Summary. Aberrant promoter methylation and subsequent silencing of cancer-related genes has been recognized as an important pathway involved in gastric carcinogenesis. In fact, several factors are believed to contribute to its induction in gastric epithelia, including aging, diet, chronic inflammation and infection of Helicobacter pylori (H. pylori) and Epstein-Barr virus (EBV). However, the underlying mechanisms are not completely identified, despite the belief that increased expression or activity of DNA methyltransferases (DNMTs), or decreased demethylation activity may contribute to the excessive methylation. A great number of genes with promoter methylation have been observed in gastric cancer (GC), among which p16INK4A (p16), Mut L homologue 1 (MLH1), Epithelial-cadherin (E-cadherin), Runt-related transcription factor 3 (RUNX3), adenomatous polyposis coli (APC), O(6)-methylguanine-DNA methyltransferase (MGMT), Ras association domain family 1A (RASSF1A) and Death-associated protein kinase (DAPK) have been extensively studied. Unlike the distinct methylation characterization in single genes, methylation analysis of multiple genes may provide more information in risk prediction, early detection, prognosis assessment and chemotherapy choice for GC. Specifically, particular monitoring and screening should be performed on those over 45 years old, with precancerous gastric disease or infection of H. pylori or EBV. As an alternative to tumor tissues, methylation detection in patient sera or gastric washes may also be used in risk prediction and early detection. However, what still poses a great challenge as well as a puzzle is the determination of the very genes that should be used in methylation analysis. Because epigenetic alterations are normally reversible, drugs or chemical compounds with demethylating activity, such as 5-aza-2'-deoxycytidine (5-aza-dC) could be used in the treatment of patients with multiple gene methylation. In view of the adverse effects of 5-aza-dC, DNMT-targeted strategy has been proposed and may prove to be more effective than demethylating agents.

Key words: Promoter methylation, Gastric cancer, DNA methyltransferase

Introduction

DNA methylation, a common epigenetic phenomenon in organisms, occurs when a methyl group is added into the carbon-5' position in CpG dinucleotide residues by some DNA methyltransferases (DNMTs). Primarily, it is needed to maintain the normal conformation and function of chromosomes in normal cells, and it is also essential for embryogenesis and fetal development. However, the process may get disturbed, for CpGs in CpG islands in gene promoters are normally unmethylated. Actually, aberrant methylation of promoters usually results in inhibition of gene expression, especially tumor-related ones, which serves as an important pathway recognized for carcinogenesis. As is also known, mechanisms of gene silencing by aberrant promoter methylation are related to the failure of transcription factors to bind their CpG containing recognition sites, due to cytosine methylation of such sites directly, or by some methyl-DNA-binding proteins, indirectly.

Mechanisms underling the promoter methylation of gastric epithelia

Some mechanisms are proposed to explain aberrant promoter methylation in carcinogenesis, such as increased expression of DNMTs. In fact, overexpression of DNMT1 was frequently observed in gastric cancer
(GC), and significantly associated with excessive DNA methylation (Kanai et al., 2001; Etoh et al., 2004; Mutze et al., 2011). Moreover, DNMT1 suppression was found to induce re-expression and demethylation of some methylated genes (Jung et al., 2007). Clinical analysis showed that low DNMT1 expression was correlated with good prognosis and chemotherapy response. Another member in the DNMT family, DNMT3b was also found to be expressed in GC, though its role in aberrant methylation has not been identified (Mutze et al., 2011; Jung et al., 2007). All these findings indicate that DNMT1 plays a crucial role in aberrant DNA methylation in gastric carcinogenesis; and DNMT1-targeted inhibition may represent a promising strategy for methylation-related GC. In addition, a reduction of DNA demethylation activity may also be responsible for excessive DNA methylation (Kanai et al., 1999), and promoter methylation in gastric epithelia could also be subject to some inhibitors of DNMTs (such as polyphenolic compounds in green tea).

Factors inducing promoter methylation in gastric epithelia

The exploration of how promoter methylation is triggered in normal and pre-cancerous cells remains one of the most important subjects in tumorigenesis. Therefore, any insightful understanding of aberrant methylation and subsequent gene silencing is essential for cancer prediction, prevention, treatment and prognosis evaluation. So far, several contributing factors have been identified in the aberrant methylation process in gastric epithelia, such as aging, diet, chronic inflammation and microbial infection.

Aging

Promoter methylation of tumor-related genes was rarely found in nonneoplastic gastric epithelia from young people, which, in contrast, was frequently observed in older people (Waki et al., 2002, 2003). Some genes showed a general progressive increase in the methylation frequency as a function of aging, whereas other genes seemed resistant to this and were rarely methylated in normal gastric tissues, even in older people (Kang et al., 2003a; So et al., 2006). Genes with aging-related methylation showed distinct time profiles. Namely, Epithelial-cadherin (E-cadherin), p16INK4A (p16) and Death-associated protein kinase (DAPK) were methylated in nonneoplastic gastric epithelia of persons who were 45 years or older, while Runx-related transcription factor 3 (RUNX3) methylation in nonneoplastic gastric epithelia was restricted to individuals aged 77 years or older (Waki et al., 2002, 2003). This pattern indicates that age-related promoter methylation is progressively accumulated, which may pose a risk of GC. However, its mechanism in gastric epithelia is not yet clear, and its potential link to increased expression or activity of DNMTs needs further exploration and determination. In fact, DNMT3b activity was found to increase in aging WI-38 human fetal lung fibroblasts, while DNMT1 seemed to be unrelated to this process, and its overexpression was involved in aging-related genomic hypomethylation (Lopatina et al., 2002). Likewise, roles of age-related promoter methylation in gastric carcinogenesis are not fully identified either, as GC does not develop in most people with age-related methylation.

Diet

Dietary factors play important roles in carcinogenesis for their apparent links to aberrant promoter methylation of tumor-related genes. They may take effect by altering the supply of methyl donors and DNMT inhibitors. Surprisingly, there were few studies focusing on the correlation, though stomach is obviously one of the organs most susceptible to dietary modification and aberrant promoter methylation. However, methylation of caudal type homeobox 2 (CDX2) and bone morphogenetic protein 2 (BMP-2) was found to be associated with the decreased intake of green tea, which contains several polyphenolic compounds inhibiting DNMT activity (Yuasa, et al., 2005, 2009). In fact, a large body of tumor-related genes was methylated in gastric epithelia, and it is therefore essential to pin down whether and which diet factors are relevant. Equally important is the thorough understanding of the underlying mechanisms in these cases so as to deter potential gastric carcinogenesis.

Chronic inflammation

Chronic inflammation is also a factor leading to aberrant DNA methylation. In fact, gastritis tissues present a far greater prevalence of promoter methylation than normal ones, and the samples with marked infiltration of mononuclear cells displayed higher numbers of genes methylated than those with mild or moderate infiltration of mononuclear cells, which suggested that chronic inflammation is closely associated with increased methylation in nonneoplastic gastric mucosa samples (Kang et al., 2003b). Inflammatory mediators, such as TNF-α, IL-1β and reactive nitrogen species are thought to be involved in the aberrant DNA methylation of gastric epithelia, though the fundamental mechanisms need further explorations.

Helicobacter Pylori

Helicobacter Pylori (H. pylori) is a definite carcinogen of gastric cancer, however, the roles of H. pylori in gastric carcinogenesis are not yet identifiable. Methylation of a series of tumor-related genes was found to be associated with H. pylori infection in human precancerous gastric lesions or normal mucosa (Maekita et al., 2006; Kang et al., 2008; Dong et al., 2009;
Gene methylation in gastric carcinogenesis

Peterson et al., 2010). H. pylori eradication led to a significant decrease of gene methylation, which also suggested that the process may delay or reverse H. pylori-induced gastric carcinogenesis (Leung et al., 2006; Perri et al., 2007). On the other hand, H. pylori infection did not induce mRNA and protein expression of DNMTs (Nakajima et al., 2009; Hur et al., 2011). In fact, animal studies showed upregulation of IL-1β, NOS2 and TNF-α was related to methylation induction (Niwa et al., 2010; Hur et al., 2011), which was blocked significantly by using the immunosuppressive drug cyclosporine to suppress the inflammation (Niwa et al., 2010). These findings indicated that it is the infection-associated inflammatory response, rather than H. pylori itself, that is responsible for inducing the aberrant promoter methylation (Niwa et al., 2010; Hur et al., 2011).

Epstein-Barr virus

As an oncogenic virus, Epstein-Barr virus (EBV) was found to be associated with promoter methylation of various tumor-related genes (Kang et al., 2002a; Sakuma et al., 2004; Chang et al., 2006; Kawamura et al., 2008; Maruyama et al., 2008). Both methylation frequency and average number of methylated genes in EBV-positive GC are higher than those in EBV-negative GC (Kang et al., 2002a; Sakuma et al., 2004; Chang et al., 2006), suggesting that aberrant methylation may be an important mechanism of EBV-related gastric carcinogenesis. However there have been few studies on this. Like H. pylori, EBV-associated promoter methylation is also gene-specific. One pathway may be related to DNMT1 overexpression, which was induced by latent membrane protein 2A (LMP2A) through the phosphorylation of STAT3 (Hino et al., 2009). LMP1, another latent membrane protein, is also involved in the aberrant methylation of tumor-related genes in cancers by DNMT1 through different signal pathways.

Genes with promoter methylation in GC

Promoter methylation and subsequent silencing of tumor-related genes in human cancer has been one of the focuses of study in the last decade. A large number of genes with promoter methylation have been observed in GC. And the list of methylated genes is still growing. Silencing of expression of tumor-related genes is thought to be an important pathway leading to carcinogenesis, therefore analysis of promoter methylation may be a useful marker for early cancer detection or prediction. Of the majority of methylated genes, p16, Mut L homologue 1 (MLH1), E-cadherin, RUNX3, adenomatous polyposis coli (APC), O(6)-methylguanine-DNA methyltransferase (MGMT), Ras association domain family 1A (RASSF1A), DAPK have been extensively studied (table 1) and will be discussed in this review. In addition, more than fifty other tumor-related genes are listed in Table 2 and Table 3 (with methylation frequency more than 20% in human primary GC), which have been reported with promoter methylation in primary GC.

Table 1. Methylated genes which have been extensively studied in GC.

<table>
<thead>
<tr>
<th>Genes</th>
<th>major function</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16</td>
<td>p16INK4A</td>
<td>CDK inhibitor</td>
</tr>
<tr>
<td>MLH1</td>
<td>Mut L homologue 1</td>
<td>Mismatch repair</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Epithelial-cadherin</td>
<td>Invasion/metastasis suppressor</td>
</tr>
<tr>
<td>RUNX3</td>
<td>Runt-related transcription factor 3</td>
<td>TGF-beta signaling</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
<td>Cell adhesion regulator</td>
</tr>
<tr>
<td>MGMT</td>
<td>O(6)-methylguanine-DNA methyltransferase</td>
<td>DNA repair</td>
</tr>
<tr>
<td>DAPK</td>
<td>Death-associated protein kinase</td>
<td>Apoptosis regulator</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>Ras association domain family 1A</td>
<td>G1/S inhibitor</td>
</tr>
</tbody>
</table>
with the malignant transformation of gastric precursor lesions.

**MLH1**

Microsatellite instability (MSI), mainly caused by mismatch repair defect, is a common phenomenon in human cancers. In sporadic GC, MSI is frequently observed, while mutations of mismatch repair genes have been rarely described. Several studies have shown that methylation and aberrant expression of MLH1 is responsible for MSI in sporadic GC, and the loss of MLH1 protein is a significant event in gastric carcinogenesis (Leung et al., 1999; Fleisher et al., 1999, 2001). In fact, the frequency of MLH1 promoter methylation is above 70 percent in patients with high MSI (MSI-H), which is usually defined as the occurrence of MSI in two or more of the five loci (or >30% of loci, if more than five loci are tested), and there is almost no MLH1 promoter methylation in microsatellite stable (MSS) patients (Fleisher et al., 1999; Leung et al., 1999). Methylation of MLH1 is also frequently present in the non-neoplastic surrounding mucosa of methylated tumors (Endoh et al., 2000), and there is a marked increase in methylation frequency from premalignant lesions to carcinomas (Kang et al., 2001). All the above suggest that MLH1 methylation occurs early in multistep gastric carcinogenesis and tends to accumulate along the process; hence, though there is not enough evidence to support the idea that MLH1 methylation itself can be used as a predictor of carcinogenesis or prognosis, it may serve as a useful approach in early detection of GC as an initial, vital event in the development of tumors of the stomach.

**E-cadherin (CDH1)**

Mutation of E-cadherin, a potential invasion/metastasis suppressing gene, is quite common in GC, but E-cadherin mutation is limited to undifferentiated-scattered (diffuse) type, and it is observed infrequently in other histological types of GC. Some GC without E-cadherin mutation also display diminished expression, which is mainly associated with E-cadherin promoter methylation (Grady et al., 2000; Machado et al., 2001). The frequency of E-cadherin methylation is about 40~50% (Grady et al., 2000; Tamura et al., 2000; Leung et al., 2001; Machado et al., 2001; Graziano et al., 2004a), being similar between early and advanced GC (Tamura et al., 2000). In chronic gastritis, intestinal metaplasia, gastric adenoma and GC, the methylation frequency is also comparable (Kang et al., 2003a). These data show E-cadherin promoter methylation associated with decreased expression may not necessarily occur in advanced GC but do in early phases. Therefore, a safe conclusion can be made that E-cadherin methylation has

### Table 2. Other genes in GC with methylated frequency no less than 50%.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Freq.(%)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>RELN</td>
<td>100</td>
<td>Dohi et al.</td>
<td>2010</td>
</tr>
<tr>
<td>PDX1</td>
<td>100</td>
<td>Ma et al.</td>
<td>2010</td>
</tr>
<tr>
<td>NID2</td>
<td>95</td>
<td>Ulazzi et al.</td>
<td>2007</td>
</tr>
<tr>
<td>NID1</td>
<td>90</td>
<td>Ulazzi et al.</td>
<td>2007</td>
</tr>
<tr>
<td>ITGA4</td>
<td>85</td>
<td>Park et al.</td>
<td>2004</td>
</tr>
<tr>
<td>PCDH10</td>
<td>82</td>
<td>Yu et al.</td>
<td>2009</td>
</tr>
<tr>
<td>MAL</td>
<td>80</td>
<td>Buffart et al.</td>
<td>2008</td>
</tr>
<tr>
<td>KLF4</td>
<td>80</td>
<td>Wei et al.</td>
<td>2005a</td>
</tr>
<tr>
<td>SPINT2</td>
<td>75</td>
<td>Dong et al.</td>
<td>2010</td>
</tr>
<tr>
<td>DCC</td>
<td>75</td>
<td>Sato et al.</td>
<td>2001</td>
</tr>
<tr>
<td>SFRP2</td>
<td>73</td>
<td>Cheng et al.</td>
<td>2007</td>
</tr>
<tr>
<td>ALX4</td>
<td>73</td>
<td>Ebert et al.</td>
<td>2006</td>
</tr>
<tr>
<td>ADRA1B</td>
<td>71</td>
<td>Noda et al.</td>
<td>2007</td>
</tr>
<tr>
<td>UCHL1</td>
<td>70</td>
<td>Tokumaru et al.</td>
<td>2008</td>
</tr>
<tr>
<td>BVES</td>
<td>69</td>
<td>Kim et al.</td>
<td>2010</td>
</tr>
<tr>
<td>XAF1</td>
<td>69</td>
<td>Zou et al.</td>
<td>2006</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>67</td>
<td>Tomii et al.</td>
<td>2007</td>
</tr>
<tr>
<td>SFRP5</td>
<td>65</td>
<td>Nojima et al.</td>
<td>2007</td>
</tr>
<tr>
<td>POPDC3</td>
<td>64</td>
<td>Kim et al.</td>
<td>2010</td>
</tr>
<tr>
<td>TSPYL5</td>
<td>64</td>
<td>Jung et al.</td>
<td>2008</td>
</tr>
<tr>
<td>OPCML</td>
<td>64</td>
<td>Cui et al.</td>
<td>2008</td>
</tr>
<tr>
<td>PLCD1</td>
<td>62</td>
<td>Hu et al.</td>
<td>2009</td>
</tr>
<tr>
<td>LRP1B</td>
<td>61</td>
<td>Lu et al.</td>
<td>2010</td>
</tr>
<tr>
<td>NMDAR2B</td>
<td>61</td>
<td>Liu et al.</td>
<td>2007</td>
</tr>
<tr>
<td>AKAP12</td>
<td>56</td>
<td>Choi et al.</td>
<td>2004</td>
</tr>
<tr>
<td>SLC19A3</td>
<td>51</td>
<td>Liu et al.</td>
<td>2009</td>
</tr>
<tr>
<td>PRDM5</td>
<td>50</td>
<td>Watanabe et al.</td>
<td>2007</td>
</tr>
<tr>
<td>DFN A5</td>
<td>50</td>
<td>Akino et al.</td>
<td>2007</td>
</tr>
</tbody>
</table>
RUNX3

RUNX3, a Runt domain transcription factor involved in TGF-beta signaling, plays important roles in gastric epithelium proliferation, differentiation and apoptosis. Mutations in RUNX3 turned out to be rare, and aberrant methylation of the CpG island is the major cause of the inactivation of RUNX3 expression (Guo et al., 2002; Li et al., 2002). RUNX3 methylation is cancer-specific, except for very old individuals. With the methylation frequency in neoplastic gastric epithelia reaching about 40%–71% (Kim et al., 2004; Oshimo et al., 2004), far above that in the corresponding non-neoplastic gastric epithelia, chronic gastritis, intestinal metaplasia and gastric adenomas, it is therefore reasonable to consider RUNX3 methylation a possible molecular diagnostic marker and malignancy predictor. In addition, with a more frequent occurrence of RUNX3 methylation in intestinal and diffuse-adherent type tumors than in diffuse-scattered type tumors (Oshimo et al., 2004), the detection of it in remnant gastric mucosa may serve as a predictor of the risk of carcinogenesis in the remnant stomach (Nakase et al., 2005). Likewise, RUNX3 was reasonably viewed as an independent prognostic factor and a potential therapeutic target for gastric cancer (Wei et al., 2005b), because its decreased expression was significantly associated with poor prognosis.

APC

Despite the fact that mutation and promoter methylation of APC were frequently observed in colorectal cancers, no APC mutations were found in gastric carcinomas (Tamura et al., 2001). Notably, methylation of APC promoter 1A was very common in GC (53%–84%) (Tsuchiya et al., 2000; Sarbia et al., 2004; Ksiaa et al., 2009). APC 1A is methylated with high, similar methylation frequency in chronic gastritis, intestinal metaplasia, gastric adenoma and GC, and it is also highly methylated in normal stomach samples or corresponding noncancerous gastric mucosa (Tsuchiya et al., 2000; Eads et al., 2001). In contrast, promoter 1B was surprisingly not found to be methylated in gastric cancer and matching noncancerous samples (Tsuchiya et al., 2000). Therefore, no final conclusion can be made as to the actual role of promoter 1A methylation, because the same APC protein is coded by two transcripts from two promoters, 1A and 1B.

MGMT

Promoter methylation of MGMT was found in relatively low frequency (16%–23%) in GC, similar to chronic gastritis, intestinal metaplasia and gastric adenoma, and rarely found in nonneoplastic gastric mucosa samples (Oue et al., 2001a,b; Park et al., 2001). GC patients with MGMT methylation were reported to display distinct molecular and clinical features, as

Table 3. Other genes in GC with methylated frequency less than 50%.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Freq.(%)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNIP3</td>
<td>49</td>
<td>Murai et al.</td>
<td>2005</td>
</tr>
<tr>
<td>SOCS6</td>
<td>47</td>
<td>Lai et al.</td>
<td>2010</td>
</tr>
<tr>
<td>IQGAP2</td>
<td>47</td>
<td>Jin et al.</td>
<td>2008</td>
</tr>
<tr>
<td>HPP1</td>
<td>47</td>
<td>Shibata et al.</td>
<td>2002</td>
</tr>
<tr>
<td>DAB2IP</td>
<td>46</td>
<td>Dote et al.</td>
<td>2005</td>
</tr>
<tr>
<td>CMTM3</td>
<td>44</td>
<td>Wang et al.</td>
<td>2009</td>
</tr>
<tr>
<td>SFRP1</td>
<td>44</td>
<td>Zhao et al.</td>
<td>2007</td>
</tr>
<tr>
<td>ADAM23</td>
<td>44</td>
<td>Takada et al.</td>
<td>2005</td>
</tr>
<tr>
<td>BMP-2</td>
<td>43</td>
<td>Wen et al.</td>
<td>2006</td>
</tr>
<tr>
<td>SFN</td>
<td>43</td>
<td>Suzuki et al.</td>
<td>2000</td>
</tr>
<tr>
<td>PRKCDBP</td>
<td>42</td>
<td>Lee et al.</td>
<td>2008</td>
</tr>
<tr>
<td>CHFR</td>
<td>39</td>
<td>Satoh et al.</td>
<td>2003</td>
</tr>
<tr>
<td>PTEN</td>
<td>39</td>
<td>Kang et al.</td>
<td>2002b</td>
</tr>
<tr>
<td>RIZ1</td>
<td>37</td>
<td>Tokumaru et al.</td>
<td>2003</td>
</tr>
<tr>
<td>DLEC1</td>
<td>34</td>
<td>Ying et al.</td>
<td>2009</td>
</tr>
<tr>
<td>FBP1</td>
<td>33</td>
<td>Liu et al.</td>
<td>2010</td>
</tr>
<tr>
<td>LOX</td>
<td>33</td>
<td>Kaneda et al.</td>
<td>2004</td>
</tr>
<tr>
<td>TMS1</td>
<td>32</td>
<td>Kato et al.</td>
<td>2008</td>
</tr>
<tr>
<td>CACNA2D3</td>
<td>30</td>
<td>Wanajo et al.</td>
<td>2008</td>
</tr>
<tr>
<td>RASSF2A</td>
<td>30</td>
<td>Maruyama et al.</td>
<td>2008</td>
</tr>
<tr>
<td>SCLCAS8</td>
<td>30</td>
<td>Ueno et al.</td>
<td>2004</td>
</tr>
<tr>
<td>DLC-1</td>
<td>30</td>
<td>Kim et al.</td>
<td>2003</td>
</tr>
<tr>
<td>ID4</td>
<td>28</td>
<td>Chan et al.</td>
<td>2003</td>
</tr>
<tr>
<td>SOCS1</td>
<td>27</td>
<td>To et al.</td>
<td>2004</td>
</tr>
<tr>
<td>RAB32</td>
<td>27</td>
<td>Shibata et al.</td>
<td>2006</td>
</tr>
</tbody>
</table>
MGMT methylation was found to be associated significantly with point mutations of K-ras, lymph node invasion, tumor stage, and survival (Park et al., 2001).

DAPK

DAPK is a serine/threonine kinase and a positive mediator of apoptosis. Promoter methylation of DAPK was detected in high frequency in GC tissues (41~70.3%) as well as in the corresponding non-neoplastic gastric epithelia (Lee et al., 2002; Chan et al., 2005; Sugita et al., 2011), and GC patients with DAPK methylation were also reported to display distinct clinical features. In fact, the response to chemotherapy was significantly lower in patients with it than in those without (Sugita et al., 2011), and patients with excessive DAPK methylation showed significantly worse survival (Chan et al., 2005; Sugita et al., 2011).

RASSF1A

The tumor suppressor RASSF1A induces cell cycle arrest through inhibition of cyclin D1 accumulation. RASSF1A methylation was commonly observed in primary tumors, including GC. Methylation frequency of RASSF1A in GC tissues (59%-67%) was significantly higher than that in corresponding normal tissues (Ye et al., 2007; Guo et al., 2009). RASSF1A is not methylated in gastric adenoma, intestinal metaplasia and chronic gastritis, suggesting that methylation and inactivation of RASSF1A may be a later event in malignant transformation, and detection of aberrant RASSF1A methylation may serve as a reliable diagnostic and prognostic marker. In addition, methylation and decreased expression of RASSF1A was found to be correlated with tumor stage (Byun et al., 2001; Guo et al., 2009).

Concurrent methylation of multiple genes in GC

Compared to the studies which focused on the single gene, many other studies have shown that concurrent methylation of many genes is associated with the process of tumorigenesis. To determine whether a tumor is associated with CpG island methylation phenotype (CIMP), a term used to describe the colorectal cancers with a 3-5 fold elevated frequency of aberrant gene methylation (Toyota et al., 1999a), a group of separated genes, including MLH1, p16 and E-cadherin, were examined. These CIMP-positive cancers show distinct genetic profiles and clinical features from those without CIMP (Toyota et al., 2000), and have independent predictive significance for the survival benefit from 5-FU chemotherapy in colorectal cancers.

Concurrent methylation of multiple genes was also observed in GC (Lee et al., 2004; Leung et al., 2001). Though concurrent methylation is also present in the normal tissues adjacent to cancer, the methylation is much less than that in cancers. Studies show a marked increase in methylated genes from chronic gastritis to intestinal metaplasia, as well as from adenomas to carcinomas (Kang et al., 2001, 2003b; Lee et al., 2004), which indicates that the CIMP seems to be an early event in GC. Multiple gene methylation was found to be associated with tumor stage (Oue et al., 2006) and poor prognosis in GC (Napieralski et al., 2007; Al-Moundhri
et al., 2010). However, unlike in colorectal cancers, concurrent methylation was not found to be statistically significantly associated with the response to cisplatin/5-fluorouracil-based therapy (Napieralski et al., 2007). These results suggest that CpG island methylation occurs early in multistep gastric carcinogenesis and tends to accumulate along the process, and methylation of multiple genes may be one of the major pathways that contribute to tumorigenesis in GC. Multiple gene analysis of CIMP can give essential help in cancer risk prediction, early detection and prognosis evaluation. However, the promising prospect is dampened by the obstacles involved in the choice of the candidate genes. In fact, the absence of an accurate panel has posed a barrier to current research and further explorations in this aspect.

Methylation in the serum and washes of GC patients

Some studies showed that aberrant methylation in serum DNA was accompanied by methylation in the corresponding tumor samples. Aberrant promoter methylation in serum can be detected in a substantial proportion of GC patients (Lee et al., 2002). Single gene analysis indicates that aberrant methylation of certain genes could be a potential biomarker for early detection of gastric cancer. The potential genes include Reprimo (RPRM) (Bernal et al., 2008), RUNX3 (Tan et al., 2007), sulfatase 1 (SULT1) (Chen et al., 2009), secreted frizzled-related protein 2 (SFRP2) (Cheng et al., 2007) and p16 (Kanyama et al., 2003). Some studies show that multiple genes are also concurrently methylated in the serum of gastric cancer patient, just like tissues (Koike et al., 2005). The combined use of APC and E-cadherin methylation markers in the serum identified a subgroup of cancer patients with worse prognosis (Leung et al., 2005). Multivariate analysis showed that global DNA methylation in peripheral blood was a significant independent predictor of worse survival (Al-Moundhri et al., 2010). In addition, gene methylation can also be detected in gastric washes. Breast cancer 1 (BRCA1) and RASSF1A promoter methylation can be detected in the peritoneal fluid DNA from patients with ovary tumors (Ibanez de Caceres et al., 2004). With a close correlation between methylation levels in tumor biopsy and gastric washes (Watanabe et al., 2009), DNA methylation in gastric washes may serve as a new method for GC detection.

Prospective and conclusion

It is acknowledged that promoter methylation of cancer-related genes is an important pathway in gastric carcinogenesis, with numerous factors involved in this process, though the underlying mechanisms have not yet been conclusively identified. Single gene methylation usually has distinct characteristics, therefore it may be more plausible to employ methylation analysis of multiple genes in risk prediction, early detection, prognosis assessment and choice of chemotherapy for GC. Especially, approaches such as monitoring or screening should be properly practiced on patients more than 45 years old, with chronic precancerous gastric disease or infection of H. pylori or EBV. Though analysis of DNA methylation in the serum or washes is a promising method for risk assessment in the development of GC, there is still substantial work to be done to determine the panel of the candidate genes.

Another commitment consists in the proper and reasonable treatment of these patients with promoter methylation pathway. Demethylation proves to be an ideal strategy because epigenetic alterations are normally reversible. Thus, drugs or compounds with demethylating activity, such as 5-aza-2′-deoxycytidine could be legitimately used in the treatment of these patients. With DNA methylation and histone modification interacting with each other, a complete understanding of these epigenetic modifications and their cross-talk will lead to the development of the most effective therapies, such as by combining DNMT inhibitors with histone methyltransferase inhibitors. However, due to its incorporation into DNA during DNA synthesis, 5-aza-2′-deoxycytidine can cause DNA damage, mutagenesis and cytotoxicity. In view of these adverse effects, DNMT-targeted strategy has been proposed and may prove to be a more effective approach. All in all, there are still some important questions that must be convincingly answered, with regard to the underlying mechanisms of aberrant promoter methylation and its precise roles in gastric carcinogenesis, before a comprehensive and effective treatment package is established and secured.

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