Epigenetic mechanisms in gastric cancer

Cancer is considered one of the major health issues worldwide, and gastric cancer accounted for 8% of total cases and 10% of total deaths in 2008. Gastric cancer is considered an age-related disease, and the total number of newly diagnosed cases has been increasing as a result of the higher life expectancy. Therefore, the basic mechanisms underlying gastric tumorigenesis is worth investigation. This review provides an overview of the epigenetic mechanisms, such as DNA methylation, histone modifications, chromatin remodeling complex and miRNA, involved in gastric cancer. As the studies in gastric cancer continue, the mapping of an epigenome code is not far for this disease. In conclusion, an epigenetic therapy might appear in the not too distant future.

KEYWORDS: chromatin remodeling complex ▪ DNA methylation ▪ epigenetics ▪ gastric cancer ▪ histone acetylation ▪ histone methylation ▪ hypermethylation ▪ miRNA ▪ phosphorylation

Carolina Oliveira Gigek*[1], Elizabeth Suchi Chen[1], Danielle Queiroz Calcagno[1], Fernanda Wisnieski[1], Rommel Rodriguez Burbano[2] & Marilia Arruda Cardoso Smith[1]

[1]Disciplina de Genética, Departamento de Morfologia e Genética, Escola Paulista de Medicina/Universidade Federal de São Paulo, Rua Botucatu 740, Ed. Leitão da Cunha – 1º andar – CEP 04023-900, São Paulo, SP, Brazil

[2]Laboratório de Citogenética Humana, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil

**Author for correspondence:*
Tel.: +55 11 5576 4848 ext 2369
Fax: +55 11 5579 8378
carolina.gigek@unifesp.br

Cancer is considered one of the major health issues worldwide, responsible for about 12.7 million cases and 7.6 million deaths in 2008 [201]. Moreover, over 70% of new cases and deaths of this type of cancer occur in developing countries [1].

Gastric cancer is considered an age-related disease, as are most solid tumors, with high incidence in the seventh decade of life, being relatively rare in individuals under 45 [2,3]. Its incidence is influenced by geographic, ethnic and cultural factors [4] and by *Helicobacter pylori* infection, a gram-negative bacteria that commonly infects the mucosa of the stomach and causes inflammation [5].

Although stomach cancer incidence has been decreasing in most parts of the world, in part due to factors related to the increased use and availability of refrigeration, consumption of fresh fruit and vegetables, and decreased intake of salted and preserved foods [1], the total number of newly diagnosed cases has been increasing as a result of higher life expectancy [6]. It is estimated that at least one third of new gastric cancer cases in the world could be prevented [7].

Gastric cancer is largely resistant to radio/chemotherapy, and the main treatment consists of performing a gastrectomy; however, a study showed that only 30–50% of patients underwent surgery expecting a full recovery [8]. Therefore, the knowledge about alterations involved in cancer progression or predisposition is important as this could increase the ability to predict prognosis and establish the most effective therapeutic regimen [9].

Although there are a rising number of studies in gastric cancer and risk factors for this disease, mechanisms underlying gastric carcinogenesis are still unclear. Since Boveri's theory of cancer, that the "primordial cell of a tumor contains, as a result of an abnormal process, a definite and wrongly combined chromosome complex" [10], scientific researchers have focused on genetic and molecular models of cancer.

Indeed, the two histologic types of gastric adenocarcinoma, which is a tumor originating in the glandular cells of stomach mucosa, accounts for 90–95% of all gastric malignancies. They vary widely in their proposed molecular mechanisms. According to Laurén's classification, which describes the gastric tumor based on microscopic observation and growth pattern, there are intestinal and diffuse types [5]. Intestinal-type gastric cancer develops following a multistep process, from chronic gastritis to dysplasia, before becoming malignant. Mutations, chromosomal instability, microsatellite instability and loss of heterozygosity have been described in intestinal-type gastric cancer. By contrast, diffuse-type gastric cancer is often related to mutations or inactivation of the important tumor-suppressor gene *CDH1* [5].

Several studies have demonstrated genomic instability, such as, chromosome 17 aneusomy [11] and chromosome 8 alterations [12–14]; chromosomal rearrangements, such as characteristic MYC insertions in diffuse type [15];

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mutations and functional polymorphisms in oncogenes and tumor-suppressor genes, such as the Survivin gene [16], *PTEN* [17], *TP53* and *WRN* [18]. However, genic imbalance does not occur solely by genetic mechanisms in gastric cancer. Pathologic epigenetic modifications are alternative processes to mutation and chromosomal alterations that provide abnormal gene function [19].

The term epigenetics was coined by Waddington, defining the development process of gene-expression control, merging both embryogenesis and genetics [20]. However, this definition evolved to refer to a variety of biological processes. Nowadays, epigenetics refers to the study of heritable alterations that promote gene-expression variation, without changes in the DNA sequence [21].

Most of these alterations are established during differentiation and maintained through multiple cellular cycles, therefore playing a fundamental role in normal development [21]. Thus, abnormalities in the epigenetic control of normal processes could lead to diseases such as cancer.

Epigenetic mechanisms include DNA modifications and/or associated factors, such as DNA methylation, histone modifications, chromatin remodeling and miRNA. Those mechanisms are linked to processes that affect stability, folding, positioning and DNA organization [22].

In this review, we provide an overview of the main recently described epigenomic mechanisms involved in gastric carcinogenesis.

DNA methylation

DNA methylation is the most studied epigenetic mechanism and is observed almost exclusively in CpG dinucleotides that tend to cluster in regions called CpG islands and are present in about 70% of human gene promoters [23].

CpG islands are typically in a nonmethylated state when picturing global DNA methylation. In general, when these regions become methylated, they are associated with gene silencing. Therefore, abnormal DNA methylation is an alternative process to mutation or allelic loss, or gene amplification that can cause alterations in gene function. However, there are known examples of CpG islands that become methylated during normal development, leading to stable silencing of the associated promoter [24].

DNA methylation is mediated by a family of enzymes known as DNA methyltransferases (DNMT). In humans, DNMT1 is responsible for maintenance of pre-existent methylation patterns during the replication of DNA, whereas DNMT3A and 3B are *de novo* methyltransferases [25].

In gastric cancer, little is known about DNMT expression and clinical significance. A functional polymorphism of *DNMT3A* was implicated in increasing its activity and therefore contributing to susceptibility of gastric cancer, with a sixfold increased risk for homozygotes for this polymorphism in a studied Chinese population [26]. DNMT3B has one polymorphism associated with decreased risk of gastric cancer, which has also been studied in a Chinese population [27].

Recently, Yang *et al.* described DNMT1, DNMT3A and DNMT3B overexpression in gastric neoplastic tissue [28]. Furthermore, DNMT3A was associated with tumor stage and lymph node metastasis, indicating a significant role in aberrant promoter methylation during the tumorigenesis process [28,29]. DNMT3B levels were higher in cases with lymph node metastasis [30] and low DNMT1 levels present a better histopathological/clinical response [29].

DNMT1 and 3A expression were enhanced when gastric cancer cell lines were cocultured with *H. pylori*, indicating that infection by this agent might promote aberrant DNA methylation of CpG islands, such as *WWOX*, a tumor-suppressor gene, recently described as hypermethylated in gastric neoplastic tissue [31]. However, Oue *et al.* have previously observed no correlation between levels of DNMTs and the DNA methylation status of *hMLH1*, *p16(INK4a)* and *CDH1* [32]. Therefore, the DNMT family appears to be involved in carcinogenesis in different stages and through different mechanisms.

DNA hypermethylation of CpG islands, provided by DNMTs, results in a stable transcriptional silencing mechanism that plays an important role in regulation of gene expression and, as a consequence, in loss of protein expression [33]. DNA methylation mapping across normal genomes and cancer genomes confirms that almost all cancer types present hundreds of genes with abnormal gain or loss in CpG island methylation [34]. The stomach has been described as the organ with the highest CpG island hypermethylation frequency that is age-associated and possibly inflammation-mediated [35].

In fact, several genes have been described as containing hypermethylated CpG islands in nontumoral gastric mucosa [36]. In gastric cancer, the growing number of publications regarding DNA methylation is remarkable. Recent publications have reviewed a large number of genes involved in gastric carcinogenesis that undergo

aberrant DNA hypermethylation [37,38]. A number of tumor-suppressor genes are inactivated by promoter methylation, such as *hMLM1*, *CDH1*, *COX-2*, *RUNX3*, *TIMP-3*, *RASSF* and *SOX2*, acting in cell cycle, apoptosis, cell adhesion, invasion and also related to *H. pylori* infection. Indeed, connections between *H. pylori* infection and epigenetic changes in gastric cancer were reported in promoter genes related to growth, such as *p16* and *p14*, DNA repair genes, as well as E-cadherin [35] and *DCC*, *CRK*, *MOS* and *VAV1* [39]. *H. pylori* also influences methylation status of miRNAs, leading to higher DNA methylation in both healthy and gastric cancer patients mucosa when compared with healthy individuals and patients without infection [40].

Aberrant methylation of CpG islands, such as in *IGFBP-3*, was previously described in other types of cancer before in gastric tissue. *IGFBP-3* hypermethylation was confirmed in both neoplastic and non-neoplastic samples; however, no correlation between promoter hypermethylation and protein levels was observed, although high protein expression was present in tumor samples, and might be a useful marker for this malignancy [41].

CpG island methylation analysis of tumor suppressor *FHIT* also showed an elevated methylation frequency in the stomach and was not associated with gastric cancer development [42]. *FHIT* is inactivated in about 60% of human tumors, and is most commonly altered in cancer and precancerous conditions. Although decreased *FHIT* gene expression in gastric tumors has not been correlated to aberrant DNA methylation control, it appears to be associated with hereditary factors and *H. pylori* infection [43].

A well-studied gene involved in gastric tumorigenesis, *CDH1*, presented CpG island hypermethylation in almost 100% of gastric adenocarcinoma samples [42], and 90% in normal gastric mucosa [Gigek CO, Leal MF, Silva PNO et al. Epigenetic pattern, mRNA and protein expression of E-cadherin and caveolin-1 in gastric adenocarcinoma (2012), Manuscript In Preparation]. A mapping of the *CDH1* promoter revealed a positive association between hypermethylation and increased age, as well a significant correlation between DNA hypermethylation and the A allele of -160C/A polymorphism. The A allele has been described as increasing the risk of development of gastric cancer and seems to act together with methylation status [44]. However, aberrant CpG island promoter hypermethylation does not always result in alteration of mRNA or protein levels. Our group observed that E-cadherin mRNA and protein levels differed between neoplastic and non-neoplastic adjacent mucosa from the same patient, as well as between normal mucosa of healthy individuals [Gigek CO, Leal MF, Silva PNO et al. Epigenetic pattern, mRNA and protein expression of E-cadherin and caveolin-1 in gastric adenocarcinoma (2012), Manuscript In Preparation]. Thus, another CpG island, or even another epigenetic marker may have an influence in this gene expression.

According to Deaton and Bird, CpG islands that acquire aberrant methylation in cancer are not always associated with tumor-suppressor genes [24]. Profiling DNA methylation near the CpG islands suggested that cancer-specific methylation patterns resemble those occurring in normal tissues [45]. However, it has been suggested that some cancer-specific CpG island methylation can be distinguished from that in normal tissues [46].

Hypermethylated DNA status of the CpG island of the catalytic subunit of telomerase gene (*hTERT*) was more frequently observed in neoplastic than in non-neoplastic gastric mucosa; therefore, the clear difference in status might be useful for diagnosis of gastric cancer and/or have an impact on the antitelomerase strategy for cancer therapy. Most normal human somatic cells lack telomerase activity due to transcriptional repression of *hTERT*. This study observed a poor relationship between this CpG island promoter and protein expression; therefore, other CpG islands might be looked at more carefully to establish better epigenetic relation [47].

The *CDKN2A* gene also presents higher frequency of DNA hypermethylation in about 30% of neoplastic gastric mucosa, while none of the normal mucosa showed methylation, or association with histological subtype [48]. This epigenetic mark was recently associated with tumor location and *H. pylori* infection in gastric cancer development [49]. These observations lead to the possibility that epigenetic alterations may also occur at different stages of gastric tumorigenesis and malignant progression. The studied CpG island of *PDCD4* was also hypermethylated in 36% of gastric cancer tissue; however, no statistically significant association with gene silencing was found [50].

DNA hypermethylation is also observed in gastric cancer culture. After treatment with 5-aza-2′-deoxycytidine, gastric cell culture that underwent DNA methylation array with six normal mucosa samples of healthy patients showed 82 hypermethylated gene promoters.

The authors investigated 15 candidate genes by methylation-specific PCR, and confirmed five highly methylated promoters of *BX141696*, *WT1*, *CYP26B1*, *KCNA4* and *FAM84A*. All of them, except *FAM84A*, also showed DNA hypermethylation in serum of gastric cancer patients, suggesting that serum DNA offers a readily accessible bioresource for methylation analysis [51].

A similar study conducted by Jee *et al.* describes 11 selected genes validated in three gastric cancer cell lines and in normal gastric tissue by bisulfite sequencing [52]. Differential DNA hypermethylation was observed in *GPX1*, *IGFBP6*, *IRF7*, *GPX3*, *TFPI2* and *DMRT1* CpG islands, but not in normal tissue. However, the only gene related with survival was *TFPI2*; a poor survival rate was observed in those individuals with higher methylation status of this gene. Therefore, it has been proposed that inactivation of this gene might be implicated in human carcinogenesis and metastasis [53].

Cancer-associated DNA hypomethylation is often associated with increased expression of oncogenes and occurs as much as cancer-linked hypermethylation [23]. *TP53* is one of the most studied tumor-suppressor genes and acts in cell cycle arrest and induction of apoptosis. The studied CpG islands in *ANAPC1* and *TP53* promoter regions were unmethylated in 100% of gastric cancer samples. Therefore, the DNA methylation status of the studied CpG island is not correlated with inactivation of the *TP53* [48].

Another two candidate genes, *MTAP* and *PLAGL1,* thought to be involved in gastric carcinogenesis by epigenetic alterations, have been evaluated in gastric cancer tissue. The authors observed hypomethylated promoters for both genes in neoplastic and in non-neoplastic gastric tissues. Therefore, the methylation status of the studied CpG islands is not part of the mechanism involved in gastric carcinogenesis. Other CpG islands within promoter regions of these genes might have an abnormal methylation status [42].

TRF2, a telomere-binding protein with a role in telomere protection, has been described as highly expressed in gastric neoplastic tissue due to hypomethylation of its promoter and exon 1 regions when compared with non-neoplastic gastric tissue [53]. However, it is known that hypomethylation of repetitive sequences, such as Alu, could influence methylation status of promoters and regions between promoters, as observed for the *MLH1* gene [54].

Detection of DNA methylation status of certain genes in blood as biomarkers for gastric cancer is of great interest and could be a useful tool for diagnosis or prognosis. Indeed, some studies have described possible serum markers proven to be aberrantly methylated in patients: *KCNA4* and *CYP26B1* [51]; *p16* [55]; RARβ and *CDH1* [56]; *RASSF1A* [57]; *FAM5C* and *MYLK* [58]; *TFPI2* [59]; *RPRM* [60]; and even *RUNX3* [61].

Histone modifications

Chromatin is composed of eight core proteins, two each of H2A, H2B, H3 and H4, wrapped around by 146 bp of DNA. The nature of the interaction between DNA and histones alters the accessibility of DNA transcriptions sites to RNA polymerase II and other transcription factors. The interaction between histones and DNA is thought to be under epigenetic control, as specific amino acid residues on specific histone core proteins can undergo a range of post-translational modifications, such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, proline isomerization, deimination and ADP ribosylation [62].

Table 1 describes some post-translational modifications and specific amino acid residues involved in this process [63]. These post-translational modifications to histone tails are reversible and govern the structural status of chromatin and the resulting transcriptional status of genes within a particular locus [64].

Histone acetylation

The status of histones acetylation is controlled by the activity of two enzymes: histone acetyltransferases (HATs), which adds an acetyl group to lysine residues on the histone tails and promote DNA interaction in the nucleosome, resulting in open chromatin and subsequent trans-activation of specific genes; and histone deacetylases (HDACs) responsible for removing the acetyl group of lysine, resulting in transcriptional inactivation [65].

HATs are recruited as coactivators of transcription by transcriptional factors, usually in the context of large chromatin remodeling complexes [66]. One major HAT family, Gcn5 related acetyltransferase (GNAT), targets histone H3 as its main substrate. The MOZ/YBF2/SAS2/TIP60 (MYST) family targets mainly histone H4. Third, the CREB binding protein (CBP)/p300 family targets both H3 and H4 [67]. In addition, HATs such as PCAF, p300 and CBP acetylate multiple nonhistone proteins, which have prominent roles in oncogenesis [67–69].

Altered HAT activity has been reported in solid cancers. Inactivation of HAT activity through gene mutation or through deregulation of HAT activity by viral oncoproteins has been described [70,71]. Missense mutations and loss of heterozygosity of p300 have also been identified in gastric cancer [68,72]. PCAF expression was found to be downregulated in gastric cancer cell lines and intestinal type gastric cancer tissues when compared with immortalized gastric cell lines and with adjacent noncancerous tissue from the same patient, respectively. Furthermore, downregulated PCAF expression was correlated to gastric wall invasion, tumor size, tumor node metastasis stage, p21, pRb and PCNA in intestinal type gastric cancer specimens. Reduced PCAF protein expression correlated significantly with mutant type p53 protein expression. Patients with high-PCAF/wild-type p53 expression have a significantly better overall survival [73].

HDAC enzymes fall into four catalytic groups, which are referred to as class I (HDAC 1–3 and 8), II (HDAC 4–7, 9 and 10), III (Sir-2 related – SIRT1–7) and IV enzymes (HDAC 11) (Table 2) [74]. Classes I, II and IV HDACs share homology in both sequence and structure; by contrast, class III HDAC share no similarities in sequence or structure with the other classes, and requires NAD^+ for catalytic activity [67,75].

Deregulation of HDAC activity by chromosomal translocations has also been strongly implicated in aberrant gene silencing and tumorigenesis promotion [66]. In addition to aberrant gene silencing, altered expression of individual HDACs in tumor samples, such as overexpression of HDAC1 and HDAC2, has also been reported in gastric carcinoma [62,76]. At present, there is some experimental evidence to suggest that increased HDAC expression can play a role in tumorigenesis and provides a molecular rationale for targeting HDAC activity in tumors (Table 2) [66].

Manipulation of the balance between acetylation and deacetylation of histones by specific HDAC inhibitors is a useful tool to delineate functional role(s) of histone hyper/hypoacetylation in various cellular activities [65]. Trichostatin A (TSA) is a potent HDAC inhibitor, and has been widely used in histone acetylation studies [77,78] and in gastric cancer cell lines [79,80]. Therefore, TSA lead to accumulation of acetylated histones in cells, of which it is reversible. In addition to a direct inhibition of HDAC catalytic activity, TSA has recently been shown to accelerate degradation of HDAC1 [65].

Table 1. Types of covalent histone post-translational modifications.

Modification	Transcription	Histone-modified sites
Small chemical groups		
Acetylation	Activation	H3 (K9,K14,K18,K56)
		H4 (K5,K8,K12,K16)
		H2A
		H2B (K6,K7,K16,K17)
Methylation	Activation	H3 (K4,K36,K79)
	Repression	H3 (K9,K27)
		H4 (K20)
Phosphorylation	Activation	H3 (S10)
Larger peptides		
Ubiquitylation	Activation	H2B (K 1 2 3)
	Repression	H2A (K 1 1 9)
Sumoylation	Repression	H3 (?)
		H4 (K5,K8,K12,K16)
		H2A (K 1 2 6)
		H2B (K6,K7,K16,K17)

Regardless of the focus on class I and II HDACs and cancer, the class III HDACs (sirtuins) also play an important role in cell survival through deacetylation of key cell cycle molecules and apoptosis regulatory proteins, including p53, p73, pRb, NF-κβ, Ku 70 and the FOXO family of proteins [81–83]. Overexpression of SIRT1, SIRT2, SIRT3 and SIRT7 has been reported in a range of tumors [84]. SIRT1 has been involved in tumorigenesis through its antiapoptotic activity and its upregulation inactivates p53 by deacetylation allowing cell proliferation [85]. Cha *et al.* reported that in gastric carcinoma samples SIRT1 expression is correlated to tumor stage, lymph node metastasis, tumor invasion, histologic types, p53 expression and shorter overall survival [86].

Histone acetylation has been clinically associated with pathological epigenetic alterations in cancer cells. Loss of acetylation of specific residues in core histones H3 and H4 has been identified as an epimarker of tumor cells [87]. Hypoacetylation of histone H3 has been reported to reduce the expression of the tumor-suppressor gene *p21* (WAF/CIP1) in gastric carcinoma specimens [88] and attenuates *RUNX3* expression in gastric cancer cells [83]. On the other hand, Song *et al.* have demonstrated that histone H3 acetylation of *ZNF312b* promoter region function as a switch for its transcriptional activation in gastric cancer, contributing to the progression of this disease [89]. Similarly, Wang *et al.* found increased expression of S100A6, which plays a

Table 2. Histone modification genes altered in gastric cancer.

	Alterations	Ref.
Histone deacetylases		
HDAC1	Upregulation/downregulation	[62,123]
HDAC2	Upregulation/mutation	[76,123]
HDAC3	Upregulation	[123]
HDAC8	Upregulation	[123]
SIRT1	Upregulation	[124]
Histone acetyltransferases		
P300	Mutation/mutation and loss of heterozygosity	[68,72]
Tip60	Downregulated	[125]
PCAF	Downregulation	[73]
HBO1	Upregulation	[126]

role in cell growth and differentiation, in gastric cancer samples associated with high levels of acetylated H3 histone [90].

Acetylated histone H4 levels were also shown to be reduced in 70% of gastric carcinomas in comparison with non-neoplastic mucosa, indicating global hypoacetylation in gastric cancer [91,92]. Reduced histone H4 acetylation levels were also found in some gastric lesions exhibiting intestinal metaplasia, a condition predisposing to gastric cancer [92]. Furthermore, reduced expression of acetylated histone H4 was correlated with advanced tumor stage, deep tumor invasion and lymph node metastasis [91,92]. These authors suggested that low levels of histone acetylation may be closely associated with the development and progression of gastric carcinoma, possibly through alteration of gene expression.

Histone methylation

It is well known that histone methylation can alter chromatin remodeling and is thought to decrease transcription of DNA close to the histone complex [93,94]. The methylation of histone tails is regulated by two groups of enzymes: histone methyltransferases (HMT) and histone demethylases. Depending on the residue and the level of methylation, the chromatin might be closed – transcriptionally inactive – or opened – transcriptionally active. For example, trimethylation at H3K27, H3K9 and H4K20, and dimethylation at H3K9 are associated with repression of gene expression, whereas trimethylation at H3K4 and H3K36 are associated with activation of gene expression [95]. Furthermore, lysine residues might present different levels of methylation – mono- (me), di- (me2) or tri-methylation (me3) – leading to different status of activation [93,94].

Histone modifications abnormalities leading to gene-expression alterations have been described in several types of cancer; however, the methylation status of histones is still unclear in gastric cancer. In gastric cancer samples, there were identified candidate genes with significant differences in H3K27me3 levels, which included oncogenes, tumor-suppressor genes, cell cycle regulators and cell adhesion [96].

In recent years, the number of studies looking for epimarkers in gastric cancer has been growing. Some of these epimarkers have also been correlated to clinicopathological variables. The levels of H3K9me3 were shown to be associated with higher T stage, lymphovascular invasion and recurrence in gastric adenocarcinoma. In addition, patients with higher H3K9me3 levels presented worse prognosis, suggesting that methylation levels in H3K9 may inactivate some tumor-suppressor genes, and thus, H3K9me3 is an independent prognostic factor [97].

In 2011, two studies revealed epigenetic alterations in cell adhesion genes in gastric cancer. Kwon *et al.* investigated in gastric carcinoma the epigenetic mechanisms which regulate CLDN4 expression, a tight junction protein that seems to be aberrantly upregulated in gastric cancer [98]. Histone demethylation at CLDN4 was associated with gene overexpression in gastric cancer cells, suggesting that CLDN4 may be a promising target for the treatment of gastric cancer.

In the other study, an overexpression of laminin-5 chain subunit genes, *LAMB3* and *LAMC2*, was observed in gastric cancer samples in relation to their normal adjacent tissue. In gastric cancer cell lines, the authors demonstrated that the overexpression of *LAMB3* and *LAMC2* was a result of the enrichment of H3K4me3 in the gene promoter region, although the authors only observed it in one cell line [99]. Together, these findings suggest that other epimarkers might be acting in the process of gastric carcinogenesis.

Few genes with histone methylation levels have been described in gastric cancer; however, more studies have analyzed the machinery of histone methylation: HMTs and histone demethylases [100]. Since histone modifications are reversible, a great deal of effort has been made in order to find epimarkers in histone modification machinery and, as a result, a potential therapeutic target.

EZH2, a HMT, plays a role in the trimethylation of H3K27 and is overexpressed in several types of cancer, including gastric cancer,

leading to the silencing of important genes in carcinogenesis, such as oncogenes [101]. When EZH2 was silenced by siRNA in gastric cancer cells, lower H3K27me3 protein levels were observed and correlated to higher levels of E-cadherin expression. Moreover, the authors showed that E-cadherin expression was associated with histone alterations but not with DNA methylation [102].

To better understand the mechanisms of histone methylation, studies have been performed using cultured cell lines treated with 5-Azacytidine (5-Aza) or 5-Aza-2′-deoxycytidine after treatment, Meng *et al.* [103] showed that a gastric cancer cell line presented a complete reversal of histone modification at the *p16* and *MLH1* promoter region, with increased levels of H3K4 methylation and reduced H3K9me2. Another study by the same group observed reduced H3K9me2 levels correlated with DNA methylation at the *p16* promoter region, leading to reactivation of *p16* expression, confirming their previous study [104].

Histone phosphorylation, ubiquitylation & sumoylation

A correlation between increased gene expression and H3 phosphorylation has been described. H3 Serine 10 (H3S10) is an important phosphorylation site for transcription from yeast to humans [63]. In gastric adenocarcinoma, overexpression of phosphorylated histone H3 was reported and correlated to intestinal type, vessel invasion and lymph node metastasis. Moreover, cases in which phosphorylated histone H3 was overexpressed showed a poorer prognosis than cases with low expression [105].

Like methylation and unlike acetylation, phosphorylation, and possibly, sumoylation, ubiquitylation can be either repressive or activating, depending on the specific sites. Ubiquitylation at H2AK119 was correlated with transcriptional repression, while, conversely at H2BK123 it was associated with transcriptional activation [63]. Deubiquitylation at the H2BK123 site is involved in both gene activation and maintenance of heterochromatic silencing through the action of two distinct proteases: Ubp8 and Ubp10. The sequence of H2B ubiquitylation followed by deubiquitylation is required to establish the appropriate levels of H3K4 and H3K36 methylation [106].

Sumoylation is the only histone post-translational modification described in yeast as repressive and is conserved in mammals [107], and may be generally negative-acting to prevent activating histone post-translational modifications. Its active inhibition occurs through two mechanisms: SUMO-histone may directly block lysine substrate sites that are alternatively acetylated or sumoylated; and sumoylated histones may recruit HDACs both to chromatin and via a SUMO group that occurs on DNA-bound repressors [63].

The SWI/SNF chromatin remodeling complex

Chromatin remodeling is a fundamental process in several key biological activities such as nucleotide synthesis, transcriptional regulation, DNA repair, methylation and recombination [108].

In humans, chromatin remodeling often works in concert with activating chromatin-modifying enzymes, and can generally be categorized into two families: the ISWI and the SWI/SNF family. The ISWI family mobilizes nucleosomes along the DNA [109,110], whereas the SWI/SNF family transiently alters the structure of the nucleosome, thereby exposing DNA. This process requires an ATPase subunit of the chromatin remodeling complex, which utilizes ATP hydrolysis to generate energy needed to alter the chromatin architecture at the nucleosomal level [86].

There is evidence that proteins of ISWI family, comprising of hSNF2L (SMARCA1) and hSNF2H (SMARCA5), have an elevated expression in several human tumors [75,81,85]. However, little is known about the functional importance of these proteins in cancer. In gastric cancer, Gigek *et al.* showed higher expression of hSNF2H protein in gastric tumors compared with non-neoplastic gastric tissue [82]. Furthermore, an inverse association was observed between *hSNF2H* promoter methylation and protein expression.

The SWI/SNF chromatin remodeling complex constitutes a highly related family of multisubunit complexes. SWI/SNF interacts with various oncogenic and tumor-suppressor proteins, such as MYC, BRCA1 and p53, suggesting that SWI/SNF is involved in multiple processes associated with formation and suppression of tumors. However, the mechanisms by which mutations in these complexes lead to carcinogenesis are unclear [111].

Mutations in *ARID1A*, which encodes a member of the SWI/SNF chromatin remodeling family, have recently been identified in several tumor types [111]. Jones *et al.* reported frequent mutation in this gene in gastric tumor displaying microsatellite instability, and that these mutations were

out-of-frame insertions or deletions at mononucleotide repeats [112]. Furthermore, Wang *et al.* showed that mutation spectrum for *ARID1A* differs between subtypes of gastric cancer, and mutation prevalence is negatively associated with mutations in *TP53* [113].

Sentani *et al.* reported that the increased expression of BRG1, a component of the SWI/SNF complex that regulates gene transcription through chromatin remodeling, might be associated with the development and progression of gastric cancer [114]. BRG1 is one of two mutually exclusive catalytic ATPase subunits present in SWI/SNF complexes, the other being the highly homologous BRM. In 2007, Yamamichi *et al.* observed that the epigenetic suppression of *BRM* would probably occur over multiple steps during gastric carcinogenesis, but never occurs in the non-neoplastic gastric tissue [115].

miRNA

ncRNAs have an important role in several biological processes, including cell differentiation, proliferation and apoptosis. Thus aberrant ncRNA expression is involved in various pathological conditions, such as tumorigenesis. The most studied class of ncRNA is an approximately 22-nucleotides long RNA, called miRNA, responsible for mediating post-transcriptional gene silencing of more than 60% of protein-coding genes [116]. miRNAs regulate their targets through either cleavage of the target mRNA or translational repression [117].

In human cancer, miRNA expression differs between normal and tumor tissues, and can act in promoting or suppressing carcinogenesis. Furthermore, miRNA dysregulation can occur through epigenetic modifications, such as DNA hypermethylation, affecting production of primary transcript, their processing to mature miRNAs and/or interaction with their target [116].

As biomarkers candidates, miRNAs have some advantages over mRNA and proteins, owing to its smaller size, stability in archival human tissues (formaldehyde fixed-paraffin embedded samples and body fluids) and its crucial translational regulatory function [118]. Furthermore, miRNA levels in plasma/serum have been demonstrated as potential signatures in cancer diagnosis. Endogenous circulating miRNAs are described as well protected from RNases, highly stable and usually associated with miRNA derived from tumors [119].

From a genome-wide miRNA profile approach in serum from patients with gastric cancer and healthy individuals, Liu *et al.* described a higher expression level of miR-187, miR-371–5p and miR-378 in serum of patients than in control [119]. After analysis, the miR-378 revealed highest sensitivity (87.5%) and specificity (70.7%). The authors suggested that the differences in miR-378 levels between serum from patients and controls could be detected at early stages of the disease. In this study, the authors also describe that patients with all types of gastric cancer, such as adenocarcinoma, signet-ring cell and mucinous carcinoma were included [119]. Therefore, they did not reproduce findings by another group, which used mostly cases of adenocarcinoma. In this second report, five miRNAs were observed to be upregulated in serum of patients than in control group. Sensitivity and specificity of these miRNAs are 80 and 81%, respectively. Functionally, these miRNAs are implicated in immune response (miR-20a and -423–5p), growth and cell cycle (miR-27a and -34) and tissue specific miRNA (miR-1). miR-27 was previously described as upregulated in tissue of the digestive tract, such as gastric and colon tissue, whereas miR-20a and -34a were upregulated in colon and pancreatic cancer, and hepatocellular carcinoma [120].

Aberrant miR-21 levels were also found in both the plasma and gastric cancer tissue of patients when compared with controls [121]. Furthermore, miR-17–5p, -106a and -106b presented higher levels in patients' plasma than in controls, whereas let-7a was lower in the same case. However, different patterns of miR-106b and let-7a levels were observed in patients' gastric tissue, as they showed lower and higher levels than in controls, respectively. Although circulating miRNA are considered to have been released from the tumor, the normal tissue may have the most influence on the plasma levels of these markers [121]. Therefore, these discrepancies remain to be better explained.

Regarding tissue-specific miRNAs, miR-145, miR-27a and miR494 have been identified as differently expressed between intestinal and diffuse-type gastric cancer. Furthermore, miR-32, miR-182 and miR-143 have expressive dysregulation related to pathological stage and therefore might be considered potential diagnostic biomarkers for intestinal-type gastric cancer [122].

Song and Ju reviewed altered miRNAs and their relation with colorectal, liver, pancreatic and gastric cancer [118]. The recently identified miRNAs in gastric tissue and whether epigenetic mechanism controls its expression is provided in Table 3.

Table 3. miRNAs in gastric tissue.

miRNA	Alteration	Target	Target function	Material	Epigenetic control?	Clinicopathological parameter	Other relevant information	Ref.
miR-43c		VEZT	Adherens junction's transmembrane protein	Neoplastic tissue and gastric cancer cells	–	–	–	[127]
miR-124a	Downregulated	*CDK6*	Oncogene – cell cycle progression and differentiation	Gastric cancer cell line	Hypermethylation	–	–	[40]
miR-215	Downregulated	–	–	Gastric cancer cells	–	Increased tumor size and advance tumor	No differences between tumor and nontumor gastric tissue	[128]
miR-203	Downregulated	–	–	Neoplastic tissue and gastric cancer cells	Methylation control in HCC samples	Tumor size and advanced pT stage	–	[129,130]
miR-429	Downregulated	Myc	Proto-oncogene	Neoplastic tissue and gastric cancer cells	–	Lymph node metastasis	Member of miR-200 family	[131]
miR-212	Downregulated	Myc	Proto-oncogene	Neoplastic tissue and gastric cancer cells	Hypermethylation	Metastasis	–	[132]
miR-101	Downregulated	EZH2, Cox-2, Mcl-1 and Fos	Inhibits cellular proliferation, migration and invasion of gastric cancer cells	Neoplastic tissue and gastric cancer cells	–	–	Suppress tumor growth *in vivo*	[133]
miR-192	Downregulated	–	–	Gastric cancer cells	–	Increased tumor size, advance tumor and higher stage	No differences between tumor and nontumor gastric tissue	[128]
miR-148a	Downregulated	ROCK1	Regulates the migration of vascular smooth muscle cells	Neoplastic tissue	–	TNM stage and lymph node metastasis	Overexpression suppressed gastric cancer migration and invasion *in vitro*	
	Downregulated	DNMT1	DNA methyltransferase	Neoplastic tissue and gastric cancer cells	Hypermethylation	–	–	[134]
miR-148b	Downregulated	CCKBR	Acts in the human GI tract mediating the normal physiological function of gastrin	Neoplastic tissue and gastric cancer cells	–	Tumor size	Inhibits cell proliferation *in vitro* and suppresses tumorigenicity *in vivo*	[135]
miR-34b	Downregulated	–	–	–	Hypermethylation	Poor features, such as infiltration and differentiation	–	[136]

HCC: Hepatocellular carcinoma; pT: Pathological tumor; TNM: Tumor node metastasis.

Table 3. miRNAs in gastric tissue (cont.).

miRNA	Alteration	Target	Target function	Material	Epigenetic control?	Clinicopathological parameter	Other relevant information	Ref.
miR-129	Downregulated	SOX4	Transcription factor	–	Hypermethylation	Poor features, such as infiltration and differentiation	–	[136]
miR-449	Downregulated	SIRT1, GMNN, MET, CCNE2	Cancer-associated cell-cycle regulator	Neoplastic tissue	No evidence for loss or hypermethylation	No	Overexpression in gastric cancer cells inhibits growth rate	[137]
miR-10b	Downregulated	*MAPRE1*	Oncogene	Neoplastic tissue and gastric cancer cells	Hypermethylation	Age and diffuse type	–	[138]
miR-182	Downregulated	*CREB1*	Oncogene	Neoplastic tissue	–	Tumor size	Overexpression suppresses proliferation and colony formation	[139]
miR-7	Downregulated	IL1β and TNF-α	Inflammatory response	Gastritis, neoplastic tissue and gastric cancer cells	No methylation control	*Helicobacter pylori*	Transfection of miR-7 suppressed cell proliferation and colony formation	[140]
miR-29a	Downregulated	p42.3	Involved in cell proliferation and tumorigenesis in gastric cancer	Neoplastic tissue and gastric cancer cells	–	–	–	[141]
miR-125a-3p	Downregulated		–	Neoplastic tissue and gastric cancer cells	–	Tumor size and invasion, lymph node and liver metastasis, advanced clinical stage and poor prognosis	Suppresses proliferation of gastric cancer cells *in vitro*	[142]
miR-125a-5p	Downregulated	ERBB2	EGF receptor family of receptor tyrosine kinases	Neoplastic tissue and gastric cancer cells	–	Tumor size and invasion, liver metastasis and poor prognosis	Suppresses proliferation of gastric cancer cells *in vitro*, enhanced by trastuzumab	[143]
miR-375	Downregulated	PDK1 and 14-3-3ζ	Regulation of homeostasis and signal transduction	Neoplastic tissue	–	–	–	[144]
let-7	Upregulated	*RAS* and *HMGA2*	Oncogenes	Metastatic gastric cancer cells	–	–	–	[145]
miR-9	Upregulated	CDX2	Development, maintenance and proliferation of intestinal epithelial cells	Neoplastic tissue and gastric cancer cells	–	–	–	[117]

HCC: Hepatocellular carcinoma; pT: Pathological tumor; TNM: Tumor node metastasis.

Table 3. miRNAs in gastric tissue (cont.).

miRNA	Alteration	Target	Target function	Material	Epigenetic control?	Clinicopathological parameter	Other relevant information	Ref.
miR-16	Upregulated	–	–	Nicotine-treated gastric cancer cells	–	–	Activated via NF-κB	[146]
miR-21	Upregulated	–	–	Nicotine-treated gastric cancer cells	–	–	Activated via NF-κB	[147]
	Upregulated	–	–	Neoplastic tissue	–	No	–	[147]
	Upregulated	PDCD4	Regulates proteins involved in tumor progression, cell cycle and differentiation	Neoplastic tissue	–	No	–	[50]
	Upregulated	PTEN	Tumor suppressor	Neoplastic tissue and gastric cancer cells	–	Differentiation, invasion and lymph node metastasis	*In vitro* downregulation inhibits biological behavior of gastric cancer cells	[96]
miR-192	Upregulated	–	–	Neoplastic tissue and gastric cancer cells	–	–	Exert cell growth and migration-promoting effects *in vitro*	[148]
miR-215	Upregulated	ALCAM	Implicated cell adhesion and migration	Neoplastic tissue and gastric cancer cells	–	–	Exert cell growth and migration-promoting effects *in vitro*	[148]
miR-126	Upregulated	SOX2	Transcription factor	Neoplastic tissue and gastric cancer cells	–	No	–	[149]
miR-146a	Upregulated	SMAD4	Signal transduction proteins	Neoplastic tissue	–	–	Improve cell proliferation and inhibits apoptosis *in vitro*	[150]
miR-200	Upregulated	ZEB1 and ZEB2	Transcriptional repressors of E-cadherin	Gastric cancer cells	–	–	SMAD3 regulates miR-200 family members	[138]
miR-451	Upregulated	MDR-1	Drug resistance	Neoplastic tissue	–	Poor prognosis	–	[151]
miR-199a-3p	Upregulated	–	–	Neoplastic tissue	–	Poor prognosis	–	[151]
miR-195	Upregulated	–	–	Neoplastic tissue	-	Poor prognosis	–	[151]

HCC: Hepatocellular carcinoma; pT: Pathological tumor; TNM: Tumor node metastasis.

Future perspective

It is well known that gene expression is regulated by epigenetic mechanisms both in normal homeostatic and pathological processes. Although a growing number of studies regarding epigenomics in gastric cancer have been published, the complete understanding and interplaying among these marks is not yet clear. Thus, the knowledge of these epigenetic signatures might lead to the development of tissue- and/or serum-specific epimarkers, which may be a useful tool for diagnosis, prognosis and development of new target of therapies.

Two classes of drugs have been more consistently studied: HDAC inhibitors and demethylating agents. As presented in this review, TSA and 5-Aza were shown to have the ability to reverse the abnormalities found in gastric cancer cell lines. Indeed, higher treatment efficiency was achieved when combined treatment – TSA and 5-Aza – was applied into cell cultures. These findings suggest that alterations in gene expression might be restored to the normal conditions.

In fact, TSA and 5-Aza were approved by the US FDA for treatment of hematologic malignancy. As the studies in gastric cancer continue, the mapping of an epigenome code is not far for this disease. In conclusion, an epigenetic therapy might appear in the not too distant future.

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Executive summary

DNA metylation

- Methylation of CpG islands in promoter regions is an important marker associated with cancer initiation and progression. In gastric cancer, several genes have been described with aberrant DNA methylation, with or without alterations in gene function. Hyper- or hypo-methylation of CpG islands can also be associated with gastric cancer type, tumor staging and prognosis. Moreover, expression of DNA methyltransferase family, aging and chronic inflammation by *Helicobacter pylori* might induce abnormal DNA methylation. Although methylation is a tissue-specific marker, detection of DNA methylation status in blood could be a useful as biomarkers.

Histone modification

- Histones are subject to post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation. The interaction between histones and DNA represents epigenetic control, as specific amino acid residues on specific histone core proteins undergo post-translational modifications are able to establish differential expression of associated genes.

Chromatin remodeling complex

- Chromatin remodeling is a fundamental process in several key biological activities, and in humans often works in concert with activating chromatin-modifying enzymes. These enzymes essentially belong to two families: the ISWI and the SWI/SNF family. Mutations, interactions with protein related to carcinogenesis and even epigenetic marks have been described in gastric cancer.

miRNA

- miRNAs have an important role in several biological processes, including cell differentiation, proliferation and apoptosis. Thus, aberrant expression is involved in various pathological conditions, such as gastric tumorigenesis. miRNAs regulate their targets through either cleavage of the target mRNA or translational repression and can also be epigenetic controlled. Furthermore, miRNA levels in plasma/serum have been demonstrated as potential signatures in gastric cancer diagnosis, due to being highly protected from RNases, highly stable and usually associated with observed in miRNA derived from tumor.

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