We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

DNA Methylation
in Aggressive Gastric Carcinoma

Chung-Man Leung, Kuo-Wang Tsai and
Hung-Wei Pan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52135

1. Introduction

Gastric cancer remains a common cancer type in humans to dates, especially in the Andean region of South America and in the Far East. Various factors contribute to cause of stomach cancer, including *Helicobacter pylori*, smoking and diet. Most patients are diagnosed with advanced gastric cancer, therefore, detailed elucidating mechanisms mediate gastric cancer progression and improving gastric cancer clinic strategies are helpful.

The complex interaction among different etiological factors leads to genetic and epigenetic alterations of proto-oncogenes and tumor-suppressor genes. Epigenetic regulation includes histone modification and DNA methylation, which involved in regulation of cell growth and development in mammals. Global DNA hypomethylation events were discovered in the human tumor in the early 1980s, and promoter hypermethylation of tumor suppressor genes were identified in cancer cells in mid 1990s.

Alteration of DNA methylation in the genome is found in almost types of cancer and can lead to change gene expression, such as over-expression of oncogenes and down-regulation of tumor suppressor genes during cancer progression. Promoter methylation is an alternative mechanism of gene silencing in human tumorigenesis. Although a number of methylated genes have been found in gastric cancer, useful methylation markers for early diagnosis and prognostic evaluation of cancer.

2. Clinical features, epidemiology, pathogenesis and progression of gastric cancer

2.1. Epidemiology

The incidence of stomach cancer is declining in most parts of the world, although it is ranked fourth after lung, breast, and colorectal cancer. A total of 989,600 new stomach cancer cases and 738,000 deaths are estimated to have occurred in 2008, accounting for 8% of total cancer cases and 10% of total cancer-related deaths [1]. The declining incidence is associated with factors related to the increased availability of refrigerated fresh foods and a decline in the consumption of those preserved using salt. The incidence rate varies substantially among countries. High incidence rates occur in East Asia, Eastern Europe, and South America. Regional variations reflect differences in dietary patterns (e.g., low intake of fruits and vegetables, and high intake of salt, nitrates, salt-cured fish, and smoked meat). Several other risk-implicated factors include *Helicobacter pylori* infection, hypochlorhydria, polyps, genetic alteration (e.g., type-A blood, pernicious anemia, *CDH1* mutation, familial gastric cancer, Li-Fraumeni syndrome, and *BRCA1* and *BRCA2*), previous radiation exposure, and prior gastrectomy.

2.2. Pathology

More than 95% of stomach cancers are adenocarcinomas. Other malignant tumors are rare and include carcinoid tumors, squamous cell carcinoma, adenoacanthoma, small cell carcinoma, mucinous carcinoma, and leiomyosarcoma. Although malignant lymphoma of the stomach is a relatively rare stomach neoplasm, it is the most common extranodal site for lymphomas of the gastrointestinal tract. It is potentially associated with *H. pylori* infection because the lymphoid tissue is often stimulated in response to colonization of the lining by *H. pylori* [2]. Furthermore, almost all patients with gastric MALT lymphoma exhibit signs of *H. pylori* infection.

2.3. Staging

There are currently 2 classification systems in use for staging stomach cancer. The Japanese classification is based on anatomic locations and the extent of the regional lymph [3]. The other staging system was developed by the International Union against Cancer and the American Joint Commission on Cancer. Tumor stage is determined based on tumor invasion depth, whereas nodal stage is determined by the number of positive lymph nodes [4]. Advances in diagnostic modalities such as endoscopic ultrasound, computed tomography (CT), positron emission tomography, magnetic resonance imaging (MRI) and laparoscopy have improved preoperative clinical staging. Classification provides useful information for tailoring initial treatment strategies.

2.4. Treatment

Surgery–Complete surgical resection is the primary treatment of early-stage stomach cancer. Gastrectomy and lymphadenopathy are the most widely used approaches, although superficial cancers can occasionally be treated by local endoscopical excision. Resection type (total

or subtotal gastrectomy) and the extent of lymphadenectomy depend on the extent, location, and stage of the disease.

Adjuvant treatment–Even patients who present the most favorable condition and undergo curative surgical resection frequently expire from disease recurrence. Adjuvant therapy is commonly conducted using chemotherapy, radiation therapy, or a combination of the two. A significant survival benefit of postoperative adjuvant combined modality therapy using radiotherapy and fluorouracil-based chemotherapy has been shown in several randomized trials [5-7].

Neoadjuvant treatment–Data from several uncontrolled series indicate that some patients with initially unresectable locally advanced disease may respond sufficiently to chemotherapy or chemoradiotherapy and are able to undergo potentially curative surgery. The benefits of preoperative therapy include an increased resectability rate, reduced rate of local and distant recurrences, and improved survival. However, this approach has not been widely adopted, primarily because of a lack of randomized trials that examine its advantages.

2.5. Prognosis

Gastric cancer (GC) is frequently diagnosed at an advanced stage. The prognosis of advanced cancer remains poor. Prognosis has improved only modestly during the previous two decades, attributable to advances in surgical treatment, postoperative care, and multimodal therapy. In the United States, the 5-year survival rate for all stages was 27% between 2001 and 2007, compared to 15% between 1975 and 1977 [8]. Local recurrence and distant metastases are the 2 primary areas of treatment failure in patients. After attempting curative resection, recurrence was local or regional in 40% of cases and distant in 60% [9].

Recent advances in genomic science have enabled the identification of detailed molecular mechanisms of stomach carcinogenesis and its progression. These techniques have been used to identify markers for early detection of stomach cancer. A better knowledge of the molecular bases will lead to new paradigms and potential therapeutic improvements. It can provide better information on potential tumor aggressiveness and assist in the personalization of treatment strategies for better outcomes.

3. Principle of DNA epigenetic modification, DNA methylation and detection

3.1. Genomic DNA methylation/demethylation

3.1.1. DNA methylation

Epigenetic regulation, including histone modification and DNA methylation, has a critical role in regulating cell growth and development in mammals [10, 11]. DNA methylation involves the regulation of gene expression by establishing and maintaining DNA methylation status at the promoter of critical genes. DNA methyltransferases (DNMTs) catalyze the covalent addition of methyl groups to 5-position of cytosine (5-methylcytosine; 5mC) bases in newly synthesized DNA (Fig. 1. Cytosine of CpG dinucleotides can be methylated by

DNMTs to form 5mC, which use S-adenosyl methionine as a donor for the methyl group. In mammalian cells, DNMTs genes are classified into de novo (DNMT3A and DNMT3B) and maintenance (DNMT1), and function in printing methylation genome maps [11]. DNMT1 is highly expressed in differentiated cells and efficiently hemi-methylated DNA during DNA replication. DNMT3A and DNMT3B are most abundant in embryonic stem cells and have low expressions in differentiated cells [12].
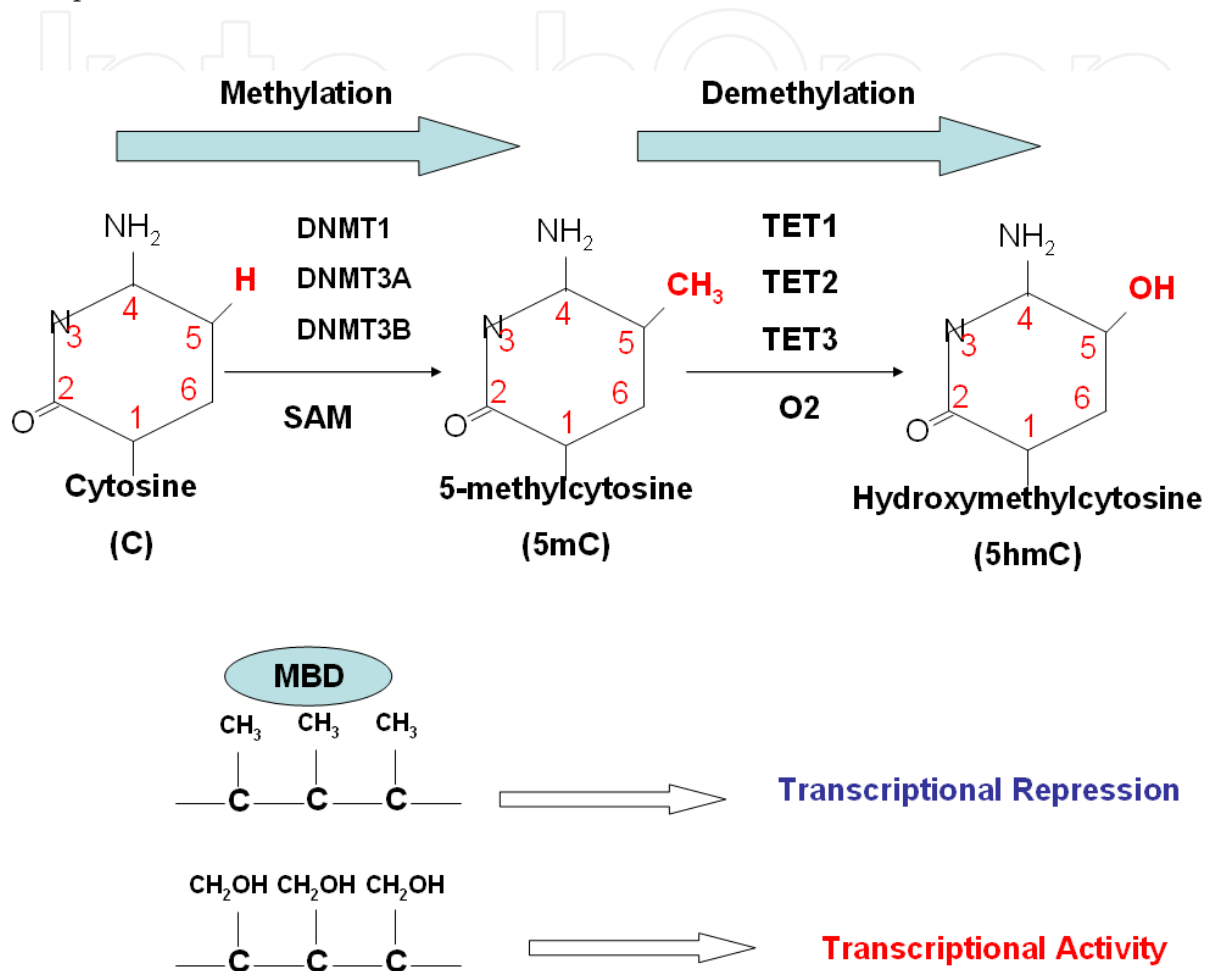

Figure 1. Schematic diagram depicting genomic DNA methylation and demethylation in cytosine

3.1.2. DNA demethylation

Tahiliani et al. [13] identified the leading enzyme (ten-eleven-translocation, TET) that can convert 5mC to 5-hydroxymethylcytosine (5hmC). Three TET proteins (TET1, TET2, and TET3) can convert 5mC to (5hmC), leading to DNA demethylation [14]. 5hmC is a potentially key intermediate in a possible active DNA demethylation process through DNA repair mechanisms. 5hmC is generated from oxidized 5mC, and has a critical role in stem/progenitor cell differentiation [11, 15-23]. The role of 5hmC in gene regulation is a crucial issue that is potentially associated with gastric cancer progression; however, its biological function in gastric cancer is unknown.

3.2. DNA methylation-regulated genes in gastric cancer

3.2.1. Protein coding genes

Global DNA hypomethylation events that occur primarily at DNA-repetitive regions and hypermethylation at specific promoter CpG islands of tumor suppressor genes are frequently observed in human tumors [10]. In gastric cancer, DNA methylation contributes to cancer progression and leads to aberrantly silencing expression of tumor suppressor genes, or oncogene reactivation [24]. Park et al.[25] profiled a global DNA methylation of gastric cancer using a methylated DNA enrichment technique and performed an analysis using a next-generation sequence approach. Gastric cancer was associated with hypermethylation of 5' CpG islands and the 5'-end of protein-coding genes, as well as hypomethylation of DNA-repetitive elements. During recent decades, a gain or loss of DNA methylation at the promoter of protein-coding gene events has been continuously studied. Numerous studies have implicated an aberrant expression of methylation-associated genes involved in the pathogenesis of gastric cancer (Table 1). E (epithelial)-cadherin gene promoter hypermethylation has frequently been observed in human gastric cancers, and methylation status has been associated with deceased expression in gastric carcinogenesis [26]. Sudo et al. also reported that promoter methylation-mediated silencing of the E-cadherin gene was closely associated with the development of Epstein-Barr virus-associated gastric carcinoma [27]. Similarly, several studies have shown that the accumulation of DNA methylation in promoter regions of tumor suppressor genes may alter cell cycle, growth, and motility, as well as adhesion molecules by silencing critical gene expression (including p16, p15, DAPK, RUNX3, MLH1, Table 1). In contrast to tumor suppressor genes, loss of DNA methylation has frequently occurred in oncogene promoter regions and leads to aberrant overexpression in gastric cancer, such as S100A6, S100A4, VEGF-C, PAR2, SNCG, and MAGE-A1-3 (Table 1).

	Gene name	Ref.
Protein-coding genes	CXCL12, CDH1, ZNF331, EDNRB, SOX9, PTPN6, MOS, DCC, CRK, VAV1, MLF1, MGMT, p16, RASSF2, hMLH1, HAND1, HRASLS, TM, FLNc, ALX4, TMEFF2, CHCHD10, IGFBP3, NPR1, GKN1, RASAL1, PAX5, SFRP1, GPX3, ADAMTS9, S100A6, EphA1, p14, DAPK, WWOX, TCF4, RUNX3, CHFR, RECK, BMP3, HACE1, PGP9.5, APC, VIM, MGMT, PTCH1a, RASSF2A, S100A4, PKD1, TMS1, RUNX3, ER, p15, EphA7, NID1, NID2, HHIP, VEGF-C, FHIT, MTAP, PLAGL1, PAR2, DFNA5, RASSF1A, CTNNB1, MTSS1, LIMS2, SNCG, MAGE-A1, MAGE-A2, MAGE-A3, CASP1, COX-2, Syk, ITGA1, SOCS-1, SERPINB5, PTEN	[40, 45-47, 51, 54, 57, 60, 61, 65, 75-112]
Small nonprotein-coding genes	miR-1,miR-9, miR-10b, miR-18b, miR-34b/c, miR-124a, miR-129, miR-137, miR-148a, miR-152, miR-155, miR-181c, miR-196b, miR-203, miR212, miR512, miR-516a	[29, 34, 35, 38, 39, 113-123]

The underline indicates that genes overexpressed with promoter hypomethylation in gastric cancer.

Table 1. Genes aberrantly expressed with hypo/hypermethylated promoter in gastric cancer.

3.2.2. microRNA

MicroRNAs (miRNAs) are endogenous non-protein-coding RNAs of short 21-23 nucleotides [28]. Abnormal miRNA expression has a critical role in gastric cancer progression. However, miRNA transcription mechanisms are similar to classic protein-coding genes; the hypermethylated promoter region of tumor-suppressive miRNAs may result in gastric cancer formation and progression. Our previous studies identified several methylation-associated miRNAs through AGS treated with a demethylation agent [29, 30]. Among these miRNAs, we first observed a primate-specific miRNA cluster (C19MC) comprising 46 pre-miRNAs, which could be co-regulated depending on the methylation status of its distal CpG-rich domain in placenta tissue [30, 31]. C19MC expression has been shown to display a maternal-specific methylation imprint acquired in oocytes [31]. We also recently identified several tumor-suppressive miRNA that were regulated with aberrant DNA methylation in gastric cancer (Figure 2). Expression of miR-1, miR-9, miR-129, and miR-34b/c was suppressed by DNA hypermethylation, and miR-196b was overexpressed with hypomethylation in gastric cancer [29, 32-35]. Numerous other studies have shown that several tumor-suppressive miRNAs contain the aberrant hypermethylation of their promoter regions in gastric cancer, including miR-9, miR-34b/c, miR-129, miR-137, miR-181c, miR-199a, miR-212, miR-512, and miR-516 [29, 30, 34-40].
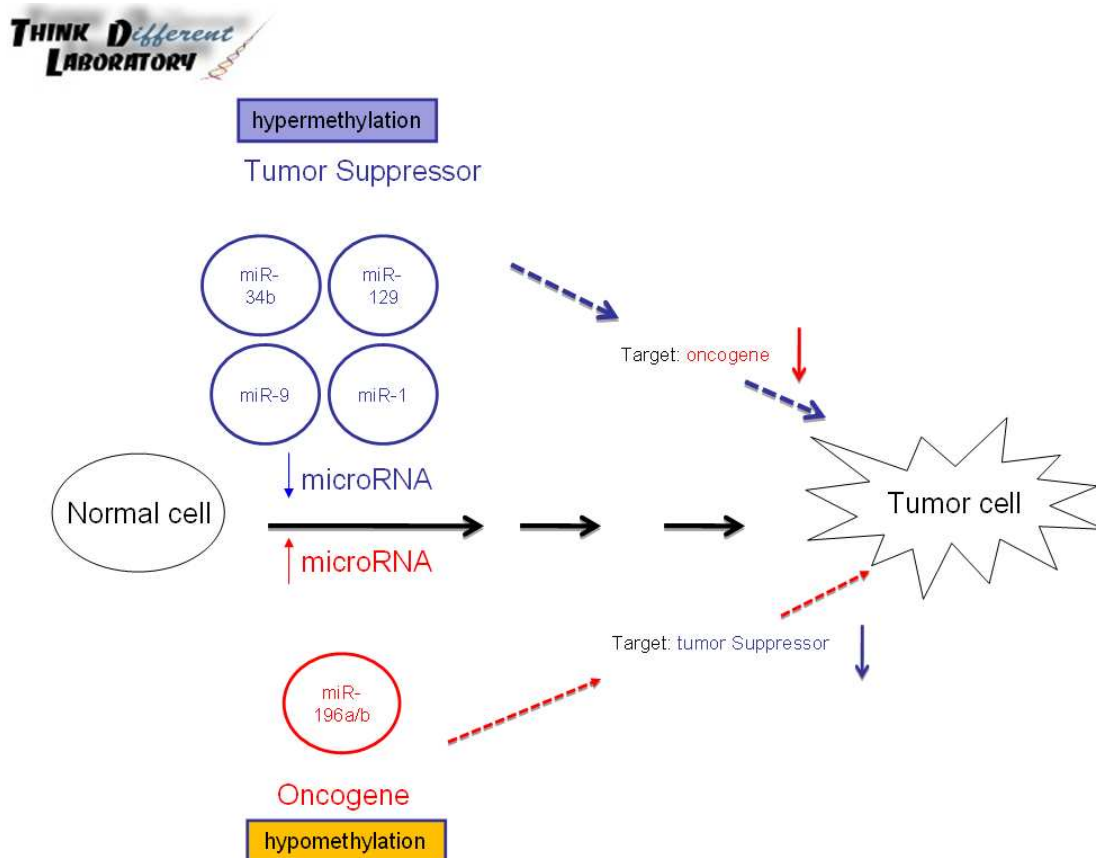

Figure 2. Schematic diagram depicting DNA hypo-/hypermethylation resulted miRNAs dysregulation in gastric cancer according our recent studies.

4. Promoter methylation of given genes versus clinical significance and prognostic values and therapeutic applications

Promoter methylation is an alternative mechanism of gene silencing in human tumorigenesis. Although a number of methylated genes have been observed in gastric cancer, useful methylation markers for early diagnosis and prognostic evaluation of gastric cancer remain unknown [41, 42]. Although the clinical outcome of gastric cancer has gradually improved, the prognosis of patients at the advanced stage remains poor. The prognosis varies widely in gastric cancer patients for undetermined biologic reasons. Thus, a greater understanding of the pathogenesis and molecular mechanisms of gastric cancer may lead to novel diagnostic, therapeutic, and preventive strategies [41, 43]. Gastric carcinogenesis is a multistep process that includes numerous genetic and epigenetic alterations, such as activation of oncogenes, overexpression of growth factors and receptors, and inactivation of tumor suppressor genes. In addition to genetic alterations, epigenetic alterations such as DNA methylation of CpG islands are involved in cancer development and progression. Promoter methylation is regarded as one of the primary mechanisms to inactivate tumor-related genes, along with gene mutation and deletions, ultimately leading to carcinogenesis. Promoter methylation is a critical hallmark of cancer cells, and has a significant role in tumor transformation and progression, impacting the clinical course of the disease. Although promoter methylation of a number of cancer-related genes, including tumor suppressor genes, has been observed in gastric cancer and precancerous lesions, epigenetic inactivation of genes related to tumor initiation and progression has not been well studied in gastric cancer outcome [41].

4.1. Gene methylation and its impact on clinical outcome in gastric cancer

Using methylation-specific polymerase chain reactions (MSP) and quantitative methylation-specific polymerase chain reactions (Q-MSP), the promoter methylation of specific genes is examined, as well as their association with clinical outcomes of gastric cancer. Inactivation of tumor suppressor genes and activation of oncogenes caused by genetic and epigenetic alterations are known to play a significant role in carcinogenesis. An increasing amount of evidence shows that epigenetic silencing of the tumor suppressor genes, particularly caused by hypermethylation of CpG islands in promoters, is critical to carcinogenesis and metastasis. Here, we detail recent progress in the study of methylations of tumor suppressor genes involved in the pathogenesis of gastric cancer.

CDH1 E-cadherin is a cell adhesion molecule considered a potential invasion/metastasis suppressor and is mutationally inactivated in almost half of all undifferentiated-scattered (diffuse-type) gastric carcinomas. In addition, silencing of E-cadherin by CpG methylation within its promoter region has been reported in several gastric carcinoma cell lines. Hypermethylation of the E-cadherin promoter was evident in 30%-55% of primary gastric carcinomas [26, 44-47] and occurred more frequently in carcinomas of the undifferentiated-scattered type (in 15 of 18, 83%) than in other histologic subtypes (34%), and it was present at similar rates in early (60%) versus advanced (49%) carcinomas [26]. E-cadherin methyla-

tion was present in 31% of gastric mucosae from dyspeptic patients, and was associated with *H. pylori* infection, although this is independent of the age of the patient or presence or absence of gastritis. E-cadherin methylation was present in 0% of normal mucosa, 57% of intestinal metaplasias, and 58% of primary and 65% of metastatic cancers. E-cadherin methylation status was concordant in 92% of intestinal metaplasias and primary cancers, and in 85% of primary and metastatic cancers from the same resected specimen. E-cadherin methylation in gastric cancer was associated with depth of tumor invasion and regional nodal metastasis [48]. By examining the relationship between molecular changes in E-cadherin and metastasis in early gastric carcinoma (EGC), Yi Kim et al. showed that 45.0% of 60 primary EGCs exhibited methylation in the CpG island of E-cadherin. Abnormal expression of E-cadherin was significantly correlated with patient age, tumor size, Lauren classification, differentiation, and lymph node metastasis [49]. Therefore, the E-cadherin promoter frequently undergoes hypermethylation in human gastric cancers, particularly those of the undifferentiated-scattered histologic subtype. E-cadherin promoter hypermethylation is associated with decreased expression and may occur during early stages of gastric cancer. Inactivation of E-cadherin might be involved in metastasis in EGC and play an important role in microscopic differentiation.

DAPK Death-associated protein kinase (DAP-kinase) is a serine/threonine kinase and a positive mediator of apoptosis. Downregulation of DAP-kinase is associated with an increased metastatic potential of tumors. Gene promoter hypermethylation could lead to downregulation of DAP-kinase. Methylation status was assessed by MSP. In total, 69.2% of GC demonstrated promoter methylation of DAP-kinase. Methylation of DAP-kinase was observed in intestinal, diffuse, and mixed types of GC. It also occurred in similar frequencies among antral, body, and cardiac gastric cancer. No association between methylation status and age or sex was demonstrated. However, the methylated cases were correlated with the presence of nodal metastasis, advance stage of disease, and a poorer event-free survival. DAP-kinase promoter methylation as a potential prognostic marker for gastric cancer patients deserved further evaluation [50]. Aberrant methylation of DAPK genes was detected in 22% of tumors. Kato et al. examined 43 patients treated by 5-fluorouracil-based chemotherapy, who had distant metastasis or recurrence after radical resection, to determine the relation between chemosensitivity and methylation. The response rate was lower in patients with either DAPK methylation than without (21% vs. 45%). Overall survival tended to be shorter in patients with both methylations compared with either or no methylation. The time to progression of patients with methylation of DAPK was significantly shorter than of patients without methylation. In conclusion, DAPK methylation might predict the prognosis and response to chemotherapy in gastric cancer [51].

CHFR Checkpoint with fork head-associated and ring finger (CHFR) governs the transition from prophase to prometaphase in response to mitotic stress. MSP and combined bisulfite restriction analysis (COBRA) are both used in detecting aberrant methylation of the CHFR gene in gastric cancer. The methylation rates of the CHFR gene promoter were significantly higher in gastric cancer samples than in the corresponding para cancer normal gastric mucosa by MSP (52% vs. 19%). However, there was no significant correlation between methyla-

tion status of the CHFR gene and the clinicopathologic parameters of gastric cancer, including age, sex, tumor size, clinical stage, Borrmann type, tumor invasion depth, differentiation, and lymph node metastasis. Aberrant methylation of the CHFR gene was detected in 42% of gastric cancer specimens using COBRA and MSP. Therefore, aberrant methylation of the CHFR gene is a frequent event in the carcinogenesis of gastric cancer. Detecting the methylation of the CHFR gene in gastric mucosa may be conducive to the diagnosis of gastric cancer [52, 53]. However, the frequency of DAPK and CHFR methylation in cancer tissues was significantly associated with the extent of differentiation and lymph node metastasis. DAPK and CHFR promoter hypermethylation may be critical in evaluating the differentiation grade and lymph node status of gastric cancer. Weak gene expression and loss of gene expression caused by promoter hypermethylation may be a cancer-specific event [54, 55].

RUNX3 Runt-related transcription factor 3 (RUNX3) is a novel tumor suppressor gene that is frequently silenced by promoter hypermethylation in gastric cancer. Sakakura et al. observed significant downregulation of RUNX3 through methylation on the promoter region in primary tumors (75%), as well as in all clinical peritoneal metastases of gastric cancers (100%), compared with normal gastric mucosa. Stable transfection of RUNX3 inhibited cell proliferation slightly, and modest transforming growth factor-beta (TGF-beta)-induced anti-proliferative and apoptotic effects were observed. RUNX3 significantly inhibited peritoneal metastases of gastric cancers in animals. Microarray analysis identified approximately 28 candidate genes under the possible downstream control of RUNX3, some of which were considered to be potentially involved in peritoneal metastases, which were related to signal transduction, apoptosis, immune responses, and cell adhesion. Some of the genes are involved in the TGF-beta signaling pathway. These results indicate that silencing of RUNX3 affects the expression of important genes involved in aspects of metastasis, including cell adhesion, proliferation, apoptosis, and promoting the peritoneal metastasis of gastric cancer. Identification of such genes could indicate novel therapeutic modalities and therapeutic targets [56]. In other studies, overall, 55% of GC demonstrated methylation of the RUNX3 promoter; 82% of GC was classified as stable microsatellite instability, 5% as low-level microsatellite instability and 13% as high-level microsatellite instability (MSI-H); and mitochondrial microsatellite instability (mtMSI) was detected in 11% of GC. A significant association was found between mtMSI and tumor-node-metastasis staging. Furthermore, an interesting association among the MSI-H status, mtMSI, and RUNX3 methylation. These data suggest that RUNX3 is an important target of methylation in the evolution of mtMSI and nuclear microsatellite instability (nMSI-H) [57].

p16 The INK4a/ARF locus encodes 2 cell cycle-regulatory proteins: p16INK4a and p14ARF. Silencing of p16INK4a and p14ARF expressions by aberrant methylation of the CpG islands in the promoter regions has recently been observed to be an alternative mechanism that inactivates possible tumor suppressor functions in various tumors. Of 10 cell lines studied, silencing of the expression of p16INK4a and p14ARF caused by the detection of promoter methylation by MSP and RT-PCR in 6 (60%) and 2 (20%) cell lines, respectively. p14ARF silencing was detected only in cell lines derived from gastric cancer of

the diffuse type, whereas p16INK4a silencing was found in cell lines derived from both diffuse and intestinal types. In primary gastric cancers, promoter methylation of p16INK4a and p14ARF was found in 17% and 24% of the tumors independently. Where-as p14ARF methylation was observed more frequently in intestinal type cancers in an early stage and in diffuse type cancers in an advanced stage, MSI tended to be related especially to p14ARF methylation in cancers of the intestinal type. Thus, the significance of p14ARF methylation differed between intestinal and diffuse types, and such a difference was not observed in p16INK4a methylation [58]. Aberrant p16 methylation was observed in 38% of primary gastric cancers, but in none of the corresponding gastric mucosae [59]. When carcinoma specimens were compared with adjacent normal gastric mucosa samples, a significant increase in promoter methylation of p16, Runx3, DAPK, and CHFR was observed, whereas all 30 histologically normal gastric specimens were methylation-free for all 4 genes. The methylation rate of the 4 genes increased from normal stomach tissue to tumor-adjacent gastric mucosa to gastric cancer tissue [54].

4.2. Hypermethylation profiling

DNA methylation has been studied extensively in gastric cancer. However, most studies have focused on aberrant methylation in a single gene. Because methylated genes rarely occur more frequently in groups than in isolation, the concept of a CpG island methylator phenotype (CIMP) in gastric. CIMP has been defined as a subset of malignancies that show widespread hypermethylation of multiple promoter CpG island loci [60].

More recently, microarray technology has made it possible to comprehensively analyze gene expression profiles [56, 61-64]. Representational difference analysis (RDA) is also used to screen differentially methylated DNA sequences between gastric primary tumor and metastatic lymph nodes [65, 66]. By using these techniques, the expression levels of thousands of genes can be analyzed in a single experiment. These technologies are a powerful tool for analyzing gene expression profiles related to the development and progression of specific diseases. Although there have been significant improvements in the analysis of genetic alterations for gastric cancer, there is insufficient information on understanding a common pathway for the development and progression of gastric cancer. Gastric cancer has diverse clinical properties such as histological type, metastatic status, race, and sex. Thus, further exploration to search for genetic alterations in gastric cancer is required.

5. Cirulating DNA methylation as biomarkers

Previous studies have demonstrated that tumor cells can release DNA to peripheral blood and enriched circulating DNA level can be observed in the serum of cancer patients, several times higher than the reference range. Previous studies have detected methylated DNA of multiple gene promoters in blood plasma, urine, sputum and peritoneal washes in several different cancers, and high-frequency hypermethylation of tumor suppression is mostly cancer-specific; therefore, it may be used as a molecular diagnostic marker of cancer [67-74].

Numerous studies have attempted to detect circulating methylated DNA from body fluid as a good biomarker for prognosis and diagnosis of gastric carcinoma (Table 2). Detection of promoter regions hypermethylation of candidate genes FAM5C, MYLK, RUNX3, TFP12, RASSF1A, p16 and CDH1 in the serum have been applied to predict the clinical features of gastric cancer patients. Furthermore, DNA methylation of BNIP3, CHFR, CYP1B1, MINT25, SFRP2, RASSF2, p16, RUNX3, CDH1, hMLH1, ABCG2, BNIP3, and RECK in peritoneal fluid form gastric cancer patients has been analyzed using quantitative methylation-specific polymerase chain reaction and as a good biomarker for the diagnosis and detection of gastric cancer. Thus, circulating methylated DNA can reflect the real methylation status of candidate gene promoters in gastric cancer tissue by examining body fluid. Therefore, releasing methylated DNA fragments has a high potential as a novel biomarker for the detection and recurrence monitoring of gastric cancer.

Body fluid	Gene name	Ref.
serum	FAM5C, MYLK, RUNX3, TFP12, RASSF1A, p16, CDH1, DAPK, GSTP1, p15	[120, 124-127]
Peritoneal fluid	BNIP3, CHFR, CYP1B1, MINT25, SFRP2, RASSF2, p16, RUNX3, CDH1, hMLH1, ABCG2, BNIP3, RECK	[55, 120, 128, 129]

Table 2. The aberrant DNA methylation of gene promoter in body fluid is a promising biomarker for gastric cancer

6. Conclusion

Gastric cancer is one of the leading causes of cancer-related death in China. Although the molecular mechanisms of gastric carcinogenesis are unclear, epigenetic silencing of tumor-related genes by promoter hypermethylation has recently emerged as a crucial mechanism of tumorigenesis. The promoter hypermethylation profile differs among cancer types and within each gene, providing tumor type- and gene-specific hypermethylation profiles that may be involved in the corresponding molecular mechanism of tumorigenesis. The identification of a novel gene targeted by promoter hypermethylation may provide insights into mechanisms for the inactivation of tumor-suppressive pathways and is critical for the identification of tumor markers in gastric cancer [42, 43]. Currently, DNA methylation markers have been used in early detection, prognosis, and prediction of response to cancer therapy.

Acknowledgements

[12] Goll, M.G. and T.H. Bestor, Eukaryotic cytosine methyltransferases. Annu Rev Biochem, (2005). , 481-514.

[13] Tahiliani, M., et al., Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science(2009). , 930-935.

[14] Cliffe, L.J., et al., JBP1 and JBP2 are two distinct thymidine hydroxylases involved in J biosynthesis in genomic DNA of African trypanosomes. Nucleic Acids Res, 2009. 37(5): p. 1452-62.

[15] Cimmino, L., et al., TET family proteins and their role in stem cell differentiation and transformation. Cell Stem Cell(2011). , 193-204.

[16] Ficz, G., et al., Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature(2011). , 398-402.

[17] Gu, T.P., et al., The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature(2011). , 606-610.

[18] Guo, J.U., et al., Emerging roles of TET proteins and 5-hydroxymethylcytosines in active DNA demethylation and beyond. Cell Cycle(2011). , 2662-2668.

[19] Zhao, J.J., et al., Identification of LZAP as a new candidate tumor suppressor in hepatocellular carcinoma. PLoS One, (2011). , e26608.

[20] Ito, S., et al., Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature(2011). , 1129-1133.

[21] Ito, S., et al., Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science(2011). , 1300-1303.

[22] Jin, S.G., et al., Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Res, (2011). , 5015-5024.

[23] Pastor, W.A., et al., Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. Nature(2011). , 394-397.

[24] Gigek, C.O., et al., Epigenetic mechanisms in gastric cancer. Epigenomics, 2012. 4(3): p. 279-94.

[25] Park, J.H., et al., Identification of DNA methylation changes associated with human gastric cancer. BMC Med Genomics, (2011). , 82.

[26] Tamura, G., et al., E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst, (2000). , 569-573.

[27] Sudo, M., et al., Promoter hypermethylation of E-cadherin and its abnormal expression in Epstein-Barr virus-associated gastric carcinoma. Int J Cancer, (2004). , 194-199.

[28] Kim, V.N., Small RNAs: classification, biogenesis, and function. Mol Cells, 2005. 19(1): p. 1-15.

[29] Tsai, K.W., et al., Epigenetic regulation of miR-196b expression in gastric cancer. Genes Chromosomes Cancer, (2010). , 49(11):969-980.

[30] Tsai, K.W., et al., Epigenetic control of the expression of a primate-specific micro-RNA cluster in human cancer cells. Epigenetics (2009). , 4(8):587-592.

[31] Noguer-Dance, M., et al., The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum Mol Genet, (2010). , 3566-3582.

[32] Chen, W.S., et al., Silencing of miR-1-1 and miR-133a-2 cluster expression by DNA hypermethylation in colorectal cancer. Oncol Rep, (2012). , 1069-1076.

[33] Liao, Y.L., et al., Transcriptional regulation of miR-196b by ETS2 in gastric cancer cells. Carcinogenesis(2012). , 760-769.

[34] Tsai, K.W., et al., Aberrant hypermethylation of miR-9 genes in gastric cancer. Epigenetics, (2011). 6(10):1189-97

[35] Tsai, K.W., et al., Epigenetic regulation of miR-34b and miR-129 expression in gastric cancer. Int J Cancer, (2011). , 2600-2610.

[36] Cheung, H.H., et al., Methylation of an intronic region regulates miR-199a in testicular tumor malignancy. Oncogene(2011).

[37] Saito, Y., et al., Chromatin remodeling at Alu repeats by epigenetic treatment activates silenced microRNA-512-5p with downregulation of Mcl-1 in human gastric cancer cells. Oncogene(2009). , 2738-2744.

[38] Shen, R., et al., Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 in gastric cancer. Biochem Biophys Res Commun, (2010). , 1047-1052.

[39] Suzuki, H., et al., (2010). Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. Carcinogenesis, 2066-2073.

[40] Chen, X.R., et al., Role of BMP3 in progression of gastric carcinoma in Chinese people. World J Gastroenterol, (2010). , 1409-1413.

[41] Yao, D., et al., Quantitative assessment of gene methylation and their impact on clinical outcome in gastric cancer. Clin Chim Acta, (2012). , 787-794.

[42] Lee, J.H., et al., Frequent CpG island methylation in precursor lesions and early gastric adenocarcinomas. Oncogene(2004). , 4646-4654.

[43] Kang, G.H., et al., Profile of aberrant CpG island methylation along multistep gastric carcinogenesis. Lab Invest, (2003). , 519-526.

[44] Corn, P.G., et al., Frequent hypermethylation of the 5' CpG island of E-cadherin in esophageal adenocarcinoma. Clin Cancer Res, 2001. 7(9): p. 2765-9.

[45] Du, P., et al., Methylation of PTCH1a gene in a subset of gastric cancers. World J Gastroenterol, (2009). , 3799-3806.

[46] Tahara, T., et al., CpG island promoter methylation (CIHM) status of tumor suppressor genes correlates with morphological appearances of gastric cancer. Anticancer Res, (2010). , 239-244.

[47] Tahara, T., et al., Chronic aspirin use suppresses CDH1 methylation in human gastric mucosa. Dig Dis Sci, (2010). , 54-59.

[48] Chan, A.O., et al., Promoter methylation of E-cadherin gene in gastric mucosa associated with Helicobacter pylori infection and in gastric cancer. Gut, (2003). , 502-506.

[49] Yi Kim, D., et al., E-cadherin expression in early gastric carcinoma and correlation with lymph node metastasis. J Surg Oncol, (2007). , 429-435.

[50] Chan, A.W., et al., Promoter hypermethylation of Death-associated protein-kinase gene associated with advance stage gastric cancer. Oncol Rep, (2005). , 937-941.

[51] Kato, K., et al., Methylated TMS1 and DAPK genes predict prognosis and response to chemotherapy in gastric cancer. Int J Cancer, (2008). , 603-608.

[52] Cheng, Z.D., et al., Promoter methylation of CHFR gene in gastric carcinoma tissues detected using two methods. Chin J Cancer, (2010). , 163-166.

[53] Hiraki, M., et al., Aberrant gene methylation in the lymph nodes provides a possible marker for diagnosing micrometastasis in gastric cancer. Ann Surg Oncol, (2010). , 1177-1186.

[54] Hu, S.L., et al., Promoter methylation of p16, Runx3, DAPK and CHFR genes is frequent in gastric carcinoma. Tumori, (2010). , 726-733.

[55] Hiraki, M., et al., Aberrant gene methylation in the peritoneal fluid is a risk factor predicting peritoneal recurrence in gastric cancer. World J Gastroenterol, (2010). , 330-338.

[56] Sakakura, C., et al., Possible involvement of RUNX3 silencing in the peritoneal metastases of gastric cancers. Clin Cancer Res, (2005). , 6479-6488.

[57] Gargano, G., et al., Aberrant methylation within RUNX3 CpG island associated with the nuclear and mitochondrial microsatellite instability in sporadic gastric cancers. Results of a GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study. Ann Oncol, (2007). Suppl 6: , vi103-vi109.

[58] Tsujimoto, H., et al., Promoter methylations of p16INK4a and p14ARF genes in early and advanced gastric cancer. Correlations of the modes of their occurrence with histologic type. Pathol Res Pract, (2002). , 785-794.

[59] Kanyama, Y., et al., Detection of p16 promoter hypermethylation in serum of gastric cancer patients. Cancer Sci, (2003). , 418-420.

[60] Chen, H.Y., et al., High CpG island methylator phenotype is associated with lymph node metastasis and prognosis in gastric cancer. Cancer Sci, (2012). , 73-79.

[61] Zhang, X., et al., An 8-gene signature, including methylated and down-regulated glutathione peroxidase 3, of gastric cancer. Int J Oncol, 2010. 36(2): p. 405-14.

[62] Chen, J., et al., Microarray analysis of gene expression in metastatic gastric cancer cells after incubation with the methylation inhibitor 5-aza-2'-deoxycytidine. Clin Exp Metastasis, (2004). , 389-397.

[63] Yamashita, S., et al., Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2'-deoxycytidine treatment and oligonucleotide microarray. Cancer Sci, (2006). , 64-71.

[64] Terashima, M., et al., Gene expression profiles in human gastric cancer: expression of maspin correlates with lymph node metastasis. Br J Cancer, (2005). , 1130-1136.

[65] Wang, J.F. and D.Q. Dai, Metastatic suppressor genes inactivated by aberrant methylation in gastric cancer. World J Gastroenterol, (2007). , 5692-5698.

[66] Wang, J.F. and D.Q. Dai, [Difference in methylation of genomic DNA between gastric primary cancer and lymph nodes with metastatic gastric cancer]. Zhonghua Yi Xue Za Zhi(2006). , 536-539.

[67] Balch, C., et al., Minireview: epigenetic changes in ovarian cancer. Endocrinology, 2009. 150(9): p. 4003-11.

[68] Bremnes, R.M., R. Sirera, and C. Camps, Circulating tumour-derived DNA and RNA markers in blood: a tool for early detection, diagnostics, and follow-up? Lung Cancer, 2005. 49(1): p. 1-12.

[69] Fleischhacker, M. and B. Schmidt, Circulating nucleic acids (CNAs) and cancer--a survey. Biochim Biophys Acta, 2007. 1775(1): p. 181-232.

[70] Giasuddin, A.S., K.A. Jhuma, and A.M. Haq, Applications of free circulating nucleic acids in clinical medicine: recent advances. Bangladesh Med Res Counc Bull, (2008). , 26-32.

[71] Patel, A., J.D. Groopman, and A. Umar, DNA methylation as a cancer-specific biomarker: from molecules to populations. Ann N Y Acad Sci, 2003. 983: p. 286-97.

[72] Vlassov, V.V., P.P. Laktionov, and E.Y. Rykova, Circulating nucleic acids as a potential source for cancer biomarkers. Curr Mol Med, (2010). , 142-165.

[73] Xue, X., Y.M. Zhu, and P.J. Woll, Circulating DNA and lung cancer. Ann N Y Acad Sci, 2006. 1075: p. 154-64.

[74] Ziegler, A., U. Zangemeister-Wittke, and R.A. Stahel, Circulating DNA: a new diagnostic gold mine? Cancer Treat Rev, 2002. 28(5): p. 255-71.

[75] Tao, K., et al., Quantitative analysis of promoter methylation of the EDNRB gene in gastric cancer. Med Oncol, (2012). , 107-112.

[76] Shi, J., et al., Prognostic significance of aberrant gene methylation in gastric cancer. Am J Cancer Res, (2012). , 116-129.

[77] Yoon, J.H., et al., Inactivation of the Gastrokine 1 gene in gastric adenomas and carcinomas. J Pathol, (2011). , 618-625.

[78] Seto, M., et al., Reduced expression of RAS protein activator like-1 in gastric cancer. Int J Cancer, (2011). , 1293-1302.

[79] Li, X., et al., Epigenetic inactivation of paired box gene 5, a novel tumor suppressor gene, through direct upregulation of p53 is associated with prognosis in gastric cancer patients. Oncogene, 2012. 31(29): p. 3419-30.

[80] Kinoshita, T., et al., Decreased expression and aberrant hypermethylation of the SFRP genes in human gastric cancer. Hepatogastroenterology, (2011). , 1051-1056.

[81] Zhang, C., et al., High-resolution melting analysis of ADAMTS9 methylation levels in gastric, colorectal, and pancreatic cancers. Cancer Genet Cytogenet, (2010). , 38-44.

[82] Wang, X.H., et al., S100A6 overexpression is associated with poor prognosis and is epigenetically up-regulated in gastric cancer. Am J Pathol, (2010). , 586-597.

[83] Wang, J., et al., Expression of EphA1 in gastric carcinomas is associated with metastasis and survival. Oncol Rep, (2010). , 1577-1584.

[84] Maeda, N., et al., Loss of WW domain-containing oxidoreductase expression in the progression and development of gastric carcinoma: clinical and histopathologic correlations. Virchows Arch, (2010). , 423-432.

[85] Joo, J.K., et al., CpG methylation of transcription factor 4 in gastric carcinoma. Ann Surg Oncol, (2010). , 3344-3353.

[86] Goto, T., et al., Methylation of the p16 gene is frequently detected in lymphatic-invasive gastric cancer. Anticancer Res, (2010). , 2701-2703.

[87] Du, Y.Y., D.Q. Dai, and Z. Yang, Role of RECK methylation in gastric cancer and its clinical significance. World J Gastroenterol, 2010. 16(7): p. 904-8.

[88] Sakata, M., et al., (2009). Methylation of HACE1 in gastric carcinoma. Anticancer Res, . 29(6): p. ., 2231-3.

[89] Mizukami, H., et al., PGP9.5 was less frequently methylated in advanced gastric carcinoma. Hepatogastroenterology, (2009). , 1576-1579.

[90] Kitamura, Y.H., et al., (2009). Frequent methylation of Vimentin in well-differentiated gastric carcinoma. Anticancer Res, . 29(6): , 2227-2229.

[91] Hibi, K., et al., Methylation of the MGMT gene is frequently detected in advanced gastric carcinoma. Anticancer Res, (2009). , 5053-5055.

[92] Wang, Y.C., et al., Detection of RASSF1A promoter hypermethylation in serum from gastric and colorectal adenocarcinoma patients. World J Gastroenterol, (2008). , 3074-3080.

[93] Li, Y., et al., Frequent S100A4 Expression with Unique Splicing Pattern in Gastric Cancers: A Hypomethylation Event Paralleled with E-cadherin Reduction and Wnt Activation. Transl Oncol, 2008. 1(4): p. 165-76.

[94] Kim, M., et al., Epigenetic inactivation of protein kinase D1 in gastric cancer and its role in gastric cancer cell migration and invasion. Carcinogenesis(2008). , 629-637.

[95] Zhao, C.H., X.M. Bu, and N. Zhang, Hypermethylation and aberrant expression of Wnt antagonist secreted frizzled-related protein 1 in gastric cancer. World J Gastroenterol, 2007. 13(15): p. 2214-7.

[96] Wang, J., et al., Differential expression of EphA7 receptor tyrosine kinase in gastric carcinoma. Hum Pathol, (2007). , 1649-1656.

[97] Ulazzi, L., et al., Nidogen 1 and 2 gene promoters are aberrantly methylated in human gastrointestinal cancer. Mol Cancer, (2007). , 17.

[98] Taniguchi, H., et al., Transcriptional silencing of hedgehog-interacting protein by CpG hypermethylation and chromatic structure in human gastrointestinal cancer. J Pathol, (2007). , 131-139.

[99] Matsumura, S., et al., DNA demethylation of vascular endothelial growth factor-C is associated with gene expression and its possible involvement of lymphangiogenesis in gastric cancer. Int J Cancer, (2007). , 1689-1695.

[100] Leal, M.F., et al., Promoter hypermethylation of CDH1, FHIT, MTAP and PLAGL1 in gastric adenocarcinoma in individuals from Northern Brazil. World J Gastroenterol, (2007). , 2568-2574.

[101] Arisawa, T., et al., Promoter hypomethylation of protease-activated receptor 2 associated with carcinogenesis in the stomach.J Gastroenterol Hepatol, (2007). , 943-948.

[102] Akino, K., et al., Identification of DFNA5 as a target of epigenetic inactivation in gastric cancer.Cancer Sci, (2007). , 88-95.

[103] Kim, S.K., et al., The epigenetic silencing of LIMS2 in gastric cancer and its inhibitory effect on cell migration.Biochem Biophys Res Commun, (2006). , 1032-1040.

[104] Chang, M.S., et al., CpG island methylation status in gastric carcinoma with and without infection of Epstein-Barr virus.Clin Cancer Res, (2006). , 2995-3002.

[105] Liu, H., et al., Loss of epigenetic control of synuclein-gamma gene as a molecular indicator of metastasis in a wide range of human cancers.Cancer Res, (2005). , 7635-7643.

[106] Jung, E.J., et al., Expression of family A melanoma antigen in human gastric carcinoma. Anticancer Res, 2005. 25(3B): p. 2105-11.

[107] Jee, C.D., et al., Loss of caspase-1 gene expression in human gastric carcinomas and cell lines.Int J Oncol, (2005). , 1265-1271.

[108] Wang, S., et al., (2004). Hypermethylation of Syk gene in promoter region associated with oncogenesis and metastasis of gastric carcinoma.World J Gastroenterol, 10(12): , 1815-1818.

[109] Park, J., et al., Aberrant methylation of integrin alpha4 gene in human gastric cancer cells.Oncogene(2004). , 3474-3480.

[110] Oshimo, Y., et al., Epigenetic inactivation of SOCS-1 by CpG island hypermethylation in human gastric carcinoma.Int J Cancer, (2004). , 1003-1009.

[111] Ito, R., et al., Loss of maspin expression is associated with development and progression of gastric carcinoma with p53 abnormality.Oncol Rep, (2004). , 985-990.

[112] Honda, T., et al., Demethylation of MAGE promoters during gastric cancer progression.Br J Cancer, (2004). , 838-843.

[113] Pavicic, W., et al., Altered Methylation at MicroRNA-Associated CpG Islands in Hereditary and Sporadic Carcinomas: MS-MLPA-Based Approach. Mol Med, (2011).

[114] Luo, H., et al., Down-regulated miR-9 and miR-433 in human gastric carcinoma. J Exp Clin Cancer Res, (2009). , 82.

[115] Rotkrua, P., et al., MiR-9 down-regulates CDX2 expression in gastric cancer cells. Int J Cancer, (2011).

[116] Kim, K., et al., Epigenetic regulation of microRNA-10b and targeting of oncogenic MAPRE1 in gastric cancer.Epigenetics(2011). , 740-751.

[117] Ando, T., et al., (2009). DNA methylation of microRNA genes in gastric mucosae of gastric cancer patients: its possible involvement in the formation of epigenetic field defect.Int J Cancer, 124(10): , 2367-2374.

[118] Zhu, A., et al., MicroRNA-148a is silenced by hypermethylation and interacts with DNA methyltransferase 1 in gastric cancer.Med Oncol, (2011).

[119] Hashimoto, Y., et al., Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis.Carcinogenesis(2010). , 777-784.

[120] Craig, V.J., et al., Epigenetic silencing of microRNA-203 dysregulates ABL1 expression and drives Helicobacter-associated gastric lymphomagenesis.Cancer Res, (2011). , 3616-3624.

[121] Wada, R., et al., miR-212 is downregulated and suppresses methyl-CpG-binding protein MeCP2 in human gastric cancer.Int J Cancer, (2010). , 1106-1114.

[122] Takei, Y., et al., The Metastasis-Associated microRNA miR-516a-3p Is a Novel Therapeutic Target for Inhibiting Peritoneal Dissemination of Human Scirrhous Gastric-Cancer. Cancer Res, (2011). , 1442-1453.

[123] Chen, Q., et al., (2011). miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42.Dig Dis Sci, 56(7): , 2009-2016.

[124] Lee, T.L., et al., Detection of gene promoter hypermethylation in the tumor and se‐rum of patients with gastric carcinoma.Clin Cancer Res, (2002). , 1761-1766.

[125] Chen, L., et al., Hypermethylated FAM5C and MYLK in serum as diagnosis and pre‐warning markers for gastric cancer.Dis Markers, (2012). , 195-202.

[126] Zheng, Y., et al., (2011). Analysis of the RUNX3 gene methylation in serum DNA from esophagus squamous cell carcinoma, gastric and colorectal adenocarcinoma pa‐tients.Hepatogastroenterology, 58(112): , 2007-2011.

[127] Tan, S.H., et al., Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3.Oncol Rep, (2007). , 1225-1230.

[128] Yu, Q.M., et al., CDH1 methylation in preoperative peritoneal washes is an inde‐pendent prognostic factor for gastric cancer.J Surg Oncol, (2012).

[129] Hiraki, M., et al., Aberrant gene methylation is a biomarker for the detection of can‐cer cells in peritoneal wash samples from advanced gastric cancer patients. Ann Surg Oncol, (2011). , 3013-3019.