Epigenetic Deregulations in Gastric Cancer

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Abnormal functioning of many cellular processes such as cell cycle, DNA repair, angiogenesis and cell–cell adhesion has been reported in all types of cancer. In the context of cancer, genes responsible to sustain integrity of the cells can be categorized into tumor suppressor genes and oncogenes. In normal conditions, these genes maintain a state of equilibrium and imbalance of these two groups of genes leads to a malignant form of cells known as Cancer. Genetics and epigenetics are the two main mechanisms that regulate expression of these genes. Silencing of tumor suppressor genes has been observed in different cancer while, on the other hand, silent oncogenes are active in cancer and confer growth advantage to malignant cells as compared with the contemporary normal cells. Gastric cancer (GC), like other cancers, is a complex disease. Multiple factors including bacterial infection, dietary habits, smoking and genetic polymorphisms determine the risk for GC development. Epigenetic modifications have been attributed as an initial event in the development of GC. Here, we have summarized recent findings in the field of epigenetics which correlated with GC.

Keywords: Gastric Cancer (GC), Epigenetics, Hyper-Methylation, Histone Modification, MicroRNA.

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1. INTRODUCTION

Epigenetics can be defined as system of genome wide changes excluding modifications in DNA sequence. These changes are heritable during cell division. System of epigenetic regulation involves methylation and demethylation of DNA, covalent and non-covalent modification of histone proteins and non-coding RNAs.¹ The epigenetic system regulates the expression of genes without modifying DNA sequences.² Cross talk of components of epigenetic system determines cellular behavior in normal and diseased states. The importance of epigenetics in biological research is evident and it affects diverse areas of study including cloning and transgenic techniques, mobile elements activity, somatic gene therapy, viral latency, genomic imprinting, developmental abnormalities and cancer biology.¹,³

Deregulations of epigenetic machinery work in parallel with genetic aberrations leading to abnormal expression of many genes in human cancer. There is a complex relationship between genetic and epigenetic mechanisms. Operation of epigenetic gears can function independently and also in conjunction with genetic system resulting in upregulation of oncogene and downregulation of tumor suppressor genes. Oncogenes, once activated, can promote carcinogenesis by conferring growth advantages to cancer cells.⁴ The epigenetic signatures are very important in maintaining cellular identity. Normal cells contain hyper-methylated state in dispersed CpG regions and demethylated state in local CpG islands. These epigenetic
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signatures are reversed in cancer cells.\textsuperscript{3} The initial events that are responsible for this abnormal epigenetic behavior are still not fully understood.\textsuperscript{3} Once this process is started, irregular genetic expression of tumor suppressor and oncogenes imbalance may contribute to cancer development.\textsuperscript{3} Rapidly growing field of cancer epigenetics has revealed the role of every component of epigenetic mechanism. DNA methylation and covalent and non-covalent histone modifications alter the chromatin structure that may affect DNA exposure to transcription network molecules resulting in abnormal genetic expression. Non-coding RNAs, especially microRNA regulate gene expression through post-transcriptional silencing of target genes.

GC is the fourth most common cancer in the world and the second most prevalent cause of cancer related death.\textsuperscript{6} It may arise either from precursor lesions or de novo. Gastric adenomas or flat dysplasia may lead to development of GC.\textsuperscript{3} There are 10\% chances of malignant transformation of gastric dysplasia/adenoma into GC.\textsuperscript{3} GC, like other cancers, is a complex disease. Multiple factors including bacterial infection, dietary habits, smoking and genetic polymorphisms determine the risk of GC development. Various genetic aberrations and epimutations are witnessed during the initiation and progression of GC. Abnormal functioning of many cellular processes such as cell cycle, DNA repair, angiogenesis and growth factor receptors are involved.\textsuperscript{2,7,8} Two-thirds of GC cases are diagnosed at advanced stages, when surgery can be the only option.\textsuperscript{9} Current situation necessitates carrying out research for better understanding of molecular events responsible for the malignant transformation of normal cells.

GC is particularly an epigenetic phenomenon in which more than 90\% of the heritable alterations are of epigenetic origin.\textsuperscript{10} Epigenetic alterations have been acknowledged as an important mechanism contributing to early gastric carcinogenesis.\textsuperscript{3} Many studies have characterized epigenetic abnormalities in Intestinal metaplasia (IM) and adenoma, which are precursors of GC. CpG island hyper-methylation and repetitive DNA hypo-methylation increase from the chronic gastritis to GC (during different stages of gastric carcinogenesis). Interestingly, IM samples, which are epigenetically altered lesions, displayed enhanced CpG island hyper-methylation and repetitive DNA hypo-methylation, regardless of Helicobacter pylori (HP) infection status or association with GC.\textsuperscript{11}

\section*{2. DNA METHYLATION}

CpG islands are 0.5–2 kb regions rich in cytosine-guanine dinucleotides and are present in the 5\' promoter region of app. 40–50\% of human genes. Methylation of cytosines within CpG islands is associated with loss of gene expression by repression of transcription and is observed in tumorigenesis, as well as under physical conditions such as X-chromosome inactivation and aging.\textsuperscript{7} DNA methylation is a heritable and enzyme-induced modification in humans.\textsuperscript{3} DNA methylation inhibits transcription factor binding, and thus, inhibits gene activation.\textsuperscript{12} As compared with normal cells, the malignant cells show major disruptions in their DNA methylation pattern.\textsuperscript{3} Cancer, particularly GC (GC), is prevalently an epigenetic phenomenon that is dependent on an altered DNA methylation pattern.\textsuperscript{10} DNA methylation is involved in silencing gene expression by establishing and maintaining a repressive status at gene promoters.\textsuperscript{13}

Increased and decreased methylations at specific sequences (hyper-methylation and hypo-methylation, respectively) are characteristic of tumor DNA in comparison with normal DNA and promote carcinogenesis in multiple ways including genomic instability.\textsuperscript{14,15} Promoter hyper-methylation is associated with a wide variety of genes, including genes involved in cell cycle, cell growth and proliferation, angiogenesis, apoptosis, DNA repair and metastasis.\textsuperscript{4,12,16–21} It usually results in silencing of tumor suppressor genes providing free space for oncogenes to regulate cellular functions without any check. Apparently, hyper-methylation-associated gene silencing provides cancer cells with a growth advantage similar to deletions and mutations.

Not all the DNA methylations are tumor specific. In colon tissues, studies have shown DNA methylation in certain genes due to aging is classified as Type A methylation. In contrast, tumor-specific methylation such as p16 and hMLH1 is classified as Type C methylation.\textsuperscript{9} Age-specific promoter hyper-methylations may be related to increased DNA damage and increased duration of carcinogen
exposure with aging and could predispose to carcinogenesis. The significance of the detection of the methylated gene can depend on the position of the CpG sites examined. Older GC patients had increased methylation levels at specific CpG sites within the CDH1, p53, and RUNX-3 promoters.

2.1. Hyper-Methylation

Hyper-methylation of DNA in promoter CpG islands is related to transcriptional repression of tumor suppressor genes. For example, hyper-methylation of the FBLN1 promoter is frequently detected in GC cell lines and primary gastric carcinoma tissues. Ectopic expression of FBLN1 inhibited growth of GC cells by inducing apoptosis. BNP3 and DAPK methylation is found in 31/80 gastric tumors and in 33/80 gastric tumors respectively. Significance correlation between lower BCL2L10 expression and CpG island hyper-methylation of the BCL2L10 gene promoter is observed in gastric carcinoma. Methylation of BCL6B is detected in patients with GC. Promoter methylations of RNF180, KL, LMX1A, OPCML, TSPY1.5, DCBLD2 and VEZT are noticed in 76%, 47%, 82%, 64%, 63.9%, 79% and 100% of primary GCs respectively. Proliferation, Invasion and metastasis are the characteristics of aggressive GC. Modified expression of tumor suppressor genes related to these functions is observed in aggressive GC. Methylation of PRDM5, an epigenetic modifier gene, is detected by methylation-specific PCR (MSP) in 88% of gastric tumors. Clonogenicity of tumor cells was inhibited when PRDM5 function was restored, at least partially through antagonizing WNT/b-catenin signaling and oncogene expression such as CDK4, TWIST1, and MDM2. HSF1 silencing is marked in GC due to its promoter methylation. Hyper-methylation of PAR4 and DCBLD2 promoter regions is linked with their silencing in GC. Besides these, CXCL12, PCDH10 and SOCS-1 are recently reported to be critical in tumor proliferation and metastasis and found methylated in GC.

DNA repair genes play important role in protecting biological information from modification. Any impairment in the function of these genes may have severe consequences. Methylation of X-ray repair cross-complementing gene 1 (XRCC1) in GC tissues is more frequently observed than gastritis tissues (P < 0.05). Nonhistone chromosomal proteins in concert with histones play important roles in the replication and repair of DNA and in the regulation of gene expression. Methylation of CHD5 promoter is detected in 11 out of 15 primary gastric carcinoma tissues examined. Finally, restored expression of CHD5 in GC cells led to a significant growth inhibition. DNA hyper-methylation can also indirectly silence additional classes of genes by silencing transcription factors and DNA repair genes. For example, HoxD10 promoter methylation is frequently detected in GC tissues obtained from endoscopic biopsies (85.7%) and surgically resected samples (82.6%). Reintroduction of HoxD10 transcriptionally upregulates IGFBP3, activates caspase3 and caspase8 consequently induces cell apoptosis. Methylation-specific PCR (MSP) analysis showed that the ZNF382 promoter was methylated in 15/15 GC cell lines. It inhibits NF-κB and AP-1 signaling and downregulate the expression of multiple oncogenes including MYC, MITF, HMGAA2, and CDK6, as well as NF-κB upstream factors STAT3, STAT5B, ID1, and IKBKE, most likely through heterochromatin silencing. Promoter Hyper-methylation of EBF3, Runx3 and ZIC1 is also witnessed in GC tissues.

Increased activity of Dnmt1 is associated with hyper-methylation of promoter regions in many tumor suppressor genes which lead to their silencing. For example, Runx3 methylation is found significantly correlated with increased Dnmt1 activity (r = 0.64, P < 0.01). DNMT1 was knock down using siRNA which led to decrease methylation level of miR-148a promoter and restore its expression. Enhanced expression of DNMT1 and DNMT3A was witnessed when cells were co-cultured with HP. It led to WWOX hyper-methylation in H.pylori induced GC.

2.1.1. Differential Methylation in Intestinal Type and Diffuse Type GC

Many genes are differentially methylated in diffuse and intestinal type GC. For example, Methylation at 79th CpG site of CPEB1 gene is significantly more prevalent in diffuse type GC (p = 0.007) and in cases with lymph node metastases (p = 0.04). GATA4 silencing is attributed to its promoter hyper-methylation in common-type GC whereas deacetylation of histones H3 and H4 is a silencing mechanism for GATA4 expression in AFP-producing GC cells. Promoter hyper-methylation is connected with lack of DLL1 expression in diffuse type of GC. Compared with normal mucosa, intestinal-type cancer demonstrates significant hypo-methylation, whereas diffuse-type cancer exhibits hyper-methylation of the hypoxic marker carbonic anhydrase (CA) IX expression. In addition to epigenetic changes in the RUNX3 proximal promoter, genetic changes in the distal promoter may be associated with susceptibility to intestinal-type GC by increasing promoter activity.

2.1.2. Field Defect

Field defect marks an area of abnormal tissue that is supposed to be transformed into cancer. It can be predicted by using reliable methylation markers. HIN-1 gene methylation is observed in 57.78% of GC and 42.1% of adjacent non-tumor tissues but was not shown in normal gastric mucosa. It suggests that HIN-1 methylation may represent the field defect of gastric carcinoma. Methylation of miR-34b/c has been found in a majority of primary GC specimens. Analysis of non-cancerous gastric mucosa from
GC patients and healthy individuals reveals that methylation levels are higher in gastric mucosa from patients with multiple GC than in mucosa from patients with single GC (27.3 vs. 20.8%; \( P < 0.001 \)) or mucosa from \( H. pylori \)-positive healthy individuals (27.3 versus 20.7%; \( P < 0.001 \)) suggesting that methylation of miR-34b/c is involved in an epigenetic field defect. The degree of LINE-1 methylation may constitute a “field defect” that may prompt normal tissues for cancer development.15

2.1.3. Methylation in Gastritis, IM, Dysplasia and GC

Many genes that are found methylated in GC are already methylated in precancerous lesions suggesting that epigenetic changes are the early event occurring during gastric carcinogenesis. For example, promoter methylation of HoxD10 is identified in GC tissues obtained from endoscopic biopsies (85.7%) and surgically resected samples (82.6%). IM tissues also shows a 60% methylation of this gene, but no detectable methylation in normal stomach tissues. Similarly RNF180 Promoter methylation is detected in 76% of primary GC and 55% of IM, but in none of 23 normal gastric tissues. Methylation of RUNX3, SOX9, p16, MGMT, and DAPK has been defined as an early event occurring during premalignant stages of cancer. The list of methylated genes which are silenced in GC is growing day by day. Many studies have identified methylation in genes, which were not previously attributed to GC. Hyper-methylation of CpG islands in promoter regions of FPI2 (80.9%), GPX3 (30.1%), DMRT1 (46.9%), GPX1 (16.7%), IGFBP6 (22.6%), IRF7 (32.1%), HIC1 (87%) and FGFR2 have been recognized in GC resulting in their down regulation. Reduced global and increased site-specific DNA methylation in CDH1, p16, and p53 promoters has been significantly associated with male gender. Hyper-methylation of three new genes CHRNA3, DOK1, GNMT, functioned in neurotransmission and angiogenic growth, host response to infection, maintenance of methylamine pool and methyl group supply, is reported in a recent study.

The mechanism of targeting genes for this aberrant DNA methylation is still unclear. Tumor-specific CpG island methylation may occur through a sequence-specific instructive mechanism by which DNMTs are targeted to specific genes by their association with oncogenic transcription factors. Retroelement methylation in the gastric mucosa infected with \( H. pylori \) may also initiate the concurrent methylation of multiple CpG-island genes. It is speculated that the trans-acting elements binding to sites, which are between promoter and Alu elements, may be involved in the methylation spreading from the Alu elements to the promoter region. Therefore, these trans-acting elements could behave as guardians of the methylation centers, e.g., Alu elements. For example, MLH1 promoter methylation may spread from Alu elements that are located in intron 1 of the MLH1 gene. Disturbance in the local balance between DNMTs and factors that protect DNA from aberrant methylation, such as the presence of RNA polymerase II and/or possible overexpression of Dnmt3b can be involved in methylation induction. Interestingly, Polymorphism may also influence CpG island hyper-methylation in Helicobacter pylori-infected GC. IL1B-511T/T allele is associated with enhanced hypermethylation of multiple CpG island loci.

The role of Dnmt1 in hyper-methylation of different genes has been reported. A recent study showed that Dnmt1 was upregulated in gastric mucosa of gerbils in the three Helicobacter-infected groups and the NaCl-treated group. However, the highest expression was observed in the NaCl group, where methylation was not prompted. These results indicated that expression of Dnmt1 was not associated with methylation induction but with cell proliferation. However, it is possible that Dnmt1 further needs some instructions for its functioning which were lacking in NaCl induced inflammation.

2.1.4. CpG Island Methylator Phenotype

Abnormal methylation usually occurs in many genes simultaneously during cancer, giving rise to the concept of a CpG island methylator phenotype (CIMP). The CIMP is defined as a subset of malignancies that show widespread hyper-methylation of multiple promoter CpG island loci. This concept is introduced in gastric and colorectal cancer in which five to seven or more genes are included in study for methylation status evaluation and for correlating the CIMP with tumor risk and prevention. ALX4, TMEFF2, CHCHD10, IGFBP3, and NPR1 were used to check CIMP status in GC. With CIMP as the dependent variable, CIMP-high GC tended to show more distant lymph node metastasis, higher pathologic tumor classification, more pathologic metastasis, and higher pathologic TNM status. The transitional CpG sites, rather than CpG-rich islands, are likely to serve as pivot methylation positions that reflect the concurrent methylation pattern associated with \( H. pylori \) infection and the evolution of cancer. Apparently, the increase in DNA methyltransferase activity plays an important role.

2.2. Hypo-Methylation

Cancer-associated DNA hypo-methylation is as prevalent as cancer-linked hyper-methylation, and it is often associated with increased expression of oncogenes. DNA hypomethylation at repeat sequences leads to increased genomic instability by promoting chromosomal rearrangements. Global DNA hypo-methylation is generally considered as one of the hallmarks in cancer cells because the genes vulnerable to aberrant hyper-methylation usually are overlapped by the genes targeted by hypo-methylation.
ALU and LINE-1 repetitive DNA elements comprise ~28% of the human genome. Hypo-methylation of this region is an early episode during multistep gastric carcinogenesis. A significant decrease in the ALU methylation levels is noted during the transitions from chronic gastritis to intestinal metaplasia and from gastric adenoma to GC. LINE-1 methylation decreases during the transition from intestinal metaplasia to gastric adenoma and no further decrease occurs during the transition from gastric adenoma to GC. A degree of LINE-1 methylation may create a “field defect” instigating normal tissues for cancer development. An association is observed between LINE-1 and MLH1 methylation levels.

One of the major and early effects of bacterial infection is induction of hypo-methylation. A gradual increase in Sat α relative demethylation level (RDL) relates with HP infection and cancer development. Sat α RDL is significantly raised in the non-cancerous gastric mucosa (NGM) in HP-positive compared with HP-negative (P < 0.001), and significantly elevated in cancer tissues (P < 0.001). A gradual decrease in the global DNA methylation is detected from HP-negative to HP-positive chronic gastritis (CG), HP-positive chronic atrophic gastritis (CAG) and GC cases. The LAMB3 and LAMC2 genes encode the laminin-5 b3 and c2 chains, respectively, which are parts of laminin-5, one of the major components of the basement membrane zone. LAMB3 and LAMC2 are frequently overexpressed in GC tissues and promoter demethylation and histone modifications are associated with the overexpression of both genes in GC. The activation histone mark H3K4me3 is associated with the expression of both genes.

3. HISTONE COVALENT MODIFICATIONS

The N-terminal tails of histones can undergo a variety of post-translational covalent modifications including methylation, acetylation, ubiquitylation, sumoylation and phosphorylation on specific residues. The complement of modifications is proposed to store the epigenetic memory inside a cell in the form of a ‘histone code’ that determines the structure and activity of different chromatin regions. Histone modifications mark its effects by altering the accessibility of chromatin or by recruiting and/or occluding non-histone effector proteins, which decode the messages encoded by the modification patterns.

Deacetylation of histones H3 and H4 is a silencing mechanism for GATA4 expression in AFP-producing GC cells. RhoE downregulation in GC cell lines is modulated by histone deacetylation at the epigenetic level. Histone modification plays significant role in controlling the expression of p16, MLH1 and iNOS in GC. Infection of gastric epithelial cells by wild-type HP induces time- and dose-dependent dephosphorylation of histone H3 at serine 10 (H3 Ser10) and decreased acetylation of H3 lysine 23. Altered c-jun and hsp70 gene expression is associated with the H3 Ser10 dephosphorylation. The activation histone mark H3K4me3 is associated with the expression of both LAMB3 and LAMC2 genes. H3–K9 di-methylation plays a crucial role in p16 expression in GC cells.

4. DNA METHYLATION AND HISTONE MODIFICATIONS

DNA and histone methylation likely have a mutually reinforcing relationship and both are required for stable and long-term epigenetic silencing. Histone H3–K9 methylation in different regions of the promoters correlates with DNA methylation status of each gene in GC cells. However, histone H3–K9 acetylation and H3–K4 methylation inversely correlate with DNA methylation status of each gene in GC cells. DNA methylation plays a direct role in both genes silencing and maintaining a repressive histone modification at a hyper-methylated gene promoter in cancer.

The interplay of DNA methylation and Histone modification control the expression of many genes. These two epigenetic modifications collaborated with each other to silence p16 and PDX1 in GC. The Sp1 antibody was used to reveal that the binding of transcription factor Sp1 to the ZNF312b promoter for its transcriptional activation required DNA demethylation and histone acetylation. Mice treated with N-methyl-N-nitrosourea (MNU) exhibited a field defect characterized by widespread trefoil factor 1 (TFF1) repression associated with histone H3 lysine 9 (H3K9) methylation and H3 deacetylation at the TFF1 promoter in epithelial cells. In MNU-induced advanced cancers, DNA methylation at the TFF1 promoter was observed.

5. MiRNAs

MiRNAs are small, 22 nt, non-coding RNAs that regulate gene expression through posttranscriptional silencing of target genes. Sequencespecific base pairing of miRNAs with 3′ untranslated regions of target messenger RNAs within the RNA-induced silencing complex result in target messenger RNA degradation or inhibition of translation. They are found to regulate genes involved in diverse biological functions. Single miRNA has potential to target a number of genes. For example, miR-449 targets GMNN, MET, CCNE2 and SIRT1. MiRNAs are deemed to play a crucial role in the initiation and progression of human cancer.

5.1. Tumor Suppressor MicroRNAs

MicroRNAs can function either as tumor suppressors or oncogenes depending upon their target genes.
miRNAs induce apoptosis but they are down regulated in GC. For example, miR-137 carries out its apoptotic function through inactivation of the Cdc42/ERK pathway but found methylated in 21 of 30 cancer tissues.68 miR-375 performs proapoptotic function, at least in part, through the downregulation of PDK1 and 14-3-3ζ.70 Loss of miR-449 expression has been reported in GC. Restoration of miR-449 expression activated p53 and p21 as well as the apoptosis marker, CASP3.

Many tumor suppressor miRNAs that target growth promoting genes are repressed in cancer. For example miR-331-3p inhibits cell growth and arrest cell cycle but it is down regulated in human gastric carcinoma.71 Epigenetic silencing of miR-34b and miR-34c has been described in GC. Overexpression of miR-34b and miR-34c leads to tumor growth suppression and dramatically changes the gene expression profile.46 MicroRNA-182, miR-429 and miR-212 affects cell viability and colony formation in gastric tumor. These miRNAs are downregulated in GC.5,72,73 MiR-182 inhibits gastric adenocarcinoma cell viability and colony formation by targeting cAMP-responsive element binding protein 1 gene.5 MiR-429 targets c-myc expression in GC cells and inhibits cell viability, proliferation and attachment.

A number of miRNAs reduce invasion and metastatic potential of GC. Let-7f can inhibit invasion and migration of GC cells by targeting MYH9 but its downregulation is witnessed in the highly metastatic potential GC cell lines GC9811-P and SGC7901-M.74 Let-7 miRNAs are expelled out of AZ-P7a (a metastatic GC cell line) cells via exosomes into the extracellular environment.75 Methylation regulated miR-9 (miR-9-1, miR-9-2 and miR-9-3) is silenced in GC. Enforced expression of miR-9 inhibited tumor cell proliferation, migration and invasion.13 MicroRNA-200bc/429 cluster can play an important role in the development of multidrug resistance (MDR) in both gastric and lung cancer cell lines, at least in part by modulation of apoptosis via targeting BCL2 and XIAP.76 Separate clusters can be co-expressed and have related activities. For example, miR-106b and miR-222 clusters are separately located in the genome, their expression patterns are highly similar and they play a similar role in cell cycle control by inhibiting three Cdk inhibitors.77 It would also be interesting to see if miRNA operons cooperate with each other to enhance disease phenotype.

The growing list of cancer related microRNAs shows their importance in cancer development. Upregulation of many miRNAs such as miR-223, miR-21, miR-23b, miR-222, miR-25, miR-23a, miR-221, miR-107, miR-103, miR-99a, miR-100, miR-125b, miR-92, miR-146a, miR-214 and miR-191 has been reported.81 On the other hand, miRNAs such as let-7a, miR-126, miR-210, miR-181b, miR-197, miR-30a-5p, miR-34b, miR-127-3p, miR-129-3p and miR-409 are down regulated in GC.1,2,81 MicroRNA-16 and miR-21 are directly regulated by the transcription factor NF-κB.83 The exact function of many miRNAs in cancer initiation and development has still to be determined.

5.3. Clustered MicroRNAs

MicroRNA gene loci are often found in close conjunction, and such clustered miRNA genes are transcribed from a common promoter to generate polycistronic primary transcript. MicroRNA-200bc/429 cluster can play a role in the development of multidrug resistance (MDR) in both gastric and lung cancer cell lines, at least in part by modulation of apoptosis via targeting BCL2 and XIAP.76 Separate clusters can be co-expressed and have related activities. For example, miR-106b and miR-222 clusters are separately located in the genome, their expression patterns are highly similar and they play a similar role in cell cycle control by inhibiting three Cdk inhibitors.77 It would also be interesting to see if miRNA operons cooperate with each other to enhance disease phenotype.

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6. RELATIONSHIP BETWEEN DNA METHYLATION AND MICRONRNAs

The two main components of epigenetic machinery DNA methylation and miRNAs not only target other genes but also interact with each other. MicroRNAs can affect DNA methylation by targeting DNMTs. For example, Ectopic over-expression of miR-148a in cancer cell lines causes reduction in DNMT1 expression and inhibited cell proliferation.41 On the other hand, silencing of several miRNAs is tightly associated with DNA methylation. Expression of miR-34b, 127-3p, 129-3p, 409, 212, 1-1, 124a-1, 124a-2, 124a-3, 148a,
152 and 18b is frequently lowered in GC with CpG-rich methylation. Epigenetic mechanisms, such as histone modification and DNA methylation, are closely linked with silencing of miRNAs. Hypomethylation of miR-196b, 200a and 208a elevates their expression in GC.

7. HP, EPSTEIN-BARR VIRUS (EBV) AND GC EPIGENETICS

The mechanism that Helicobacter pylori (HP) infection follows in the development of GC is still not clear. Identification of molecular changes induced by HP and response of epithelial cells is very important to understand early carcinogenesis events. DNA hypo-methylation is considered as one of early events in HP related gastric carcinogenesis.

Increased cell proliferation during inflammation characterized by specific inflammation related genes is necessary for induction of methylation. High expression of Il1b, Nos2 and Tnf has been identified in HP infection. NFkB-p65 signaling pathway mainly mediates expression of inflammatory genes in HP induced inflammation. iNOS gene activation is induced by HP infection in gastric cells. Nitric oxide (NO) overproduction as a result of IL-1b stimulation of NFkB transcriptional system may play an important role in H. pylori-induced epigenetic changes in many cancer-relevant genes. Moreover IL1B-511T/T allele may promote hyper-methylation of multiple CpG island loci which may increase the risk for GC development in HP infected individuals. Cells cultured with HP or treated with IL-1b exhibited increased DNMT activity. Methylation of WWOX, IRX1 is noticed in HP infection. Deyprophosphorylation of histone H3 at serine 10 (H3 Ser10) and decreased acetylation of H3 lysine 23 is manifested in H. pylori infected gastric epithelial cells. H3 Ser10 dephosphorylation alters the expression of c-jun and hsp70. High expression of Dnmt1 is noticed in NaCl induced inflammation as compared with HP induced inflammation but high expression of Dnmt1 has not induced methylation.

Approximately 10% of GC worldwide is associated with the presence of EBV. Hyper-methylation of tumor suppressor genes is significantly more frequent in EBV-associated GC in comparison with EBV-negative GC. The majority of EBV induced GC with p16 hyper-methylation shows p16 protein loss. Genes having diverse functions such as cell cycle regulation (IGFBP3, CDKN2A, CCND1, HSP70, ID2, ID4), DNA repair (BRCAl, TFFI), cell adhesion (ICAM1), inflammation (COX2), and angiogenesis (HIF1A) are found methylated in Epstein-Barr Virus (EBV) derived GC. Methylation of SOX9 is closely related to GC carcinogenesis and EBV-associated GC carcinogenesis.

8. CLINICAL IMPLICATIONS

Gastric precancerous lesions (GPL) which confer a high risk for GC are including chronic atrophic gastritis (CAG), intestinal metaplasia (IM) and gastric epithelial dysplasia (GED). Therefore, it is crucial to explore effective biomarkers for diagnosis and pre-warning of GC. DNA methylation is a promising biomarker for GC risk prediction and prognostication. Minimal invasive treatment is widely accepted in the early stages of GC. It is necessary to develop noninvasive and affordable methods of “serologic biopsy” for the asymptomatic population.

8.1. Diagnostic Markers

Various markers have been suggested in recent studies for the diagnosis and pre-warning against GC. Identifying DNA methylation and microRNAs in the serum of GC patients offers a promising tool for GC detection. Methylated RNF180 DNA is detected in the plasma of 56% of GC patients, but not in healthy controls (P = .003). The specificity and sensitivity of detecting RNF180 methylation in the plasma DNA are 91% and 63%, respectively. In tissues and sera of GC patients, a higher prevalence of methylation is observed for KCNA4 and CYP26B1, and KCNA4, compared with healthy people (p<0.05, respectively). Detection of the methylation prevalence of KCNA4 and CYP26B1 together in serum demonstrated the good sensitivity (91.3%) and specificity (92.1%). Detection of hyper-methylation for SLC19A3, Sox17, FAM5C and MYLK in sera has been suggested as a diagnostic marker for GC.

Serum miR-378 could serve as a novel noninvasive biomarker in GC (GC) detection. miR-378 yields a receiver operating characteristic (ROC) curve area of 0.861 with 87.5% sensitivity and 70.73% specificity in discriminating GC patients from healthy controls. A panel of three serum miRNAs (miR-221, miR-744, and miR-376c) is identified as potential biomarkers for GC detection. ROC curve-based risk assessment analysis proposed that this panel could distinguish GCs from controls with 82.4% sensitivity and 58.8% specificity. This panel could classify serum samples collected up to 5 years ahead of clinical GC diagnosis with 79.3% overall accuracy.

The methylation status in the peritoneal fluids (PFs) can be a predictive marker for peritoneal metastasis in patients with depth of cancer invasion beyond the muscularis propria (MP). Patients with positive methylation in the PF have a 12-fold increased risk of peritoneal recurrence. Methylated levels at MOS_E60 CpG site have potential usage as a marker for the development of GC. A recent study suggested that assessing global DNA methylation rather than the methylation status of a single gene may contribute to predict the individual susceptibility to develop GC.
8.2. Prognosis Markers

CIMP-high GC shows significantly worse survival compared with that of CIMP-low/CIMP-negative GC ($P < 0.001$). It spectroscopically that there is an association between CIMP status and lymph node metastasis in GC and CIMP-high is an independent prognostic factor. The LINE-1 methylation status can be used as a molecular biomarker to define a subset of GC patients with poor prognosis.

The survival of patients possessing methylated alleles of TFP12 (123/152, 80.9%) is poorer than that of patients with unmethylated alleles ($p = 0.023$). Multivariate analysis confirmed that TFP12 methylation is a significant and independent prognostic factor in gastric carcinoma. Promoter methylations of EBF3, KL, CALCA, RARBeta, RASSF1A, TIMP3, PAX6, BCL6B, MFL1, MGMT, p16, RASSF2, HMLH1, HAND1, HRASLS, TM, and FLNc are studied to be significantly related to poor survival and thus can be used as prognosis marker for GC patients.

Multivariate analysis showed that global DNA hypermethylation is a significant independent predictor of worse survival hazard ratio (HR) = 2.0, 95% CI: 1.1–3.8; $p = 0.02$) and high methylation mean values across p16 promoter sites 1–7 are associated with better survival with HR of 0.3 (95% CI: 0.1–0.8; $p = 0.02$) respectively.

Seven-miRNA signature (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p, miR-126) is identified for overall survival ($p = 0.0009$) and relapse-free survival ($p = 0.0005$) of GC patients. PDCD6 protein expression is closely associated with the prognosis of advanced GC patients. Multivariate analysis has shown that the risk signature is an independent predictor of overall survival (HR = 3.046; 95% CI, 1.246 to 7.445, $p = 0.015$) and relapse-free survival (HR = 3.337; 95% CI, 1.298 to 8.580, $p = 0.012$). The prognostic signature can be applied to future decisions concerning treatment.

Hypo-methylation of several specific gene promoter sites in whole blood DNA is associated with poor prognostic features. In particular, hypo-methylation of p53 at sites 1, 3, 4, and overall, and of RUNX3 at site 2 is associated with lymph node involvement. Methylated CDH1 in pre-operative peritoneal washes (PPW) predicts poor prognosis for GC patients.

8.3. Therapy Targets

MicroRNA-34b and miR-34c are epigenetically silenced in GC. miR-34b/c may be a useful therapeutic target in GC as their ectopic expression significantly down regulated their target genes (e.g., CDK4 and MET) and suppressed GC cell proliferation. Suppressor of cytokine signaling-1 (SOCS-1) is a negative regulator of pro-inflammatory cytokine signaling. It suppressed STAT3 phosphorylation and proliferation of NUGC-3 and AGS cells in vitro. The anti-proliferative effects of SOCS-1 are not only attributed to the inhibition of STAT3 but also to that of p38 MAPK activity. But SOC-1 is found methylated in GC. Enforced expression of SOCS-1 may represent a novel therapeutic approach for the treatment of GC. RhoE downregulation in GC cell lines is modulated by histone. The reversal of RhoE expression by HDAC inhibitors may be of therapeutic interest in GC.

S-adenosylmethionine (SAM) serves as a major methyl donor in biological transmethylation events. S-adenosylmethionine (SAM) can effectively inhibit the tumor cells growth by reversing the DNA hypo-methylation on promoters of oncogenes, thus down-regulating their expression. It can be used as a potential drug for cancer therapy.

9. CONCLUSION

Progress in the field of epigenetics has highlighted its importance in many diseases. As it is an early event in gastric cancer development, further research in this area will not only contribute our understanding of puzzling phenomena of cancer initiation, but it may also identify new therapeutic targets. It has potential to identify new markers that may help us to diagnose gastric cancer at early stages. Currently, our knowledge is very limited about gastric cancer stem cells. Future research in epigenetics will reveal its role in the birth of these cells and will identify epigenetic signatures that may be obligatory for these cells to maintain their “stem cell” status. Finally, nanotechnology has been widely applied in researches about cancer stem cells, cancer molecular imaging, DNA methylation and RNA or miRNAs RNA, which should be helpful for us to identify new markers that may help us to diagnose gastric cancer at early stages.

Acknowledgment: We acknowledge the financial supports of the National Key Program for Developing Basic Research (2010CB933903), the NSFC (60927001, 60971045, 61271056, and 21205013), Hunan Science and Technology Projects (2012SK3105 and 2010sk2003), Scientific Research Fund of Hunan Provincial Education Department (11A030).

References and Notes


Received: 3 May 2012. Accepted: 28 August 2012.