynx tissue.

The *MALT1* gene, responseible for activating nuclear factor-kappaB in lymphocyte lineages, is located in a genomic region encoding putative TSG and is expressed in the nucleus of oral epithelial cells. Absence of expression due to epigenetic inactivation during tumor progression has been associated with tumor recurrence and poor patient survival, suggesting that analysis of *MALT1* expression may be a useful predictive and prognostic marker for OSCC (Chiba et al. 2009).

In primary OSCC, aberrant methylation of the *RASSF2* gene and a high frequency of *ROBO1* methylation have been reported in early dysplastic lesions of the head and neck (Imai et al. 2008; Ghosh et al. 2009). A significant inverse correlation between *RASSF1A* promoter methylation and HPV infection in HNSCC has been shown (Dong et al. 2003).

GALR1 methylation is observed in primary HNSCC tumors and correlates with decreased *GALR1* expression, as well as increased tumor size, lymph node status, tumor stage, *CCND1* expression, *p16* methylation, and survival (Misawa et al. 2008).

Methylation of the *CDH1* gene promoter has been reported in tongue cancer (Chang et al. 2002), salivary gland adenoid cystic carcinoma (Zhang et al. 2007a), and nonmetastatic oral cancer as an early event (De Moraes et al. 2008; Maeda et al. 2007a; Yeh et al. 2002).

Expression of the *RARβ* gene has been studied in primary tissue specimens of different anatomical sites from patients with HNSCC and a strong correlation was found between hypermethylation and reduced expression of *RARβ2* (Youssef et al. 2004). In particular, significantly lower hypermethylation and higher *RARβ2* mRNA expression levels when compared to the tumors located at other sites of the head and neck were observed in tumors from the hypopharynx (Olasz et al. 2007) and salivary duct or acinic cell carcinomas displaying *RASSF1* methylation (Williams et al. 2006).

Genes with different functions

CHFR is a putative early mitotic checkpoint gene which causes a delay in chromosome condensation in response to mitotic stress. In a study, aberrant promoter methylation of the *CHFR* gene was reported in patients with OSCC, while the gene was methylated minimally in the surrounding normal mucosa and no methylation was observed in the *CHFR* promoter in a healthy cohort (Baba et al. 2009; Toyota et al. 2003).

The *HIC1* gene plays a role in the regulation of transcription. A study using MSP has reported that *HIC1* is highly (95%) methylated in HNSCC and reexpression of the gene was associated with decreased aggressiveness (Brieger et al. 2010). On the other hand, in a recent study from our group, no significant methylation of the *HIC1* gene was observed in patients with HNC using a more sensitive technique (Yalniz et al. 2011).

The *CYGB* gene was first described as an intracellular globin of unknown function (Burmester et al. 2002). *CYGB* downregulation is a key event in the familial cancer syndrome of the upper aerodigestive tract. Increased expression of the *CYGB* gene displays an inverse correlation with promoter methylation and a strong correlation with tumor hypoxia. It has been consistently associated with aggressive tumors in oral and oropharyngeal squamous cell carcinoma when compared with histologically tumor-free surgical margins (Shaw et al. 2009).

In individual studies, aberrant methylation of *RB1* (Kishi et al. 2005) and *14-3-3 sigma* (Uchida et al. 2004; Gasco et al. 2002) in salivary gland cancer, *Apaf-1* (Huang et al. 2004) and *DAPK* (Zhang and Kong 2004) in laryngeal squamous cell carcinoma, *TSC2* (Chakraborty et al. 2008), *SFRP1*, *SFRP2*, *SFRP5* (Sogabe et al. 2008), *RECK* (Long et al. 2008), *EpCAM* (Shiah et al. 2009), *MTNR1A* (Nakamura et al. 2008), *IKKalpha* (Maeda et al. 2007b), *PRTFDC1* (Suzuki et al. 2007), *LRP1B* (Nakagawa et al. 2006), *DBCCR1* (Gao et al. 2004), and *14-3-3 sigma* (Gasco et al. 2002) in OSCC, *PGP9.5* (Tokumaru et al. 2008), *C/EBPalpha* (Bennett et al. 2007), *LHX6* (Estécio et al. 2006), *STAT1* (Xi et al. 2006), *TIG1* (Tokumaru et al. 2005), *TCF21* (Smith et al. 2006), *SOCS3* (Weber et al. 2005), *p15* (Chang et al. 2004), and *ATM* (Ai et al. 2004) genes in patients with HNC have also been reported.

miR-137 plays an important role in the cell cycle control. It has been shown that *miR-137* and *miR-193a* are epigenetically silenced during oral carcinogenesis (Kozaki et al. 2008). Methylation of *miR-137* has been reported in squamous cell carcinoma tissue and oral rinse samples and was associated with gender and inversely associated with body mass index (Langevin et al. 2010).

Specific lysine residues in histone tails are methylated, providing an epigenetic marker that modulates biological functions changing the heterochromatin structure and leading to tumor development. H3K4 histone methylation was investigated in OSCC, dysplastic lesions, and normal tissue samples (Piyathilake et al. 2005). The levels of H3K4me2 and H3K4me3 displayed striking variations in OSCC when compared with normal tissue and leukoplakias. The me2 levels were increased while the me3 levels decreased in the tumors (Mancuso et al. 2009).

Nasopharyngeal carcinoma and studies of TSG gene panels

Nasopharyngeal carcinoma (NPC) is a rare malignancy with unique genetic, viral, and environmental characteristics that distinguish it from other types of head and neck carcinoma. It has a different etiology, epidemiology, prognosis, and therapy. The clinical management of NPC remains challenging largely due to the lack of early detection strategies for this tumor (Loyo et al. 2011; Razak et al. 2010).

A recent study investigating a panel of 18 marker genes in nasopharyngeal tumors has shown that the methylation status of the *AIM1*, *APC*, *CALCA*, *DCC*, *DLEC*, *DLC1*, *ESR*, *FHIT*, *KIF1A*, and *PGP9.5* genes were significantly associated with NPC when compared with other tumors or the benign nasopharyngeal biopsy samples (Loyo et al. 2011).

In an 11-gene panel, promoter methylation levels of *CDH1*, *p15*, *THBS1*, *RASSF1A*, *MLH1*, *MGMT*, *p16*, and *TP73* genes were significantly higher in the tumor samples from patients with NPC when compared with the lymphocytes from the same individuals (Wong et al. 2003a).

Using the discovery approach after expression profiling among eight potential candidate TSG, promoter methylation of only three genes (*CCNA1*, *RARRES1*, and *HRASLS*) have been significantly associated with NPC (Yanatatsaneejit et al. 2008).

Promoter methylation of the *RASSF1A*, *DAPK*, and *RARβ2* genes was analyzed by MSP in primary NPC tumors and normal nasopharyngeal epithelia. All genes were highly methylated in tumor tissue, whereas methylation was not observed in the normal nasopharyngeal tissue. Methylation of the three genes was significantly associated with lymph node involvement. *RASSF1A* and *RARβ2* methylation also correlated with age at diagnosis, T stage, and histological type (Fendri et al. 2009). Epigenetic silencing of cellular retinol-binding proteins, *CRBPI*, *CRBPIV*, and *RARβ2* are also commonly observed in NPC tumor samples (Kwong et al. 2005a, 2002).

High methylation frequencies for the *DAPK*, *RASSF1A*, *CDH1*, and *p16* genes are frequently observed in NPC tumors (Kwong et al. 2002; Chang et al. 2003a). In contrast to HNSCC (Dikshit et al. 2007), in NPC, no change has been reported in the *RUNX3* promoter region, whereas the *p16*, *RASSF1A*, *CDH1*, and *hMLH1* gene promoters were frequently methylated (Tan et al. 2006).

In the patients with NPC from Southeast Asia but not from North Africa, methylation of *p16*, *DLEC1*, *BLU*, and *CDH1* genes were found to be associated with the juvenile and adult forms of the disease. Strong correlations were observed between aberrant promoter methylation of the *CDH1* and *BLU* genes and lymph node invasion (Li et al. 2003a, b) or undifferentiated tumors, respectively (Ayadi et al. 2008).

Single-gene studies in NPC

Tumor suppressor genes

WIF1 is a highly conserved gene on chromosome 12 and encodes a protein of the sFRP family, which inhibits the Wnt signaling pathway (Kawano and Kypta 2003). Inhibition of Wnt signaling induces apoptosis and inhibits tumor growth in many cancer types (He et al. 2004). Silencing of the *WIF1* gene by hypermethylation may result in the activation of some tumors (Suzuki et al. 2004; Caldwell et al. 2004; Ai et al. 2006; Taniguchi et al. 2005). The *WIF1* promoter region is highly methylated in nasopharyngeal tumors, whereas no methylation is observed in the normal mucosa (Fendri et al. 2010; Chan et al. 2007). *WIF1* methylation has been found to be associated with tumor size, node involvement and metastasis, and age (Fendri et al. 2010).

Expression of the *MIPOL1* gene is also downregulated in some NPC tumors via promoter hypermethylation and allelic loss (Cheung et al. 2009). Likewise, *DAB2* is frequently methylated in NPC, which correlates with the loss of expression in NPC tumors (Tong et al. 2010).

Two studies investigating the *RASSF1A* gene have shown that the gene is highly methylated in primary NPC but not in normal nasopharyngeal epithelia (Wang et al. 2009; Lo et al. 2001). It has been suggested that aberrant hypermethylation of *RASSF1A* and high Epstein–Barr virus (EBV) load may play an important role in NPC carcinogenesis (Zhou et al. 2005).

Methylation of *CDH1* is more frequently observed in advanced stages of NPC (Niemhom et al. 2008). Hypermethylation of *CDH1* promoter and presence of EBV are predominantly detected in undifferentiated and nonkeratinizing NPC compared to squamous cell NPC. Most of the NPC samples demonstrating *CDH1* hypermethylation were EBV-positive, whereas the EBV genome and hypermethylation were not detected in normal nasopharyngeal tissue when *CDH1* meythylation was absent, indicating a correlation between *CDH1* hypermethylation and EBV infection (Niemhom et al. 2008; Krishna et al. 2005; Li et al. 2003a, b; Tsao et al. 2003; Kao et al. 2002).

Genes with various funcitons

14-3-3 sigma, the downstream target of *p53*, is a negative regulator of cell cycle G2–M phase checkpoint in response to DNA damage. By MSP, 100% methylation of *14-3-3 sigma* was shown in tumor tissue but not in any of adjacent normal nasopharyngeal epithelial tissue (Yi et al. 2009). Analysis by real-time PCR, Western blotting, and immunohistochemistry have revealed that *14-3-3 sigma* expression is downregulated or absent in NPC samples displaying high methylation. In addition, hypermethylation of *14-3-3 sigma* has been associated with lymph node and distant metastasis.

Methylation of the *PGP9.5* gene is frequently detected in primary NPC but only minimally observed in normal nasopharyngeal tissue, indicating that the methylation-mediated silencing of *PGP9.5* may be important in nasopharyngeal carcinogenesis (Li et al. 2010). The *EDNRB* gene, located on chromosome 13q22, was highly methylated in primary NPC tumor samples, while no methylation was observed in normal nasopharyngeal epithelia (Zhou et al. 2007; Lo et al. 2002). In patients with NPC, high methylation of the *LARS2* gene, which is located at the chromosome 3 common eliminated region-1 (*C3CER1*) on 3p21.3, has been reported (Zhou et al. 2009).

Several genes which have been studied in gene panels were also evaluated in single-gene studies. *RASFF2* (Zhang et al. 2007b), *DLC1* (Seng et al. 2007), *DAPK* (Kong et al. 2006; Wong et al. 2002), *CHFR* (Cheung et al. 2005), *TIG1* (Kwong et al. 2005b), *BLU* (Qiu et al. 2004; Liu et al. 2003a, b), and *p16* (Lo et al. 1996) are among those. The corresponding data are given in Table 3. Other newly identified TSG are *IRF8* (Lee et al. 2008), *ADAMTS18*, which is a novel gene located on 16q23 (Jin et al. 2007), *LTF* (Yi et al. 2006), *CDH13* (Sun et al. 2007), *PCDH10* (Ying et al. 2006), *TSLC1* (Hui et al. 2003), *HIN-1* (Wong et al. 2003b), and *RIZ1* (Chang et al. 2003b) The *GNAT1* gene has been shown to be methylated not only in all primary NPC tissues but also in 80% of tissue samples with chronic nasopharyngitis (Yi et al. 2007).

Conclusions

Biomarkers predicting clinical response, tumor recurrence, or patient survival are not available for HNC. Further studies are needed to identify new biomarkers for early detection and prediction of the therapeutic response or prognosis. Follow-up studies using quantitative MSP analysis, global methylation profiling, and detailed analysis of downstream DNA repair genes regulated by promoter methylation may provide new insight into the issue. Considering the great heterogeneity of HNC, a combination of multiple genes for analysis may provide a higher coverage for diverse tumors than the analysis of a single gene (Carvalho et al. 2008). Recent data indicate that promoter hypermethylation of the *KIF1A* and *EDNRB* genes is a frequent event in primary HNSCC and combining only the *KIF1A* and *EDNRB* genes provides a higher specificity and sensitivity than using a panel of 10 different genes (Demokan et al. 2010). Preferential methylation of these genes in salivary rinses from HNSCC patients may provide a promising potential biomarker for the disease. Other genes investigated frequently and found to display significant differential methylation are *RASSF1A*, *DAPK1*, *MGMT*, *RARβ*, *CDH1*, *hMLH1*, and *CHFR*, although a wide range of different methylation ratios have been reported. These variations most probably result from the differences in the sensitivity of the methods, variations in the processing of the samples, and composition of the patient cohorts.

Methylation analysis has the additional advantage that methylation patterns are not affected by external factors or temporary physiological changes. They persist and are usually increased during disease progression. The higher specificity and sensitivity provides a suitable tool to obtain predictive or prognostic information. So far, the information about DNA methylation has not translated into useful and reliable markers for HNC in the clinical practice. However, given the sensitive and high-throughput quantitative methodologies for methylation analysis, specific markers for HNC will certainly

Table 3 Methylation of different genes reported in individual studies

Gene	Name	Function	Tumor type	Method	Samples	Methylation range (%)
ADAM23	ADAM metallopeptidase domain 23	Cell–cell and cell–matrix interactions	LC (Calmon et al. 2007)	MSP (Calmon et al. 2007)	T (Calmon et al. 2007)	42
ADAMTS18	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 18	Tumor suppression	NPC (Jin et al. 2007)	MSP (Jin et al. 2007)	(T,NE) (Jin et al. 2007)	70
AIM1	Absent in melanoma 1	Tumor suppression	NPC (Loyo et al. 2011)	QMSP (Loyo et al. 2011)	T (Loyo et al. 2011)	30
APAF1	Apoptotic peptidase activating factor 1	Apoptosis	LC (Huang et al. 2004)	MSP (Huang et al. 2004)	T (Huang et al. 2004)	40
ATM	Ataxia telangiectasia mutated	Regulating DNA damage response mechanism	HNC (Ai et al. 2004)	MSP (Ai et al. 2004)	(T,NE) (Ai et al. 2004)	25
BMP2A	Bone morphogenetic protein 2A	Induction of cartilage and bone formation	HNSCC (Tokumaru et al. 2004)	BS (Tokumaru et al. 2004)	T (Tokumaru et al. 2004)	25
C/EBPalpha	Tumor suppressor CCAAT enhancer binding protein alpha	Transcriptional regulation	HNC (Bennett et al. 2007)	COBRA (Bennett et al. 2007)	(T,MN) (Bennett et al. 2007)	68
CALCA	Calcitonin-related polypeptide alpha	Calcium regulation and phosphorus metabolism	NPC (Loyo et al. 2011)	QMSP (Loyo et al. 2011)	T (Loyo et al. 2011)	44
CHD13	Cadherin 13	Calcium-dependent cell adhesion	NPC (Sun et al. 2007)	MSP (Sun et al. 2007)	(T,NE) (Sun et al. 2007)	89.7
CRABP2	Cellular retinoic acid binding protein 2	Retinoic acid-mediated regulation of human skin growth and differentiation	HNSCC (Calmon et al. 2009)	MSP (Calmon et al. 2009)	(T,L) (Calmon et al. 2009)	58
CRBPI	Cellular retinol-binding protein type I	Intracellular transport of retinol	NPC (Kwong et al. 2005b)	MSP (Kwong et al. 2005b)	T (Kwong et al. 2005b)	87.8
CRBPIV	Cellular retinol-binding protein type IV	Intracellular transport of retinol	NPC (Kwong et al. 2005b)	MSP (Kwong et al. 2005b)	T (Kwong et al. 2005b)	54
CYGB	Cytoglobin	Transfer of oxygen from arterial blood to the brain	OSCC (Shaw et al. 2006)	PS (Shaw et al. 2006)	(T,MN) (Shaw et al. 2006)	65
CYP1A1	Cytochrome p450 family 1A	Carcinogen metabolism	HNC (Sharma et al. 2010)	MSP (Sharma et al. 2010)	(T,NE) (Sharma et al. 2010)	39.7
CYP2A13	Cytochrome P450, family 2, subfamily A, polypeptide 13	Carcinogen metabolism	HNC (Sharma et al. 2010)	MSP (Sharma et al. 2010)	(T,NE) (Sharma et al. 2010)	27.4
DAB2	Human disabled-2	Signal transduction	NPC (Tong et al. 2010)	MSP (Tong et al. 2010)	T (Tong et al. 2010)	65
DBCCR1	Deleted in bladder cancer 1	Inhibition of cell proliferation, involvement in cell death, and regulation of plasminogen pathway	(OSCC, PM-OCL) (Gao et al. 2004)	MSP (Gao et al. 2004)	(T,MN) (Gao et al. 2004)	44
EpCAM	Epithelial cell adhesion molecule	Homotypic calcium-independent cell adhesion	OSCC (Shiah et al. 2009)	MSP (Shiah et al. 2009)	T (Shiah et al. 2009)	51
ESR	Estrogen receptor alpha	Hormone binding, DNA binding and activation of transcription	NPC (Loyo et al. 2011)	QMSP (Loyo et al. 2011)	T (Loyo et al. 2011)	26
FancB	Fanconi anemia, complementation group B	DNA repair	HNSCC (Smith et al. 2010)	QMSP (Smith et al. 2010)	(T,NE) (Smith et al. 2010)	31
GALR1	Galanin receptor 1	Tumor suppression	HNSCC (Misawa et al. 2008)	(MSP, BS) (Misawa et al. 2008)	T (Misawa et al. 2008)	38
GNAT1	Guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1	Signal transduction	NPC (Yi et al. 2007)	MSP (Yi et al. 2007)	(T,CINT) (Yi et al. 2007)	100

Table 3 (continued)

Gene	Name	Function	Tumor type	Method	Samples	Methylation range (%)
GSTM1	Glutathione *S*-transferase mu 1	Carcinogen metabolism	HNC (Sharma et al. 2010)	MSP (Sharma et al. 2010)	(T,NE) (Sharma et al. 2010)	58.1
HIC1	Hypermethylated in cancer 1	Transcriptional regulation	HNSCC (El-Naggar et al. 1997)	MSP (El-Naggar et al. 1997)	T (El-Naggar et al. 1997)	95
HRASLS	HRAS-like suppressor	Formation of peripheral nerve trajectories	NPC (Yanatatsaneejit et al. 2008)	MSP-COBRA (Yanatatsaneejit et al. 2008)	(T,NE,L) (Yanatatsaneejit et al. 2008)	17
IKKalpha	IkappaB kinase-alpha	NF-kappaB signaling pathway	OSCC (Maeda et al. 2007b)	BS (Maeda et al. 2007b)	(T,NE) (Maeda et al. 2007b)	63
IRF8	Interferon regulatory factor 8	Transcriptional regulation	NPC (Lee et al. 2008)	MSP (Lee et al. 2008)	T (Lee et al. 2008)	78
LARS2	Leucyl-tRNA synthetase 2, mitochondrial	The action of a catalyst charging of $tRNA^{Leu(UUR)}$ with leucine	NPC (Zhou et al. 2009)	PCR-SSCP (Zhou et al. 2009)	T (Zhou et al. 2009)	64
LHX6	LIM homeobox 6	The specification of cortical interneuron subtypes and in the migration of GABAergic interneuron precursors from the subpallium to the cerebral cortex	HNC (Estécio et al. 2006)	(MS/AP-PCR) (Estécio et al. 2006), (COBRA) (Estécio et al. 2006)	(T,NE) (Estécio et al. 2006)	65.6
LRP1B	Low-density lipoprotein receptor-related protein 1B	Process of receptor-mediated endocytosis	OSCC (Nakagawa et al. 2006)	COBRA (Nakagawa et al. 2006)	T (Nakagawa et al. 2006)	43
LTF	Lactotransferrin	Antimicrobial activity	NPC (Yi et al. 2006)	MSP (Yi et al. 2006)	(T,CINT) (Yi et al. 2006)	63.6
MALT1	Mucosa-associated lymphoid tissue 1	Signal transduction	OSCC (Chiba et al. 2009)	MSP (Chiba et al. 2009)	(T,NE) (Chiba et al. 2009)	83
MINT2	Methylated in tumor-2	Tumor suppression	OSCC (Ogi et al. 2002)	MSRE (Ogi et al. 2002)	T (Ogi et al. 2002)	8
MINT27	Methylated in tumor-27	Tumor suppression	OSCC (Ogi et al. 2002)	MSRE (Ogi et al. 2002)	T (Ogi et al. 2002)	16
MIPOL1	Mirror-image polydactyly 1	Tumor suppression	NPC (Cheung et al. 2009)	MSP (Cheung et al. 2009)	(T,MN) (Cheung et al. 2009)	63
MT1G	Metallothionein 1G	Response to heavy metals	HNSCC (Tokumaru et al. 2004)	BS (Tokumaru et al. 2004)	T (Tokumaru et al. 2004)	25
MTNR1A	Melatonin receptor 1 A	Reproductive and circadian actions of melatonin	OSCC (Nakamura et al. 2008)	(COBRA, MSP) (Nakamura et al. 2008)	(T,MN) (Nakamura et al. 2008)	62
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	Regulation of the calcium channel activity of TRPCs and activation against influenza virus and VSV, a rhabdovirus	HNSCC (Calmon et al. 2009)	MSP (Calmon et al. 2009)	(T,L) (Calmon et al. 2009)	46
NDRG2	N-*myc* downstream-regulated gene 2	Neurite outgrowth	(OSCC, PM-OCL) (Furuta et al. 2010)	MSP (Furuta et al. 2010)	T (Furuta et al. 2010)	80
NISCH	Nischarin	Membrane-associated mediator of the I1R signaling	HNSCC (Hoque et al. 2008)	QMSP (Hoque et al. 2008)	T (Hoque et al. 2008) (SA-H,NE) (Hoque et al. 2008)	36

Table 3 (continued)

Gene	Name	Function	Tumor type	Method	Samples	Methylation range (%)
NmU	Neuromedin U	Stimulation of contractions of the ileum and urinary bladder	HNSCC (Tokumaru et al. 2004)	BS (Tokumaru et al. 2004)	T (Tokumaru et al. 2004)	20
OGDHL	Oxoglutarate dehydrogenase-like	Citric acid cycle	HNSCC (Hoque et al. 2008)	QMSP (Hoque et al. 2008)	T (Hoque et al. 2008) (SA-H,NE) (Hoque et al. 2008)	8
PAK3	p21-activated protein kinase 3	Cytoskeleton reorganization and nuclear signaling	HNSCC (Hoque et al. 2008)	QMSP (Hoque et al. 2008)	T (Hoque et al. 2008) (SA-H,NE) (Hoque et al. 2008)	52
PCDH10	Protocadherin 10	Calcium-dependent cell adhesion	NPC (Ying et al. 2006)	MSP (Ying et al. 2006)	T (Ying et al. 2006)	82
PRTFDC1	Phosphoribosyl transferase domain containing 1	Tumor suppression	OSCC (Suzuki et al. 2007)	COBRA (Suzuki et al. 2007)	T (Suzuki et al. 2007)	17
RASSF4	Ras association (RalGDS/AF-6) domain family member 4	Tumor suppression	HNSCC (Steinmann et al. 2009)	COBRA (Steinmann et al. 2009)	(T,MN) (Steinmann et al. 2009)	13
RASSF5	Ras association (RalGDS/AF-6) domain family member 5	Tumor suppression	HNSCC (Steinmann et al. 2009)	COBRA (Steinmann et al. 2009)	(T,MN) (Steinmann et al. 2009)	58
RB1	Retinoblastoma 1	Tumor suppression	SGC (Kishi et al. 2005)	MSP (Kishi et al. 2005)	T (Kishi et al. 2005)	41.7
RECK	Reversion-inducing cysteine-rich protein with kazal motifs	Suppression of tumor invasion and metastasis	OSCC (Long et al. 2008)	MSP (Long et al. 2008)	(T,MN,NE) (Long et al. 2008)	52
RIZ1	Retinoblastoma protein-interacting zinc finger protein	Transcriptional activation	NPC (Chang et al. 2003a)	MSP (Chang et al. 2003a)	(T, L,SA-P) (Chang et al. 2003a)	60
ROBO1	Roundabout, axon guidance receptor, homolog 1 (*Drosophila*)	Tumor suppression	(HNSCC) (Ghosh et al. 2009) (PM-HNL) (Ghosh et al. 2009)	MSRE (Ghosh et al. 2009)	T (Ghosh et al. 2009)	21
SFRP2	Secreted protein 2	Inhibition of Wnt signaling	OSCC (Sogabe et al. 2008)	MSP (Sogabe et al. 2008)	(T, MN) (Sogabe et al. 2008)	36
SFRP5	Secreted protein 5	Inhibition of Wnt signaling	OSCC (Sogabe et al. 2008)	MSP (Sogabe et al. 2008)	(T, MN) (Sogabe et al. 2008)	16
SOCS3	Suppressor of cytokine signaling 3	Signal transduction	(HNC, PM-HNC) (Weber et al. 2005)	MSP (Weber et al. 2005)	(T,MN,LN) (Weber et al. 2005)	90
STAT1	Signal transducer and activator of transcription 1	Signal transduction	HNC (Xi et al. 2006)	MSP (Xi et al. 2006)	(T,L) (Xi et al. 2006)	75
SYK	Spleen tyrosine kinase	Signal transduction	OSCC (Ogane et al. 2009)	QMSP (Ogane et al. 2009)	(T,MN) (Ogane et al. 2009)	63
TGFBR-I	Transforming growth factor, beta receptor 1	Signal transduction	HNSCC (Muñoz-Antonia et al. 2009)	MSP (Muñoz-Antonia et al. 2009)	T (Muñoz-Antonia et al. 2009)	62
TCF21	Transcription factor 21	Epithelial–mesenchymal interactions	HNC (Smith et al. 2006)	COBRA (Smith et al. 2006)	(T,MN) (Smith et al. 2006)	76
TSC2	Tuberous sclerosis 2	Tumor suppression	OSCC (Chakraborty et al. 2008)	COBRA (Chakraborty et al. 2008)	(T,MN) (Chakraborty et al. 2008)	100
TSLC1	Tumor suppressor in lung cancer	Tumor suppression	NPC (Hui et al. 2003)	MSP (Hui et al. 2003)	(T,NE) (Hui et al. 2003)	34.2

emerge by careful evaluation and combination of different marker panels to achieve a high sensitivity for the disease. Over the next years, clinical tests based on diagnostic and therapeutic methylation markers will certainly be available and will be used for the assessment of prognosis, treatment planning, and to predict the response.

Future studies aiming to determine the causative role and significance of these epigenetic alterations may provide important clues into the mechanism and contribution of the specific events and help to establish a sequence of methylation events during tumor development associated with different stages of head and neck carcinogenesis. Since tumor-specific DNA can be easily detected in blood, serum, and saliva, methylation analysis can be used as an noninvasive method for the early detection using a panel of the genes specific for the disease. Furthermore, epigenetic silencing of the genes offers new therapeutic approaches using demethylating agents. Analysis of genome-wide methylation profiles by new high-throughput technologies which enable simultaneous analysis of thousands of genetic loci will certainly help to identify highly specific novel methylation biomarkers.

Conflict of interest The authors declare that they have no conflicts of interest.

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