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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

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2. 実用新案登録

該当無し

3. その他

該当無し

別紙４

研究成果の刊行に関する一覧表

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# Chapter 14
# Epigenetic Epidemiology of Infectious Diseases

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## Contents



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**Abstract** Cancers induced by infectious agents, such as *Helicobacter pylori*, hepatitis viruses, Epstein-Barr virus, papilloma virus, liver fluke, and Schistosoma, are often associated with frequent DNA methylation of tumor-suppressor genes. Chronic inflammation and direct effects of some infectious agents are responsible for methylation induction. Analysis of non-cancerous, thus polyclonal, tissues provides unbiased information on which genes are methylated by specific agents. Gastric mucosae with *Helicobacter pylori* infection show methylation of specific promoter CpG islands. Promoter CpG islands of genes with low transcription levels and trimethylation of histone H3 lysine 27 are susceptible to methylation induction, and those with RNA polymerase II are resistant, even if the genes are not transcribed. Methylation of specific genes is expected to remain even after its inducing agent has vanished and these cytosine methylation fingerprints may prove to be good markers of past exposure to specific agents in molecular epidemiology.

## Abbreviations

| | |
|---|---|
| CGI | CpG island |
| CIMP | CpG island methylator phenotype |
| DNMT | DNA methyltransferase |
| EBV | Epstein-Barr virus |
| ESCC | Esophageal squamous cell carcinoma |
| *H. pylori* | *Helicobacter pylori* |
| H3Ac | Acetylation of histone H3 |
| H3K27me3 | Trimethylation of histone H3 lysine 27 |
| H3K4me3 | Trimethylation of histone H3 lysine 4 |
| H3K9me3 | Trimethylation of histone H3 lysine 9 |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HPV | Human papilloma virus |
| LMP1 | Latent membrane protein 1 |
| MeDIP | Methylated DNA immunoprecipitation |
| MSP | Methylation-specific PCR |
| Pol II | RNA polymerase II |
| PRC | Polycomb repressive complex |

## 14.1 Introduction

Infectious agents are some of the most well-known inducers of aberrant DNA methylation. Infection with *Helicobacter pylori* (*H. pylori*), a bacterial strain causally involved in gastric carcinogenesis, is known to induce aberrant DNA methylation in

gastric epithelial cells [1–3]. Infection with hepatitis C and B viruses (HCV and HBV), both of which are involved in the development of hepatocellular carcinomas, is associated with aberrant DNA methylation in cancer tissues and surrounding non-cancerous tissues [4–6]. Infection with Epstein-Barr virus (EBV), associated with lymphomas, nasopharyngeal cancers, and gastric cancers, is also associated with frequent DNA methylation in tumor tissue. Aberrant methylation induced by an infectious agent is present not in random genes, but in a group of genes affected by the agent [7]. Such gene-specific methylation provides an excellent tool for molecular epidemiology revealing past exposure to specific infectious agents, even if these agents are no longer present.

In this chapter, we will introduce methylation induction by infectious agents, its mechanisms, methylation of specific genes by specific inducers (a methylation fingerprint), and its mechanisms. We will conclude with the potential application to epidemiology.

## 14.2 Infectious Agents and Methylation Induction

Cancers induced by infectious agents, such as *H. pylori*, hepatitis viruses, EBV, papilloma virus, liver fluke, and Schistosoma, tend to have frequent aberrant DNA methylation of promoter CpG islands (CGIs) of tumor-suppressor and other genes (Table 14.1). In addition, non-cancerous tissues exposed to some infectious agents also show methylation of some genes, many of which are carried over into cancer cells.

### *14.2.1 The Significance of Methylation in Cancers and in Non-cancerous Tissues*

Methylation of promoter CGIs of tumor-suppressor genes is frequently present in cancers induced by an infectious agent. This can be a result of frequent induction of methylation in the infected tissue by the agent, or a result of growth advantage of a cell with methylation of a tumor-suppressor gene and its clonal expansion. If methylation is present in non-cancerous (thus polyclonal) tissue after infection, it is unlikely that a cell with a rare event of methylation of a tumor-suppressor gene, which itself is unrelated to the infection, has been selected and expanded. Therefore, when tumor-suppressor genes or other genes whose methylation can confer growth advantage to a cell are analyzed, use of non-cancerous tissues provides more unbiased information on whether or not an infectious agent is capable of inducing DNA methylation.

Since non-cancerous tissues are polyclonal, high methylation levels of a specific gene means that more cells acquired methylation of this gene in the past. Therefore, high methylation levels can be associated with stronger tissue damage in the past

**Table 14.1** Infectious agents and genes whose methylation is associated with an agent

| | Methylated genes in cancer tissue | Methylated genes in non-cancerous tissue | Ref |
|---|---|---|---|
| *Helicobacter pylori* | | | |
| Gastric cancer | | | |
| | *CDKN2A* | *CDKN2A* | [2] |
| | *FLNc* | *FLNc* | [2] |
| | *LOX* | *LOX* | [2, 8] |
| | *THBD* | *THBD* | [2] |
| | *miR-124a* | *miR-124a* | [8] |
| | *26 genes, including BDNF and IGFBP3* | *26 genes, including BDNF and IGFBP3* | [9] |
| Hepatitis virus B (HBV) | | | |
| Hepatocellular carcinoma | | | |
| | *APC* | *APC* | [6] |
| | *CDKN2A* | *CDKN2A* | [6] |
| | *RASSF1A* | *RASSF1A* | [6] |
| Hepatitis virus C (HCV) | | | |
| Hepatocellular carcinoma | | | |
| | *APC* | *APC* | [5, 6] |
| | *CDKN2A* | *CDKN2A* | [6] |
| | *RASSF1A* | *RASSF1A* | [6] |
| Epstein-Barr virus (EBV) | | | |
| Gastric cancer | | | |
| | *CDH1* | | [10] |
| | *CDKN2A* | | [11, 12] |
| | *MGMT* | | [11] |
| | *PTEN* | | [13] |
| Papilloma virus (HPV) | | | |
| Cervical cancer | | | |
| | *RASSF1A (unmethylated)*[a] | | [14, 15] |
| Head and neck cancer | | | |
| | *RASSF1A (unmethylated)*[a] | | [16] |
| | *SFRP4* | | [17] |
| Liver fluke | | | |
| Cholangiocarcinoma | | | |
| | *MLH1* | | [18] |
| Schistosoma | | | |
| Bladder cancer | | | |
| | *APC* | | [19] |
| | *CDH1* | | [19] |
| | *CDKN2A* | | [19] |
| | *RASSF1A* | | [19] |

[a]Considered to be due to the functional interaction between *RASSF1A* inactivation and HPV infection

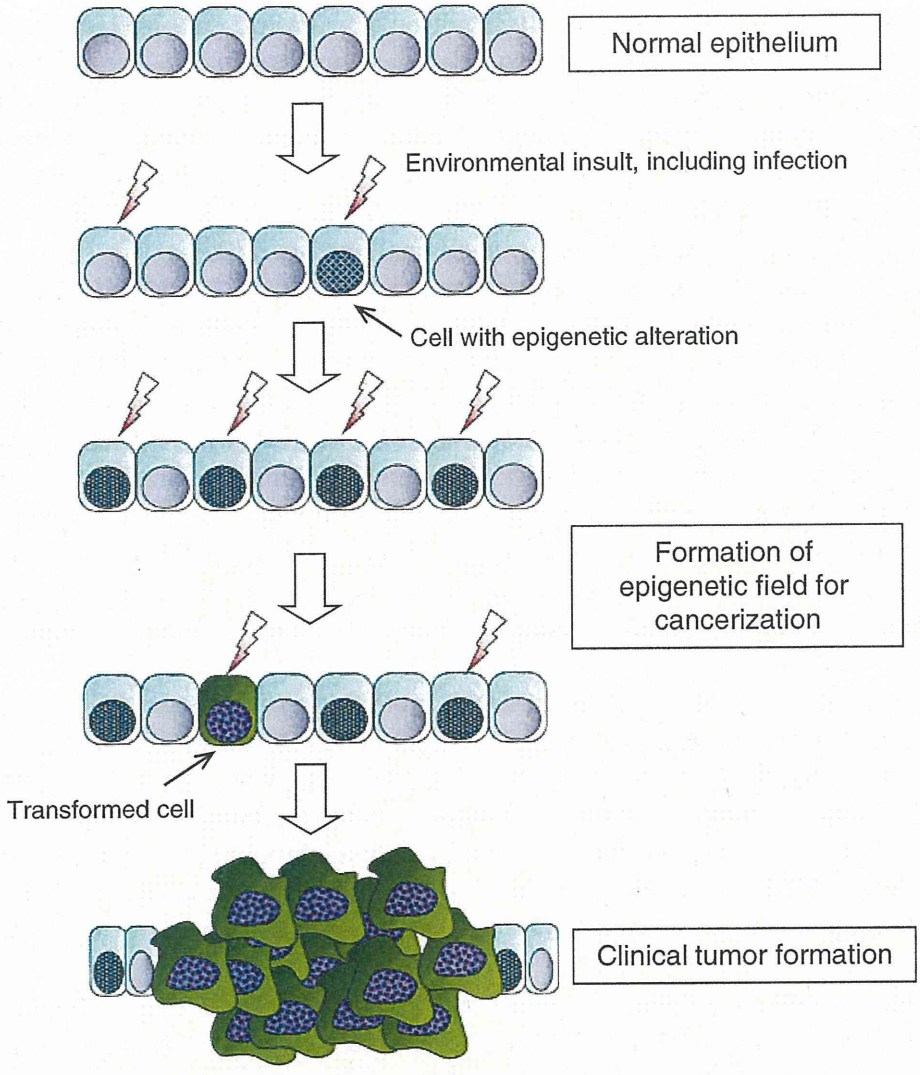


**Fig. 14.1** Formation of an epigenetic field for cancerization. Exposure to environmental stimuli, including infection, induces epigenetic alterations in a significant fraction of cells in normal-appearing tissues. When such stimuli continue, epigenetic alterations accumulate in a tissue, and an epigenetic field for cancerization is produced

and accumulation of epigenetic alterations. Indeed, for cancers like gastric cancers and hepatocellular carcinomas (HCCs), methylation accumulation in non-cancerous tissues is known to form a field from which cancers tend to develop (an epigenetic field for cancerization; Fig. 14.1) [20]. Methylation levels in gastric mucosae are associated with risk of developing gastric cancers [20, 21].

### *14.2.2 Helicobacter pylori Infection*

Gastric cancer is one of the leading causes of cancer deaths in the world, and most gastric cancer patients have a history of *H. pylori* infection [22]. *H. pylori* is a

Gram-negative bacterium, and is involved not only in gastric cancers but also in peptic ulcers and specific types of gastric B-cell lymphomas [23]. The infection itself is very prevalent, affecting nearly half of the world's population, although the prevalence is consistently decreasing [24]. *H. pylori* infection usually leads to acute and chronic gastritis, but only a small fraction of the infected individuals develop gastric cancer. Differences in *H. pylori* strains and host genetic backgrounds are considered to be responsible for the diverse clinical outcomes [25].

In gastric cancers, tumor-suppressor genes, such as *CDKN2A*, *MLH1*, *CDH1*, and *RUNX3*, are inactivated more frequently by methylation of their promoter CpG islands than by mutations [1]. Recent genome-wide analyses revealed that in addition to tumor-suppressor genes, hundreds of other genes are also methylated in gastric cancers [26, 27]. The possible association between *H. pylori* infection and methylation suggested by analysis of gastric cancers is solidified by analysis of non-cancerous gastric tissues with and without *H. pylori* infection [2, 9]. Methylation levels of eight specific CGIs in individuals with *H. pylori* infection were 5.4- to 303-fold as high as those in individuals without *H. pylori* infection ($P<0.0001$). In addition to protein coding genes, microRNAs are also methylated in *H. pylori* infected tissues [8].

The accumulation level of aberrant DNA methylation in gastric mucosae is known to correlate with gastric cancer risk [2, 21]. Among individuals without current *H. pylori* infection, methylation levels in gastric mucosae were lowest in healthy individuals, high in cancer patients with a single gastric cancer, and highest in cancer patients with multiple gastric cancers (both metachronous and synchronous gastric cancers) [21]. The correlation strongly supports that the accumulation of aberrant methylation in gastric mucosae produces an epigenetic field for cancerization.

An animal model is available for gastric cancers induced by *H. pylori* infection [28]. Mongolian gerbils infected with *H. pylori* showed increased methylation levels compared with age-matched non-infected gerbils, demonstrating a causal role of *H. pylori* infection in methylation induction [3].

### *14.2.3 Hepatitis Viruses*

HCCs are also a major cause of cancer deaths and are known to arise from liver tissues that heavily suffered from chronic inflammation, such as chronic hepatitis and liver cirrhosis [29]. As etiological agents, HCV and HBV play major roles, as well as some additional agents, such as excessive alcohol consumption and aflatoxin.

HCC tissues also have aberrant methylation of promoter CGIs of multiple tumor-suppressor genes [5, 6, 30]. Some genes, such as *CDKN2A*, are specifically methylated in HCCs while others, such as *RASSF1A*, are already methylated in surrounding liver tissues with chronic hepatitis or liver cirrhosis [30]. HCCs arising from liver cirrhosis have more methylated genes than those arising from chronic hepatitis, indicating that background tissue damage is reflected in HCCs [30]. When methylation

of 19 genes, mostly tumor-suppressor or tumor-associated genes, was analyzed, HCV-positive HCCs had more frequent methylation than had HBV-positive cancers and virus-negative HCCs [6].

Aberrant methylation of various genes is also present in surrounding non-cancerous liver tissues of patients with HCCs [6, 30, 31]. HCV-positive liver tissues show more frequent methylation than HBV-positive liver tissues and virus-negative liver tissues [6], strongly supporting HCV infection induces aberrant DNA methylation. Non-cancerous liver tissues of individuals with HBV and alcoholic hepatitis also had higher methylation levels than normal liver tissues of healthy individuals, indicating that these agents can induce aberrant DNA methylation in human liver tissues.

### *14.2.4 Epstein-Barr Virus*

EBV infection is highly prevalent in the world, and is known to affect mainly B cells [32]. Its infection is implicated as an etiology of EBV-associated B-cell, T-cell, and NK-cell lymphomas and nasopharyngeal carcinomas. A minor fraction of gastric cancers is also associated with EBV infection, and such cancers are accompanied with strong infiltration of inflammatory cells [11, 33]. Gastric cancers with EBV infection are known to be associated with highly frequent methylation of multiple genes, including *CDH1*, *MGMT*, *PTEN*, and *p16*, demonstrating the CGI methylator phenotype (CIMP) [10–13, 33]. Surprisingly, non-cancerous gastric mucosae of patients with gastric cancers with EBV infection tended to have lower levels of methylation than those of patients with gastric cancers but without EBV infection, most of whom were infected by *H. pylori* [12].

### *14.2.5 Papilloma Virus*

Human papilloma virus (HPV) is associated with cervical cancer, head and neck cancers, oral cancers, and possibly lung cancer [34–36]. In cervical cancers, HPV infection was associated with unmethylated status of *RASSF1A*, possibly due to their complementary functions in cervical carcinogenesis [14]. In contrast, in cervical dysplasia, from which cervical cancers develop, aberrant methylation of multiple genes, including *CCNA1*, *DAPK1*, *HS3ST2*, *PAX1*, and *TFPI2*, was present [37, 38], suggesting that HPV infection was associated with methylation induction.

In head and neck cancers, HPV infection was associated with unmethylated status of *RASSF1A*, again possibly due to their complementary functions in cervical carcinogenesis [16]. HPV infection was associated with *SFRP4* methylation in cancer tissues [17].

### *14.2.6 Liver Flukes*

Liver flukes are parasites that infect the biliary tract, and are known to be a risk factor for cholangiocarcinoma, a rare malignant liver tumor arising from the intrahepatic biliary tract [39]. Like in other cancers, aberrant DNA methylation of tumor-suppressor genes, such as *APC*, *CDH1*, *CDKN2A*, and *MLH1* is frequently observed in cholangiocarcinoma [40]. Especially, *MLH1* methylation is frequently observed in liver fluke-related cholangiocarcinoma in Thailand [18].

### *14.2.7 Schistosoma*

Schistosoma is a parasite that infects the urinary ducts and bladder, and Schistosoma infection is associated with risk of several malignancies, including bladder cancers [41]. Bladder cancers in general also show aberrant DNA methylation in multiple genes [42]. Notably, bladder cancers with Schistosoma infection had more methylated tumor-suppressor genes, including *APC*, *CDH1*, *CDKN2A*, and *RASSF1A*, than those without Schistosoma infection [19].

## 14.3 Mechanisms for Methylation Induction by Infectious Agents

Infectious agents are inducers of inflammation, which is known to be deeply involved in methylation induction [43, 44]. In addition, direct effects by infectious agents on cancer precursor cells need to be considered.

### *14.3.1 Role of Chronic Inflammation*

The role of chronic inflammation in methylation induction was proposed based on the observation that aberrant methylation of specific genes was present in colonic mucosae of patients with ulcerative colitis [43, 45]. In addition, the infectious agents listed above are all associated with chronic inflammation. This strongly indicates that chronic inflammation is involved in methylation induction.

Direct evidence for the role of chronic inflammation in methylation induction was provided by an animal model of methylation induction by *H. pylori* infection. When inflammation induction by *H. pylori* infection was suppressed by treating Mongolian gerbils with cyclosporin A, an immunosuppressant, methylation induction was markedly suppressed without affecting colonization of *H. pylori* (Fig. 14.2). This showed that it is inflammation, not *H. pylori* itself, that is involved in methylation induction [3].

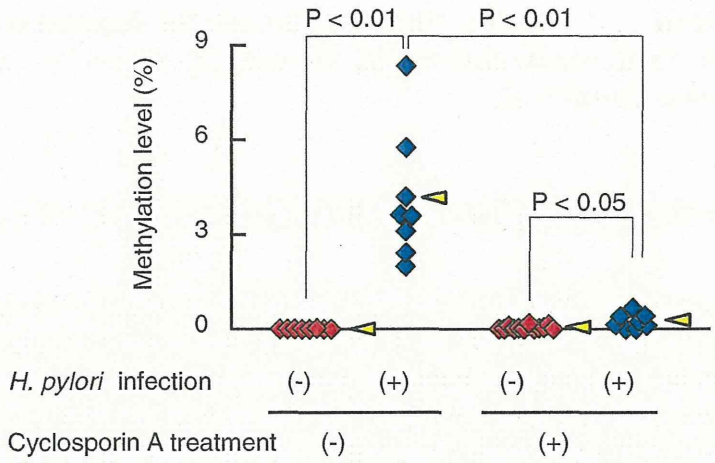


**Fig. 14.2** A critical role of inflammation in methylation induction. The methylation level of a CGI (HE6) was measured by quantitative MSP in gastric mucosae of gerbils infected with *H. pylori* with or without cyclosporin A treatment. While a high level of methylation was induced by *H. pylori* infection without cyclosporin A, the induction was markedly suppressed with cyclosporin A. It was confirmed that cyclosporin A suppressed immune reaction, but did not affect colonization of *H. pylori*. Arrow head: mean methylation level in a group (Modified from Niwa et al. [3])

### *14.3.2 Direct Effect of Infectious Agents*

In addition to signals from chronic inflammation to epithelial cells, some infectious agents have a direct effect on methylation induction. Especially, up-regulation of DNA methyltransferases by infectious agents has been reported for multiple viruses. The X protein of HBV (HBx) up-regulates DNMT1 and DNMT3A; the HCV core protein up-regulates DNMT1 and DNMT3B; HPV E7 and adenovirus E1A up-regulate DNMT1; and Kaposi's sarcoma-associated herpes virus LANA protein up-regulates DNMT3A [46–52]. The major EBV oncogene, latent membrane protein 1 (LMP1), up-regulates DNMT1, DNMT3A, and DNMT3B in nasopharyngeal carcinoma cell lines and induces methylation of the tumor-suppressor genes *RARB* and *CDH13* in these cell lines [49, 53]. LMP2A, another EBV latent gene, up-regulates DNMT1 and induces methylation of the tumor-suppressor gene, *PTEN*, in gastric cancer cell lines [11, 13]. In contrast with these viruses, DNMTs were not upregulated in human and gerbil gastric mucosae infected by *H. pylori* [3, 9].

## 14.4 Methylation Fingerprints Produced by Specific Inducers

It is likely that promoter CGIs of specific genes are methylated by a specific inducer, as described in this section. We here call this specific pattern of methylation "methylation fingerprint," as a mutation pattern specific to a mutagen is termed its