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Review

Epigenetic Field for Cancerization

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Epigenetic alterations, represented by aberrant DNA methylation, are deeply involved in human cancers. In gastric cancers, tumor-suppressor genes are inactivated more frequently by promoter methylation than by mutations. We recently showed that *H. pylori* infection, a potent gastric carcinogenic factor, induces methylation of specific genes in the gastric mucosae. When the methylation levels were analyzed in the gastric mucosae of healthy volunteers, cases with a single gastric cancer, and cases with multiple gastric cancers, who have increasing levels of risks for gastric cancers, there was a significant increasing trend in the methylation levels among the individuals without current *H. pylori* infection. This finding unequivocally showed the presence of an epigenetic field for cancerization. The degree of the field defect was measured more conveniently using methylation levels of marker genes than using those of tumor-suppressor genes. The presence of an epigenetic field for cancerization has been indicated for liver, colon, Barrett's esophageal, lung, breast, and renal cancers. Since decreased transcription is involved in the specificity of methylated genes, it is likely that specific genes are methylated according to carcinogenic factors. These findings emphasize the usefulness of DNA methylation as a marker for past exposure to carcinogens and future risk of cancer development.

Keywords: Cancer, DNA methylation, Epigenetics, Field cancerization, Field defect

Introduction

An innovation brings up a new challenge. Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) techniques were recently introduced into clinical practice to treat early gastric cancers saving a large area of the stomach (Gotoda *et al*., 2006). Now, a high incidence of second primary gastric cancers in the remaining stomach, reaching as high as 2.0% per year, is recognized (Nakajima *et al*., 2006a). The incidence is extremely high compared with the incidence (0.14% per year) in the general Japanese population (Lee *et al*., 2006). This contrast clearly shows that at least some gastric cancer cases have gastric mucosae that do not have any tumors but are already predisposed to developing gastric cancers.

The presence of mucosae that are predisposed to cancer development was initially described for oral cancers by Slaughter *et al*., using the term "field cancerization" (Slaughter *et al*., 1953). Although the predisposed mucosae can display some histological changes, such as atrophic gastritis and intestinal metaplasia in the stomach, they are essentially made of epithelial cells of polyclonal origins and have few monoclonal lesions. Nevertheless, the predisposed mucosae develop multiple cancers, and this phenomenon was denoted as "field cancerization" or the presence of "field defect" (Braakhuis *et al*., 2003). Field cancerization has been described for various organs, including the stomach (Nakajima *et al*., 2006a; Nakajima *et al*., 2006b), oral cavity (Slaughter *et al*., 1953; Partridge *et al*., 2000), the upper aerodigestive tract of smokers (Copper *et al*., 1993; Sozzi *et al*., 1995; Wistuba *et al*., 1997), the esophagus with Barrett change (Eads *et al*., 2000) or of heavy drinkers or smokers (Miyazaki *et al*., 2002), and the bladder (Hafner *et al*., 2002).

Most of the field cancerization has been explained by the presence of cells with genetic alterations (Sozzi *et al*., 1995; Wistuba *et al*., 1997; Partridge *et al*., 2000; Hafner *et al*., 2002; Braakhuis *et al*., 2003). However, involvement of epigenetic alterations in field cancerization is shown by our findings in the stomach (Maekita *et al*., 2006; Nakajima *et al*., 2006b), in addition to the reports in the liver (Kondo *et al*., 2000), colon (Hsieh *et al*., 1998; Issa *et al*., 2001; Shen *et al*., 2005), Barrett's esophagus (Eads *et al*., 2000), lungs (Guo *et al*., 2004), breasts (Yan *et al*., 2006), and kidneys (Arai *et al*., 2006).

In this review, after making a brief introduction to cancer epigenetics, I will focus on an epigenetic field defect for gastric cancers. Its presence has been documented by

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quantitative analysis of samples with established defect and those without, and its inducer is also evident. Then, I will describe the nature of field defects, including those for other cancers. Finally, I will discuss clinical applications of field defects.

Epigenetics and epigenetic alterations in cancers

Epigenetic information is defined as information other than the DNA sequence that is faithfully replicated upon somatic cell replication. It is carried by DNA methylation at CpG sites, histone modifications, and polycomb complex formation (Baylin and Ohm, 2006). Especially, DNA methylation is known to be replicated with a high fidelity in mammalian cells (Ushijima *et al*., 2003; Riggs and Xiong, 2004; Laird *et al*., 2004), and serves as a long-term memory of cells (Li, 2002). DNA methylation in promoter CpG islands very consistently represses transcription of their downstream genes (Fig. 1) (Ushijima, 2005; Baylin and Ohm, 2006), mainly by inducing changes in histone modifications, such as deacetylation of histones and methylation of lysine 9 of histone H3 (Richards and Elgin, 2002). Methylation in gene bodies does not block transcription, and is sometimes associated with active transcription (Miyamoto *et al*., 2003; Baylin and Ohm, 2006). Even when methylation of a gene body is associated with decreased transcription, such association has many exceptions, and does not have a causal role in gene silencing (Ushijima, 2005).

In cancer cells, "genome-overall hypomethylation and regional hypermethylation" are present. The "genome-overall" hypomethylation is almost always observed in cancers, and is mainly due to hypomethylation of repetitive sequences, which comprise more than 40% of the human genome and are normally heavily methylated (Kaneda *et al*., 2004a). The hypomethylation can lead to genomic instability and is considered to be involved in tumor progression (Eden *et al*., 2003). Genome-overall hypomethylation can also involve normally methylated CpG islands, which can induce aberrant transcription of their downstream genes, such as melanoma antigen genes (MAGEs) (de Smet *et al*., 1999).

Regional hypermethylation has been extensively analyzed in various cancers because methylation of promoter CpG islands of various tumor-suppressor genes can cause their inactivation (Baylin and Ohm, 2006; Ushijima, 2005). At the same time, methylation of CpG islands outside promoter regions is also present in cancers, and it is still unclear whether or not such methylation has any biological consequences. For example, in gastric cancers, *CDKN2A* (*p16*), *CDH1* (*E-cadherin*), *hMLH1*, and *RUNX3* can be inactivated by promoter methylation (Ushijima and Sasako, 2004; Li *et al*., 2002). In colorectal cancers, *CDKN2A*, *hMLH1*, *HIC1*, *SFRP1*, and many other genes can be inactivated (Baylin and Ohm, 2006). Notably, methylation of some tumor-suppressor gene, such as *SFRP1*, whose inactivation enhances Wnt signaling, was observed in very early lesions of colon carcinogenesis, aberrant crypt foci.

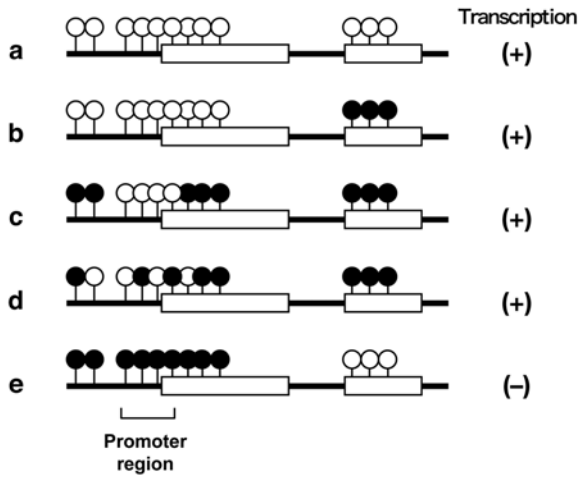

Fig. 1. Methylation of a gene region and its effect on gene transcription. Open circles, unmethylated CpG sites; and closed circles, methylated CpG sites. Methylation of exons (b, c) does not block gene transcription. Mosaic methylation of promoter CpG island also does not block transcription (d). However, dense methylation of promoter CpG islands completely blocks transcription, and is often associated with hypomethylation of downstream regions (e).

In the early 1990's, methylation of promoter CpG islands of tumor suppressor genes was discovered (Ohtani-Fujita *et al*., 1993; Baylin and Ohm, 2006). Since only a limited number of genes other than tumor-suppressor genes were analyzed, many investigators felt that most genes methylated in cancers were tumor-suppressor genes. However, as more genes were found to be silenced in various cancers by use of genome-wide screening techniques, it is now recognized that promoter CpG islands of many genes are methylated in cancers and only a fraction of them are tumor-suppressor genes (Ushijima, 2005). An extreme example of a gastric cancer cell line has as many as 421 silenced genes, and most of them cannot be tumor-suppressor genes (Yamashita *et al*., 2006).

The presence of aberrant DNA methylation in non-cancerous gastric mucosae

In gastric cancers, inactivation of *CDKN2A*, *CDH1*, *hMLH1*, and *RUNX3* due to their promoter methylation is more frequently observed than their inactivation due to mutations (Ushijima and Sasako, 2004). We applied a genome-wide screening method for differences in DNA methylation, methylation-sensitive-representational difference analysis (MS-RDA) (Ushijima *et al*., 1997; Kaneda *et al*., 2003), to gastric cancers, and identified nine silenced genes (Kaneda *et al*., 2002). One of the nine genes, Lysyl Oxidase (*LOX*), was later shown to possess a tumor-suppressive function in gastric

cancer cells (Kaneda *et al*., 2004b), as in prostate, colon, and breast cancers (Ren *et al*., 1998; Csiszar *et al*., 2002; Min *et al*., 2007).

Five of the nine genes, *THBD*, *LOX*, *HRASLS*, *FLNc*, and *HAND1*, were found to be infrequently methylated in non-cancerous gastric mucosae, in addition to their frequent methylation in cancers (Kaneda *et al*., 2002). Similar findings were reported for *CDH1* (Waki *et al*., 2002; Chan *et al*., 2003), and for *DAPK*, *CDH1*, *p14*, *THBS1*, and *TIMP-1* (Kang *et al*., 2003).

Induction of aberrant methylation in gastric mucosae by *Helicobacter pylori*

The presence of trace amounts of methylation in non-cancerous gastric mucosae suggested that some gastric carcinogens could have induced the methylation, and that the degree of methylation could be associated with gastric cancer risk. The most important gastric carcinogenic factor is *Helicobacter pylori* (*H. pylori*) infection, which increases the risk of developing gastric cancers by 2.2- to 21-fold (Uemura *et al*., 2001; Ekstrom *et al*., 2001). The presence of *CDH1* methylation was associated with *H. pylori* infection (Chan *et al*., 2003) while the number of methylated genes was not associated in the other study (Kang *et al*., 2003). All these studies, including ours, were performed using methylation-specific PCR (MSP), which can potentially overestimate methylation of small amounts of DNA molecules depending upon experimental conditions. The meaning of the methylated DNA molecules in the non-cancerous gastric mucosae could be different, depending upon the quantity of methylated DNA molecules. They could have originated from neoplastic lesions contaminated in "non-cancerous" samples, or from gastric mucosae that constituted the majority of the DNA molecules.

Therefore, we quantified the fraction of methylated DNA molecules in the gastric mucosae of healthy volunteers with (n = 98) and without (n = 56) current *H. pylori* infection by the quantitative methylation-specific PCR (quantitative MSP) method (Maekita *et al*., 2006). We also analyzed gastric mucosae of gastric cancer cases with (n = 43) and without (n = 29) *H. pylori* infection. The fraction of methylated DNA molecules was considered to reflect the fraction of cells with methylation of individual genes. Since inactivation of tumor-suppressor genes could lead to formation of neoplastic lesions, both tumor-suppressor genes (*CDKN2A* and *LOX*) and genes without evident tumor-suppressor function (*THBD*, *HRASLS*, *FLNc*, and *HAND1*) were analyzed. We also analyzed CpG islands outside promoter regions (exon 1 of *CDKN2A* and exon 8 of *p41ARC*) that were known to be susceptible to DNA methylation (Ushijima *et al*., 2003; Ushijima, 2005).

It was unequivocally shown that *H. pylori* infection potently induced aberrant methylation in gastric mucosae because methylation levels in *H. pylori*-positive healthy volunteers were 5.4- to 303-fold higher than those in *H. pylori*-negative healthy volunteers (Fig. 2) (Maekita *et al*., 2006). Although

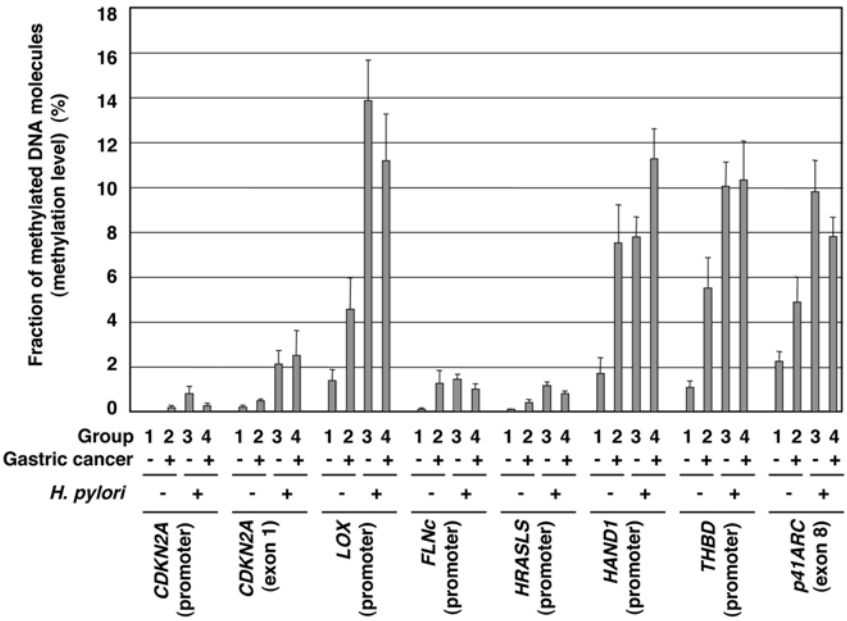

Fig. 2. Methylation levels in the non-cancerous gastric mucosae of healthy volunteers (gastric cancer: -) and gastric cancer cases (+) with and without *H. pylori* infection. Methylation levels were measured for eight regions of seven genes using DNA obtained from antral non-cancerous gastric mucosae. No or low methylation was observed in *H. pylori*-negative healthy volunteers (group 1), and high methylation levels were present in *H. pylori*-positive healthy volunteers (group 3) and cancer cases (group 4). In *H. pylori*-negative cancer cases (group 2), most of whom were considered to have past *H. pylori* infection, methylation levels were lower than individuals with current *H. pylori* infection. Error bars: standard errors. Adopted from Ushijima *et al*., 2006.

the absolute levels of methylation were also different depending upon a gene region, the same tendency was observed for all the eight regions analyzed. It was also noted that methylation levels of some genes, such as *LOX*, *THBD*, and *HAND1*, reached as high as 20-40% in healthy volunteers with *H. pylori* infection. These high fractions of cells with methylation in many healthy volunteers could never be due to the presence of neoplastic lesions in their gastric mucosae. Rather, it was shown that methylation of preferential genes can be induced in a significant fraction of gastric epithelial cells in a specific condition, such as in the presence of *H. pylori* infection.

Association between methylation levels in gastric mucosae and gastric cancer risks

Next, we compared methylation levels in the gastric mucosae of healthy volunteers and those in the non-cancerous gastric mucosae of cases with differentiated-type gastric cancers (Fig. 2). Since cases with gastric cancers are known to have higher risks of developing second primary gastric cancers (Nakajima *et al*., 2006a), the gastric mucosae of the cancer cases were considered to have higher risks of developing gastric cancers. Among the *H. pylori*-negative individuals, the cancer cases had 2.2- to 32-fold higher methylation levels than the healthy volunteers (Maekita *et al*., 2006). When methylation levels were analyzed in healthy volunteers, cases with a single gastric cancer, and cases with multiple gastric cancers, there was a significant increasing trend in the methylation levels (Nakajima *et al*., 2006b). These two studies demonstrated that the methylation levels in the gastric mucosae correlated with the risks of developing gastric cancers among individuals without current *H. pylori* infection. In contrast, among the *H. pylori*-positive individuals, methylation levels were almost the same in the cancer cases and healthy volunteers, and higher than or equal to those in the gastric mucosae of *H. pylori*-negative cancer cases.

All or the vast majority of gastric cancer cases are known to be associated with *H. pylori* infection (Uemura *et al*., 2001; Ekstrom *et al*., 2001). This indicates that the cancer cases without *H. pylori* infection at the time of analysis had past *H. pylori* infection. This was also supported by the presence of gastric atrophy in most of the *H. pylori*-negative cancer cases. Therefore, it was considered that the methylation levels in the gastric mucosae are zero or very low without *H. pylori* infection (*H. pylori*-negative healthy volunteers), increase to very high levels with current, or active, *H. pylori* infection (*H. pylori*-positive healthy volunteers and cancer cases), and decrease to certain levels after eradication or extinction of *H. pylori* infection (*H. pylori*-negative cancer cases) (Fig. 3). This "up-and-down" course was also supported by a recent study showing that CDH1 methylation can be reversed by *H. pylori* eradication by MSP (Chan *et al*., 2006).

As for the mechanism for the decrease, cell turnover was considered to be the major mechanism since DNA demethylase has not been established. The cell turnover was likely to be occurring within epithelial cells. Peripheral lymphocytes of *H. pylori*-positive individuals did not have methylation (Nakajima *et al*., 2006b), and gastric epithelial cells isolated from Mongolian gerbils infected with *H. pylori* by the gland isolation technique had methylation of specific regions (Niwa *et al*., unpublished results). We currently hypothesize two types of methylation, one being temporary methylation induced in progenitor or differentiated cells and the other being permanent methylation induced in stem cells (Fig. 3) (Ushijima *et al*., 2006). The former disappears as new cells are supplied from unmethylated stem cells while the latter does not. By assuming that *H. pylori* infection induces both the temporary and permanent methylation, the decrease in methylation levels after discontinued *H. pylori* infection can be explained. In *H. pylori*-negative individuals, only the permanent methylation remains, and their methylation levels are expected to be proportional to the fraction of stem cells with methylation, and thus to gastric cancer risks.

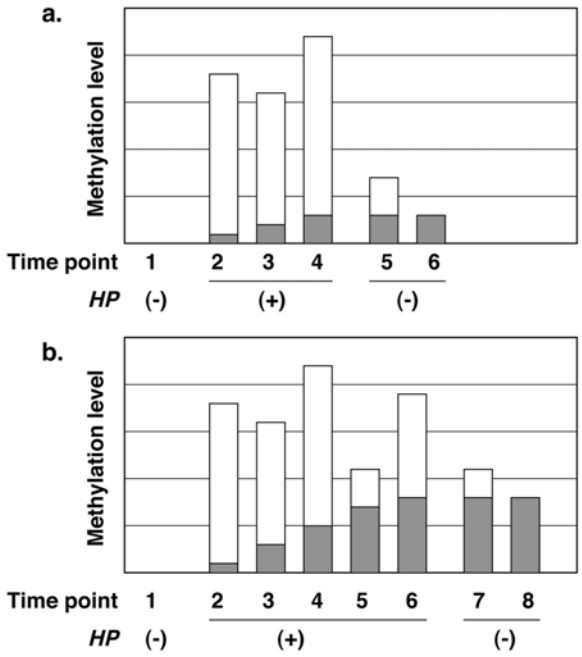

Fig. 3. A time course model of methylation levels in gastric mucosae with *H. pylori* infection. It was shown that individuals who have never had *H. pylori* infection have almost no methylation (point #1). During *H. pylori* infection, both temporary (white column) and permanent (gray column) components of methylation are induced. Although the temporary component fluctuates during the infection (points #2-4 in panel a, and #2-6 in b), the permanent component gradually increases. When *H. pylori* infection discontinues, the temporary component will disappear, leaving only the permanent component (points #5-6 in panel a, and #7-8 in b). The remaining permanent components (point #6 in panel a, and #8 in b) correlate with the risk of developing gastric cancers.

Epigenetic field for cancerization in the stomach and other organs

The clear association between the methylation levels in the gastric mucosae without any histologically malignant changes and the risk of developing gastric cancers showed that there was a field defect for gastric cancers that can be detected by DNA methylation. *LOX*, *THBD*, and *HAND1* had methylation levels as high as 5-8% in the non-cancerous gastric mucosae of *H. pylori*-negative cancer cases (Maekita *et al*., 2006), showing that this large fraction of gastric epithelial cells had their methylation. In contrast, the promoter region of *CDKN2A* had a methylation level of 0.2%, showing that its methylation was very rare. The methylation level of the promoter region of *hMLH1* was also near zero (Enomoto *et al*., manuscript submitted). These showed that the numbers of cells with methylation of tumor-suppressor genes, such as *CDKN2A* and *hMLH1*, were very small while those of cells with methylation of marker genes, such as *THBD* and *HAND1*, were large (Fig. 4). Notably, *LOX* tumor-suppressor gene had a high methylation level, and could be directly involved in the formation of field defect. Since the methylation levels of the tumor-suppressor genes correlate with those of the marker genes, the degree of field defect can be measured using marker genes whose methylation levels can be accurately measured.

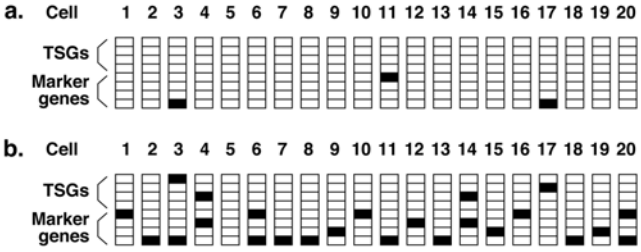

Fig. 4. Methylation of tumor-suppressor genes (TSGs) and marker genes. (a) A tissue without field defect. (b) A tissue with field defect. Open boxes, unmethylated genes; closed boxes, methylated genes. A tissue with field defect has low methylation levels of tumor-suppressor genes, and high levels of marker genes. Although methylation of marker genes is not directly involved in carcinogenesis, their methylation levels correlate with those of tumor-suppressor genes, and thus gastric cancer risks. However, since methylation of a marker gene is not requisite for carcinogenesis, a cancer arising from a mocosa with methylation of a marker gene does not necessarily have methylation of the gene.

The fractions of cells with methylation of tumor-suppressor genes, such as *CDKN2A* and *hMLH1*, were very small, but were considered to be much larger than the fraction of cells with mutations of specific genes. Although information on such a fraction of cells in the gastric mucosae is not available,

Table 1. Studies on epigenetic field for cancerization

Cancer	Inducing factor	Detection method and analysis way	Genes analyzed	Non-predisposed samples/ methylation in defect vs non-predisposed	Author
Liver cancer	HBV and HCV	COBRA/Incidence	*CDKN2A*, *hMLH1*, *THBS-1*, and five MINT loci	8 normal livers 4/5 vs 0/8	Kondo, 2000
Colorectal cancer (UC-associated)	UC	MSP/Incidence	*CDKN2A*	Not available	Hsieh, 1998
	UC	COBRA/ Quantitative	*ER, MYOD, CKDN2A*, and *CSPG2*	5 non-UC patients not significant	Issa, 2001
Barrett's cancer	Reflux esophagitis?	MethyLight/ Incidence	*APC, CDKN2A*, and *ESR1*	Not available	Eads, 2000
Lung cancer	Smoking?	MSP/Incidence	*CDKN2A, MGMT, DAPK, SOCS1, RASSF1A, COX2*, and *RARβ*	Not available	Guo, 2004
Colorectal cancer (sporadic)	Unknown	COBRA/ Quantitative	*MGMT*	33 healthy subjects 8.8% vs 2% 22/44 vs 4/33	Shen, 2005
Gastric cancer	*H. pylori*	qMSP /Quantitative	*CDKN2A, LOX, THBD, HRASLS, FLNc, HAND1*, and *p41ARC*	98 healthy subjects 2.2-to 32-fold increase in methylation levels	Maekita, 2006
Breast cancer	Unknown	qMSP/Incidence	*CYP26A1*	25 samples from reduction mammoplasty 4/5 vs 0/16	Yan, 2006
Renal cancer	Unknown	MSP/Incidence	*CDKN2A, hMLH1, THBS-1*, and five MINT loci	9 samples without renal cancers 44/60 vs 1/9 etc.	Arai, 2006

"Methylation in defect vs non-predisposed" describes the incidence (or methylation level) in histologically non-malignant, but predisposed area vs that in non-predisposed area. COBRA, combined bisulfite restriction analysis; qMSP, quantitative MSP; UC, ulcerative colitis; HBV, hepatitis B virus; and HCV, hepatitis C virus.

fractions of cells with mutations of a marker gene were in the range of 10-$10^3/10^6$ in animal models exposed to carcinogens (Nagao *et al*., 2001). Therefore, it is suggested that the number of epigenetically predisposed cells is much larger than genetically predisposed cells in the gastric mucosae after *H. pylori* infection. It was considered that the chance of suffering the next genetic/epigenetic alterations is much higher in epigenetically predisposed cells, and that the degree of field defect can be measured using DNA methylation as a marker.

Looking at cancers of other organs (Table 1), the presence of an epigenetic field for cancerization (field defect) was first suggested by the increased incidence of aberrant methylation in the non-cancerous liver tissues of cases with hepatocellular carcinomas (Kondo *et al*., 2000). Similar findings were obtained in the colonic mucosae of cases with colorectal cancers developed from ulcerative colitis (Issa *et al*., 2001), in Barrett's esophagus (Eads *et al*., 2000), and in the bronchial epithelium of lung cancer cases (Guo *et al*., 2004). It is critically important to compare predisposed and non-predisposed mucosae for demonstration of the field defect. Therefore, Shen *et al*. quantified MGMT methylation levels in the colonic mucosae of colorectal cancer cases and healthy individuals, and unequivocally showed the presence of epigenetic field defect (Shen *et al*., 2005). Our studies adopted a concept of marker genes and utilized an accurate method of quantitative MSP. Most notably, these demonstrated the presence of an inducer of the field defect, *H. pylori* (Maekita *et al*., 2006; Nakajima *et al*., 2006b). Recently, the presence of epigenetic field defect was indicated in breast cancers (Yan *et al*., 2006) and renal cancers (Arai *et al*., 2006). These multiple studies on epigenetic field defect underscore its reality and importance.

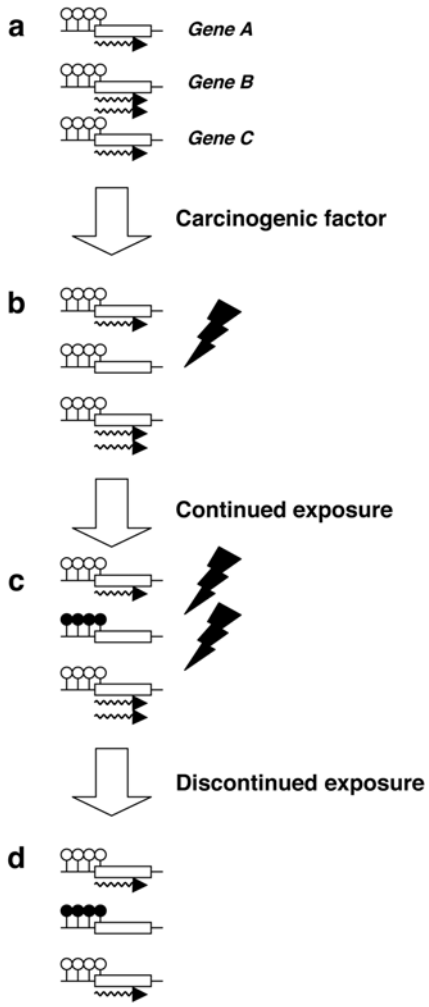

Fig. 5. A model for methylation induction in CpG islands of preferential genes. (a) Physiological transcription statuses (shown by wavy arrows) of three hypothetical genes (genes A, B, and C). (b) When a carcinogenic factor with epigenetic mechanisms is present, changes in the transcription levels (decreased gene B and increased gene C transcriptions) and abnormalities in epigenetic regulatory machineries (shown by lightning) are induced. (c) When the exposure continues, methylation of a gene (gene B) with no or low transcription can be induced. (d) The methylation alteration continues even after carcinogen exposure discontinued.

Inducing factors of methylation and target specificity

Inducing factors, except for gastric cancers, of methylation are still unclear. Although aging is well-known as an inducing factor of methylation (Issa *et al*., 1994), field defect due to aging is unknown. Rather, methylation induction by ulcerative colitis (Hsieh *et al*., 1998; Issa *et al*., 2001) and chronic hepatitis (Kondo *et al*., 2000) is likely to be involved in the formation of field defect. Ulcerative colitis, chronic hepatitis, and *H. pylori* infection all involve chronic inflammation, and at least some types of inflammation seem to lead to abnormalities in the epigenetic regulation. Actually, a proinflammatory allele of interleukin 1β, is associated with an increased risk of gastric cancers, especially when *H. pylori* infection is present (El-Omar *et al*., 2000; Lee *et al*., 2004).

Methylation of preferential genes was induced by *H. pylori* infection (Maekita *et al*., 2006). Our study using 48 genes (Yamashita *et al*., 2006) showed that some of these genes were susceptible to methylation induction by *H. pylori* while others were resistant (manuscript in preparation). We believe that a decrease or absence of transcription is deeply involved in the specificity of methylated genes. First, methylation analysis of exogenous or endogenous genes with and without transcription showed that low transcription is a trigger of methylation (Song *et al*., 2002; de Smet *et al*., 2004). Second, our extensive analysis on genes methylated in various types of cancers showed that most genes methylated in cancers are those untranscribed in normal counterpart cells (Furuta *et al*., 2006; Ushijima, 2005). At the same time, it is also true that genes with similar low transcription levels are not affected equally, and there should be some additional mechanisms for the specificity.

Taken together, abnormalities in the epigenetic regulation and a decrease or absence of transcription of target genes seem to be simultaneously involved in the methylation induction of specific genes (Fig. 5).

Clinical implication as a marker for carcinogen exposure and cancer risk

Methylation induction of specific genes by *H. pylori* infection also has clinical values. There is a possibility that other carcinogenic factors, such as Epstein-Barr virus infection, induce methylation of different sets of genes. This is because different carcinogenic factors induce expression changes of different genes, and, since low transcription is involved in methylation induction, genes specific to a factor could be methylated. Therefore, methylation patterns in the gastric mucosae, or in any other tissues, have a potential as a marker to identify carcinogens to which an individual was exposed in the past.

As a risk marker of developing a gastric cancer, methylation in the gastric mucosae also has a high potential. The methylation levels correlated with the increasing gastric cancer risks (cases with a single gastric cancer and those with multiple gastric cancers), and were independent from the degree of atrophy, another gastric cancer risk marker (Nakajima *et al*., 2006b). Clinically, prediction of metachronous cancers is important (Nakajima *et al*., 2006a), and a prospective study is necessary to make a final evaluation on the usefulness of DNA methylation levels as a risk marker. Since epigenetic field defect is present also for many other types of cancers, use as a risk marker is an important field.

Epilogue

The presence of an epigenetic field for cancerization is now evident for gastric cancers, and such a field is likely to be present also for liver, colon, Barrett's esophageal, lung, breast, and renal cancers. Aberrant DNA methylation is now shown to be involved not only in cancers but also in disorders with polyclonal origins (Mihara *et al*., 2006; Robertson, 2005). Epigenetic therapy is now actively being developed (Yoo and Jones, 2006), and its application to field defects has a potential as a preventive method for cancers and possibly other disorders. Research in this field has a strong potential to reveal new diagnostic markers and possibly therapeutic targets.

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