Epigenetic Alterations in Head and Neck Cancer: Prevalence, Clinical Significance, and Implications

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Head and neck cancers are a group of malignancies with diverse biologic behaviors and a strong, well-established association with tobacco and alcohol use. Although the hunt for genetic alterations in head and neck cancer has continued in the past two decades, with unequivocal proof of a genetic role in multistage head and neck carcinogenesis, epigenetic alteration in association with promoter CpG island hypermethylation has emerged in the past few years as one of the most active areas of cancer research. It is now firmly believed that, in cancer cells, promoter CpG island hypermethylation (epigenetic alteration) represents a bona fide alternative mechanism, as opposed to genetic factors, such as gene mutations and deletion, in the inactivation of many tumor-suppressor genes. It is also realized that epigenetic and genetic factors often work together, affecting multiple cellular pathways, such as cell-cycle regulation, DNA repair, apoptosis, angiogenesis, and cell-to-cell adhesion, during the process of tumor growth and progression.

Introduction

Head and neck cancers are a heterogeneous group of malignant neoplasms with a wide range of biologic behaviors. Cancer of the head and neck is the sixth most common malignancy worldwide, with approximately 500,000 new patients projected annually. In the United States, cancer of the head and neck accounts for 5% of the total cancer burden, with an estimated 30,000 new cases and 8000 deaths annually [1].

Head and neck cancer is primarily and causally associated with longstanding tobacco and alcohol exposure [2], and alcoholic beverages appear to interact with tobacco use to dramatically enhance head and neck cancer risk [3]. Tobacco smoke and tobacco products contain well-known carcinogens and procarcinogens, including benzo(A)pyrene, tobacco-specific nitrosamines, and arylamines. Other causative factors include smokeless tobacco use [4], betel nut chewing [4,5], Epstein-Barr virus (EBV) [6], and human papillomavirus [7]. Of particular interest is the high prevalence of oral cancer in the Indian subcontinent and large parts of southern Asia, where the habit of chewing betel nuts or smokeless tobacco is popular [4,5], and high prevalence of nasopharyngeal carcinoma in southern China, where EBV infection is endemic [6].

These factors can directly or indirectly cause damage to DNA molecules, resulting in irreversible genetic aberrations. For the past two decades, unparalleled emphasis has been placed on the characterization of genetic abnormalities that are traditionally thought to be the predominant or sole molecular basis for tumor development in human malignancies, including head and neck cancer. Among the commonly detected genetic abnormalities in head and neck cancer are loss of heterozygosity (LOH) or allelic loss on chromosome arms 3p, 5q, 6p, 8q, 9p, 9q, 11p, 11q, 13q, 17p, and 18q [8–10]. LOH analysis is a technique commonly used to identify tumor-suppressor genes (TSG). Head and neck cancers also frequently show expansion or deletion of some intronic simple repeated (CACACA)n or microsatellite sequence. Increased frequency of this change is referred to as microsatellite instability (MSI), which is caused by a defective DNA mismatch repair (MMR) system [11]. At the individual gene level, amplification of protooncogene *cyclin D1* and inactivation of two TSGs, *p53* and *p16*, are most commonly detected, being seen in over 50% of all head and neck cancer [12]. The elucidation of these genetic alterations in head and neck cancer provides support for a genetic basis for human malignancy and has in many cases confirmed Knudson's two-hit hypothesis [13].

In addition to these well-characterized genetic changes, epigenetic alterations in association with promoter CpG island hypermethylation are among the most common molecular alterations in human neoplasia [14••,15,16]. Epigenetics is referred to as a trait that is not based on a change in DNA sequence, yet is heritable. DNA methylation is a well-known epigenetic phenomenon, and growing evidence during the past decade supports promoter CpG island hypermethylation as a bona fide mechanism for

gene inactivation [17••]. Loss of gene function due to promoter hypermethylation has several characteristics that bear striking similarity to loss of TSG function by somatic mutation: First, promoter hypermethylation in one allele is frequently accompanied by deletion of the opposite allele, resulting in loss of heterozygosity of the gene. Second, gene inactivation in association with promoter hypermethylation is fully heritable. Finally, loss of gene function due to epigenetic alterations leads to selective growth advantage in a manner identical to loss of TSG function due to somatic mutation [18••].

The overall 5-year survival rate for patients with head and neck cancer has not improved much in the past several decades (approximately 50%) despite aggressive multimodality management [19]. The dismal treatment outcome for this disease may result from the lack of early detection and frequent locoregional recurrence [19]. Therefore, the development of new molecular markers is imperative for early tumor detection and prognostic prediction and to identify molecular targets for novel chemotherapeutic intervention.

Various genetic markers, such as point mutation, LOH, and MSI, have been used extensively in head and neck cancer for early tumor detection, prognostic prediction, and elucidation of the genetic progression model [8,20–23]; however, promoter hypermethylation is an alternative, attractive epigenetic marker that has recently gained in popularity. An epigenetic marker has the following advantages over a genetic marker: 1) promoter hypermethylation is much more common than genetic alterations in cancer; 2) promoter hypermethylation occurs in the same well-defined region of any given gene across all forms of cancer, whereas a wide range of mutational variations occur within a given gene, among different tumors, so that promoter hypermethylation analysis represents a much more efficient and cost-effective tumor detection approach; and 3) promoter hypermethylation constitutes a "positive signal" that can be easily detected against a background of normal cells, whereas some genetic markers, such as LOH, homozygous deletion, and MSI, represent "negative signals" and would be difficult to detect in a background of normal cells [17••,24••]. This review focuses on the prevalence of epigenetic alterations, highlighting their clinical significance and implications in head and neck cancer.

CpG Island Methylation and Mechanisms of Epigenetic Gene Silencing

In the mammalian genome, DNA methylation takes place only at cytosine bases when they reside 5′ to a guanosine in a CpG dinucleotide [25]. The CpG dinucleotide is hypermutable via spontaneous deamination to form the uracil base during evolution [26]. Consequently, a relative paucity of CpG dinucleotide is found throughout the human genome, at only about 20% of its expected frequency. However, in approximately half of the human gene promoter regions there are CpG-rich areas of 0.5 to 2 kb in length where the CpG dinucleotide frequency is as high or higher than expected [25]. These CpG-rich areas are often referred to as CpG islands. CpG islands share the following characteristics: 1) they are small stretches of DNA (0.5–2.0 kb in length) in which the total guanine and cytosine content is over 50% and the ratio of CpG to GpC is equal or over 1; 2) they are located mostly in the promoter regions of a "housekeeping" gene but rarely also in "tissue-specific" genes; 3) generally, they are free of methylation to permit gene expression in normal cells; and 4) in cancer, hypermethylation of the CpG islands can cause transcriptional silencing of genes [14••,16,25,26].

Compared with normal cells, changes in DNA methylation in cancer are complicated, encompassing a global hypomethylation and localized hypermethylation [16,17••]. In fact, global hypomethylation of the genome was initially thought to be an exclusive event in cancer [27,28]. DNA hypomethylation appears to involve primarily repetitive satellite DNA sequences and CpG sites in introns. The cause and consequences of this global DNA hypomethylation remain unknown. Although it was initially speculated that oncogenes could be overexpressed in cancer due to demethylation of CpG islands that were normally hypermethylated [29], this concept is now largely dismissed due to lack of experimental confirmation [17••,30•]. Global hypomethylation in cancer has also been linked tentatively to gross chromosomal instability, but the exact relationship remains to be consolidated [31].

In parallel to global genomic hypomethylation, frequent localized hypermethylation is also found and typically involves CpG islands of the gene promoter regions [16,30•]. Because CpG island hypermethylation is associated with transcriptional inactivation of genes and is stably inherited through mitosis, it is regarded as an alternative mechanism, as opposed to mutation, for silencing TSGs [16,17••,31].

Although epigenetic alterations in association with promoter CpG island hypermethylation were first reported in the retinoblastoma (*Rb*) gene by Greger *et al.* [32] in 1989, the role of epigenetic gene silencing was not clearly recognized until 1994, when Herman *et al.* [33] demonstrated that the Von Hippel–Lindau (*VHL*) TSG was silenced by DNA methylation in renal carcinoma. The notion of epigenetic gene silencing was elucidated further in the following year by two independent reports, in which the *p16*^{INK4a} TSG was shown to be frequently inactivated through promoter hypermethylation in many forms of human cancer [34,35].

The development of methylation-specific polymerase chain reaction (PCR) provided a user-friendly, sensitive, and highly specific method for the detection of DNA methylation [36]. This technique takes advantage of sodium bisulfite modification of DNA, in which sodium bisulfite is used to convert unmethylated cytosine residues to uracil residues, under conditions whereby methylated cytosine is

not reactive [37]. The converted DNA is then amplified by PCR, using primers specific for methylated or unmethylated sequences.

With these advances in detection and new knowledge in cancer epigenetics, the number of tumor-associated genes that undergo epigenetic silencing has grown rapidly in recent years. A partial list consists of genes involved in cell-cycle regulation (*p16*^{INK4a}, *p15*^{INK4b}, and *p14*^{ARF}), DNA repair (*hMLH1* and *MGMT*), cell-cell/cell-matrix adhesion (*E-cadherin*, *H-cadherin*, and adenomatous polyposis coli [*APC*]), apoptosis (*DAPK*, *TMS1*, and *caspase-8*), and angiogenesis (*THBS-1* and *p73*) [17••, 38•].

One should bear in mind that epigenetic alterations do not work alone. Rather, they almost always interact with genetic factors either directly or indirectly in promoting multistep carcinogenesis. Direct interaction between these two factors has been demonstrated in the inactivation of *p16*^{INK4a} and *BRCA1* TSGs in colon and breast cancers [39,40], in which one wild-type allele showed promoter hypermethylation, whereas the other unmethylated allele contained mutations. The interaction between these two epigenetic and genetic events fits perfectly into the two-hit theory proposed by Knudson [13], and both contribute to the complete silencing of *p16*^{INK4a} in colon [39] and *BRCA1* in breast cancers [40].

Examples of indirect interaction between epigenetic and genetic factors in carcinogenesis include gross genomic instability, such as increased MSI in sporadic colorectal, endometrial, and gastric cancers resulting from epigenetic silencing of *hMLH1*, a major MMR gene, and increased G:C to A:T transition mutations in the k-*ras* oncogene and *p53* TSG in colorectal cancer, caused by epigenetic silencing of *MGMT* DNA repair [17••]. *MGMT* is a repair gene for O^6-methylguanine, a promutagenic DNA base lesion induced by *N*-nitroso compound (NOC). Persistence of O^6-methylguanine in DNA due to deficient repair leads to G:C to A:T transition mutations upon DNA synthesis [17••]. In our laboratory, we detected *hMLH1* gene promoter hypermethylation, apparent loss of hMLH1 protein expression, and increased MSI in 33%, 18%, and 18% of 120 primary head and neck squamous cell carcinomas, respectively (manuscript in preparation). We further demonstrated that *hMLH1* promoter hypermethylation was significantly correlated with decreased protein expression (P=0.001) and increased MSI (P=0.01) in these tumors (manuscript in preparation), underscoring the significant role of the MMR system in maintaining global genomic integrity and stability in head and neck cancer.

One fundamental question regarding the gene inactivation in cancer in association with promoter CpG island hypermethylation is how the epigenetic DNA methylation silences transcription of the involved gene. The mechanisms whereby CpG island methylation suppresses gene transcription are now better understood. and multiple steps, starting from initiation and maintenance of methylation to protein acetylation and chromatin organization, appear to work as layers in cancer cells to achieve transcription silencing [18••,31].

Growing evidence supports the roles of increasing DNA methyltransferase (DNMT) activity in cellular transformation [18••]. Three such enzymes (DNMT1, DNMT3A, and DNMT3B) have been identified. DNMT3A and DNMT3B are implicated in establishing the de novo methylation pattern, whereas DNMT1 was thought to be responsible for maintaining the DNA methylation patterns [31]. These three DNA methyltransferase genes have been shown to be overexpressed at the mRNA and/or protein levels in many types of human cancer [31].

Mechanically, there are three main mechanisms whereby DNA methylation can repress gene transcription [41]. The first is methyl CpG binding domain (MBD)–mediated gene silencing, whereby methylated CpG islands specifically bind MBDs. At least three members of this protein family have been identified (MBD1, MBD2, and MeCP2), and all possess a transcriptional repressor domain and can thus directly repress transcription. In addition, these MBDs can recruit transcriptional corepressors, such as histone deacetylases (HDACs) and Sin3A, to methylated DNA. The deacetylation of chromatin histone results in closed or repressed chromatin configuration, which in turn leads to exclusion of transcription factors and allele-specific gene silencing. The second mechanism is DNMT-mediated gene silencing. All three DNMTs (DNMT1, DNMT3A, and DNMT3B) have a transcription repressor domain and can thus directly suppress transcription. In addition, these DNMTs can recruit cotranscriptional repressors, such as HDACs, to methylated DNA in a manner identical to MBDs. The third mechanism, CpG island hypermethylation, can sterically interrupt the binding of activating transcription factors to gene promoters.

In summary, in normal cells, the CpG dinucleotide is underrepresented throughout the genome, except in scattered segments of DNA of 0.5 to 2 kb in length where CpG dinucleotide reaches statistically expected frequency. These CpG islands are mostly located in the promoter regions and are often free of methylation in normal cells. In cancer cells, widespread hypomethylation coexists with distinct, localized hypermethylation, mostly in the promoter CpG islands in the genome. Although the role of global hypomethylation needs further definition, promoter CpG island hypermethylation has been well characterized, and its role in the transcriptional silencing of genes is incontestable.

Prevalence of Epigenetic Alterations

It has become apparent that different tumor types possess a different spectrum, profile, or clustering of gene hypermethylation, referred to as "methylotype" [17••] or "CpG island methylator phenotype" (CIMP) [30•] as opposed to the genetic terms, "genotype" or "mutator phenotype." The methylator phenotype or methylotype has been examined

in a limited number of cases of various tumors [24••]. More extensive analyses focusing on single types of tumor were also performed in colorectal [30•], breast [38•], and lung [42•] cancers.

Since the first report showing *p16*^{INK4a} promoter hypermethylation in head and neck cancer in 1995 by Herman *et al.* [34], a steady flow of studies have demonstrated promoter CpG island hypermethylation in various tumor-associated genes in head and neck cancer [43–56]. A review of the recent literature is warranted to examine the specific methylator phenotype in head and neck cancer.

The promoter hypermethylation of various tumor-associated genes in head and neck cancer is summarized in Table 1. Because of differences in patient populations, tumor locations, cancer risk factors (*ie*, smoking and alcohol use in North America and Japan, smokeless tobacco and betel nut chewing in India and southern Asia, and EBV infection in nasopharyngeal carcinoma of southern China), experimental designs, applications, and the results in each gene may not be directly comparable (Table 1). Nevertheless, the overall frequency of promoter hypermethylation of any given genes across multiple studies should provide us with a methylator phenotype that is relatively specific for head and neck cancer. Although there is some degree of variation, studies from multiple academic centers and numerous countries consistently identify certain genes with a high prevalence of epigenetic silencing (*eg*, *p16*, *MGMT*, and *E-cadherin*) and others without or with a very low proportion of epigenetic alteration (*eg*, *GSTP1* and *VHL*) in head and neck cancer.

Studies from multiple centers indicate that *E-cadherin*, *p16*, and *MGMT* are frequently altered epigenetically, with promoter methylation frequencies of 51%, 30%, and 30%, respectively (Table 1). In contrast, *GSTP1* and *VHL* gene promoters are not methylated at all (Table 1). *GSTP1* genes were previously shown to be the preferred target for epigenetic silencing in tumors derived from hormone-secreting organs, such as prostate and breast, whereas the *VHL* gene is preferentially targeted for epigenetic silencing in renal cell carcinoma.

The frequencies of promoter CpG island methylation of other genes, such as *DAPK*, *hMLH1*, *p14*, and *p15*, range from 10% to 25% (Table 1). *RARB2* and *RASSF1A* are potentially targeted for frequent epigenetic inactivation in head and neck cancer. However, the frequency of promoter methylation in these two genes (81% and 36%, respectively) was derived from only one or two studies, and confirmation is needed.

Thus, head and neck cancer has a methylotype or methylator phenotype characterized by a wide spectrum of epigenetic alterations involving multiple molecular pathways with certain genes, such as *E-cadherin*, *p16*, and *MGMT*, most frequently targeted, and others affected rarely, such as *GSTP1* and *VHL* (Tables 1 and 2). The methylator phenotype in head and neck cancer appears to be vividly different, compared with that in lung cancer [42•], a closely related and tobacco-induced cancer. The promoter methylation profile in lung cancer is characterized by a very high prevalence of epigenetic alterations in *APC*, *RARB2*, and *RASSF1A* genes (about 50%) and a low frequency of promoter methylation in *hMLH1*, *p14*, and *p15* genes (3%, 3% and 8%, respectively) (Table 2). These observations reinforce the suggestion by Esteller *et al.* [24••] that a unique methylator phenotype exists for each human tumor. It can thus be concluded that head and neck cancer is a unique type of malignant disease that is primarily induced by tobacco and alcohol use and progresses as a result of frequent epigenetic alterations interrupting primarily cell-cell interaction (*E-cadherin*), DNA repair (*MGMT*), and cell-cycle control (*p16*), in combination with perhaps equally frequent genetic changes, chiefly involving *p53* and *p16* inactivation and *cyclin D1* amplification [12].

Clinical Significance and Implications

The rapid advances in detection techniques and expansion of our knowledge in tumor epigenetics have raised expectations for transferring these techniques from the basic research laboratory to the clinic. The use of promoter CpG island hypermethylation as a biomarker or molecular target has shown great promise in the following areas: 1) early tumor detection; 2) prediction of tumor behavior or prognosis; 3) epigenetic classification of tumors; and 4) innovative cancer therapy.

Promoter hypermethylation in early detection of cancer

Promoter CpG island hypermethylation may be the most promising and robust biomarker for the early detection of tumor. The following qualities of epigenetic alterations, in association with DNA methylation, make them much better and more alluring alternatives to genetic markers (*eg*, mutation, LOH, and MSI):

1. *High informativity*. Promoter methylation occurs more frequently in tumor compared with genetic alterations in general. With several methylated loci analyzed simultaneously, the informative cases can reach 70% to 90% [24••].
2. *Superior specificity*. The promoter CpG islands of many tumor-associated genes are methylated frequently in cancer but rarely or never found to be methylated in normal tissues. This allows detection of tumor cells with high specificity in a background of normal cells.
3. *Simplicity.* Promoter methylation, unlike genetic mutations, which occur in numerous positions throughout genes with a wide spectrum of variations, almost always occurs within a well-defined region of the gene. This allows more efficient and cost-effective analyses with large numbers of cancer cases.

Table 1. Overview of genes with CpG island hypermethylation

Study	Genes	Pathway	Samples methylated, *n*	Samples analyzed, *n*	Proportion, %	Technique
Esteller *et al.* [24••]	*p16*	Cell cycle	26	95	27	MSP
Wong *et al.* [43]			30	90	33	MSP
Ogi *et al.* [44]			28	96	29	MSP
Viswanathan *et al.* [45]			23	99	23	RE
Hasegawa *et al.* [46]			26	80	32	MSP
Gonzalez *et al.* [47]			4	20	20	RE
Yeh *et al.* [48]			20	48	41	MSP
Nakahara *et al.* [50]			16	32	50	MSP
Ai *et al.* [54]			27	100	27	MSP
Total			200	660	30	
Wong *et al.* [43]	*p15*	Cell cycle	22	63	35	MSP
Ogi *et al.* [44]			6	96	6	MSP
Viswanathan *et al.* [45]			12	51	23	RE
Gonzalez *et al.* [47]			1	20	5	RE
Yeh *et al.* [48]			13	48	27	MSP
Total			54	278	19	
Esteller *et al.* [24••]	*p14*	Cell cycle	1	25	4	MSP
Wong *et al.* [43]			7	62	11	MSP
Ogi *et al.* [44]			13	96	13	MSP
Total			21	183	11	
Esteller *et al.* [24••]	*MGMT*	DNA repair for O^6-methylguanine	37	116	31	MSP
Wong *et al.* [43]			13	61	21	MSP
Viswanathan *et al.* [45]			41	99	41	RE
Zuo *et al.* [55]			19	93	20	MSP
Total			110	369	30	
Wong *et al.* [43]	*hMLH1*	Mismatch DNA repair	12	30	40	MSP
Ogi *et al.* [44]			0	96	0	MSP
Viswanathan *et al.* [45]			8	99	8	RE
Author's unpublished data			39	120	33	MSP
Total			59	345	17	
Esteller *et al.* [24••]	*GSTP1*	Carcinogen detoxification	0	106	0	MSP
Wong *et al.* [43]			0	33	0	MSP
Author's unpublished data			0	100	0	MSP
Total			0	239	0	

APC—adenomatous polyposis coli; ATM—ataxia-telangiectasia-mutated; MSP—methylation-specific polymerase chain reaction (PCR); RE—methylation-sensitive restriction enzyme digestion, followed by PCR; *VHL*—Von Hippel–Lindau.

Table 1. Overview of genes with CpG island hypermethylation *(Continued)*

Study	Genes	Pathway	Samples methylated, *n*	Samples analyzed, *n*	Proportion, %	Technique
Esteller *et al.* [24••]	*DAPK*	Apoptosis	17	92	18	MSP
Wong *et al.* [43]			48	65	73	MSP
Ogi *et al.* [44]			7	96	7	MSP
Hasegawa *et al.* [46]			19	80	24	MSP
Author's unpublished data			25	100	25	MSP
Total			116	433	26	
Wong *et al.* [43]	*E-cadherin*	Cell-cell adhesion	15	30	50	MSP
Viswanathan *et al.* [45]			35	99	35	RE
Hasegawa *et al.* [46]			29	80	36	MSP
Yeh *et al.* [48]			41	48	85	MSP
Saito *et al.* [51]			9	52	17	MSP
Nakayama *et al.* [53]			18	23	78	MSP
Author's unpublished data			74	100	74	MSP
Total			221	432	51	
Wong *et al.* [43]	*p73*	Angiogenesis and apoptosis	6	30	40	MSP
Esteller *et al.* [24••]	*APC*	Cell-cell adhesion	0	10	0	MSP
Wong *et al.* [43]	*RARB2*	Apoptosis	26	32	81	MSP
Wong *et al.* [43]	*RASSF1A*	Apoptosis	51	78	65	MSP
Hasegawa *et al.* [46]			6	80	8	MSP
Total			57	158	36	
Wong *et al.* [43]	*THBS1*	Angiogenesis	15	30	50	MSP
Wong *et al.* [43]	*Caspase-8*	Apoptosis	2	29	7	MSP
Wong *et al.* [43]	*VHL*	Tumor suppressor	0	30	0	MSP
Yeh *et al.* [48]			0	48	0	MSP
Waber *et al.* [52]			0	26	0	MSP
Total			0	104	0	
Ogi *et al.* [44]	*DCC*	Cell-cell adhesion	16	96	16	MSP
Ai *et al.* [56]	*ATM*	Cellular response to DNA damage	25	100	25	MSP

APC—adenomatous polyposis coli; ATM—ataxia-telangiectasia-mutated; MSP—methylation-specific polymerase chain reaction (PCR); RE—methylation-sensitive restriction enzyme digestion, followed by PCR; *VHL*—Von Hippel–Lindau.

Table 2. Comparison of promoter hypermethylation profiles between head and neck and lung cancers

	Head and neck*			Lung†		
Gene	Samples methylated, *n*	Samples analyzed, *n*	Proportion, %	Samples methylated, *n*	Samples analyzed, *n*	Proportion, %
p16	200	660	30	353	1106	31
p15	54	278	19	17	199	8
p14	21	183	11	13	384	3
MGMT	110	369	30	177	480	36
hMLH1	59	345	17	3	109	3
GSTP1	0	239	0	56	241	23
DAPK	116	433	26	141	513	27
E-cadherin	221	432	51	41	196	21
APC	0	10	0	312	452	69
RARB2	26	32	81	158	321	49
RASSF1A	57	158	36	193	363	53

*Data extracted from Table 1.
†Data derived from Tsou *et al.* [42•].

4. *High sensitivity.* The development of PCR-based techniques, which use differential modification of methylated versus unmethylated DNA by sodium bisulfite [36,37], enables detection of a very small amount of tumor-derived DNA from readily obtainable samples, such as saliva, sputum, serum, and stool [57••].

The most pressing problem for head and neck cancer is its dismal patient survival rate (approximately 50%), which has not improved for the past several decades, partly due to lack of effective early detection and/or reliable biomarkers for monitoring locoregional recurrence. Thus, the demonstrated capability of detecting a small amount of tumor-derived DNA from saliva and serum in patients with head and neck cancer [58,59] holds great promise for detection of early-stage cancer of the oral cavity long before it grows too large and becomes too late for operation, and for monitoring of tumor-derived DNA in serum for effectiveness of surgical therapy and evidence of tumor recurrence.

Promoter hypermethylation in prediction for tumor behavior or prognosis

Epigenetic alterations in tumors, in association with promoter CpG island methylation, have also been used effectively to predict tumor behavior or prognosis, probably because the genes that are silenced by promoter hypermethylation can affect many aspects of tumor progression and growth characteristics. For example, the presence of promoter hypermethylation in *MGMT*, a major repair gene for alkylating agent–induced DNA damage, was associated in gliomas with significantly increased overall and disease-free survival [60•], probably due to augmented responsiveness of tumor cells to alkylating chemotherapeutic agents such as carmustine. In another study involving lung carcinoma, promoter hypermethylation of the *DAPK* (death-associated protein kinase) gene was determined to be the strongest independent predictor for decreased patient survival [61]. In our laboratory, we found that promoter hypermethylation of *MGMT* and ataxia-telangiectasia-mutated (*ATM*) genes was present in 20% and 25% of 93 and 100 patients, respectively, with head and neck cancer. In addition, positive epigenetic change in these two genes was significantly correlated with decreased overall patient survival [55,56].

Promoter hypermethylation in epigenetic classification of tumor

Extensive analyses of promoter hypermethylation profiles in different tumor types reveal that each tumor has its unique epigenetic "fingerprint" [24••,30•,38•,42•]. Head and neck cancer also has its own distinct epigenetic profile (Table 1), which is different from that of lung cancer, an etiologically related malignancy (Table 2).

Characterization of tumors by their epigenetic or genetic makeup is significant because it provides invaluable information regarding the molecular basis of tumor development, biologic behavior, and predicted response to therapy [57••]. Although morphologic recognition and examination remain the golden and global standard for tumor diagnosis and classification, we should believe, given the recent rapid advance in molecular technology, that we are one big step closer to that foreseeable future in which all tumors will not be solely judged by their ever-changing appearance but by critical scrutiny of their unique epigenetic or genetic characteristics.

Promoter hypermethylation in innovative cancer therapies

In parallel with the use of DNA methylation as a biomarker for tumor detection and prognosis prediction, immense interest has been expressed in the development of novel can-

cer therapies targeting the DNA methylation process for the following reasons. First, many key tumor suppressor genes governing tumor growth and progression are silenced by promoter hypermethylation. Second, DNA methylation is a biologic modification that can be reversed by small pharmaceutical molecules. Thus, reactivation of a transcriptionally silenced gene by methylation is more amenable to a simple therapeutic approach than is the restoration of gene function due to gene mutation or deletion.

Reactivation of genes by inhibition of DNA methylation is an attractive approach for design of novel anticancer therapeutics because, in normal cells under normal physiologic conditions, genes are not subjected to regulation by DNA methylation, and therefore toxicity of DNA methylation inhibitors to normal cells is potentially lower than in conventional anticancer chemotherapeutic agents [57••].

Pharmaceutical agents that are capable of reactivating gene expression through this mechanism include specific DNMT inhibitors such as 5-azacytidine, 5-aza-deoxycytidine (decitabine), two newly discovered cardiovascular drugs (hydralazine and procainamide) and inhibitors of histone deacetylase (HDAC), such as trichostatin A and phenylbutyrate [41,57••]. In vitro and in vivo studies have shown increased sensitivity of tumor cells to such drugs as cisplatin and carboplatin after reactivation of the *hMLH1* gene by 5-aza-deoxycytidine (decitabine). This study led to a clinical trial using decitabine and carboplatin in combination for multiple tumor types [57••]. In the research laboratory it has also been demonstrated that the combination of decitabine and the HDAC inhibitor trichostatin A caused a synergistic reactivation of *hMLH1* and *TIMP3* genes in a colon cancer cell line. This study led to a clinical trial using these two agents for treatment of multiple tumor types [57••].

Reactivation of key enzymes controlling cellular response to anticancer drugs is another appealing approach. The best example of this approach was shown in a study by Esteller *et al.* [60•], who demonstrated that *MGMT* inactivation by promoter hypermethylation is critical in enhancing the responsiveness of highly aggressive glioma cells to such alkylating agents as carmustine. *MGMT* is a key cellular gene for alkylating agent–induced DNA damage, and its inactivation is primarily caused by promoter hypermethylation [18••]. Thus, conceptually, tumors with aberrant promoter hypermethylation in the *MGMT* gene should show greatly enhanced response to alkylating anticancer drugs, compared with those with intact *MGMT* gene function.

Head and neck cancer, along with lung carcinoma, shows a prevalence of *MGMT* promoter hypermethylation that is comparable (approximately 30%) with that of malignant gliomas (Tables 1 and 2) [60•]. Although alkylating agents such as carmustine are used as first-line chemotherapy in malignant gliomas, they are rarely used in head and neck cancer. In the future, clinical trials with antitumor alkylating chemotherapeutic agents should be aimed at treatment of patients with recurrent or late-stage head and neck cancers that fail all conventional therapies yet demonstrate epigenetic silencing of the *MGMT* gene.

Conclusions

The past few years have witnessed an upsurge of interest and explosive expansion of our understanding in tumor epigenetics, in particular regarding the mechanisms and targets of promoter CpG island hypermethylation in human cancer. Major developments include identification of key proteins required for DNA methylation, elucidation of the relationship between DNA methylation and chromatin structure, and an expeditious influx of candidate genes that are targeted by promoter methylation. Promoter hypermethylation has been firmly established as an alternative mechanism for transcriptional inactivation of genes, and genes targeted by epigenetic silencing affect multiple cellular pathways from cell-cycle control to apoptosis, cell-cell adhesion, and DNA repair. Aberrant methylation of promoter CpG islands has been proven to be one of the most robust biomarkers discovered by the scientific community and has great potential for use in many areas of cancer patient care, including early tumor detection, epigenetic tumor classification, prediction of tumor prognosis, and novel therapeutic intervention.

References and Recommended Reading

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