Overview on Gastric Cancer

Chapter 2

Alteration of DNA Methylation in Gastric Carcinogenesis

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Abstract

Gastric cancer (GC) is one of the leading causes of cancer death worldwide. Aberrant DNA methylation is closely associated with GC development. Understanding the comprehensive profiling of altered DNA methylation in different stages of gastric carcinogenesis will enable development of biomarkers for early detection, risk prediction, and allow for the development of novel targeted intervention strategies. Different DNA methylome mapping techniques are indispensable to realize this project in the future. We discuss in this chapter the recent published evidence and propose future perspectives on the DNA methylation changes in gastric carcinogenesis.

Keywords: DNA methylation; Gastric cancer; Gastric intestinal metaplasia; Carcinogenesis.

1. Introduction

Increasing studies have identified aberrant DNA methylation is one of the earliest molecular alterations in gastric carcinogenesis [1,2]. Generally, cancer cells exhibit two opposing aberrant DNA methylation patterns: global DNA hypomethylation and regional (gene promoter or the first exon region) hypermethylation (**Figure 1**) [3]. Therefore, genes with aberrant methylation status are attractive candidates for detection of early neoplastic events. These changes in DNA methylation status could be used as epigenetic biomarkers for

the diagnosis and prognosis of cancer patients. In this chapter, we will summarize our actual state of knowledge concerning DNA methylation alterations in gastric carcinogenesis and their clinical application as potential biomarkers.

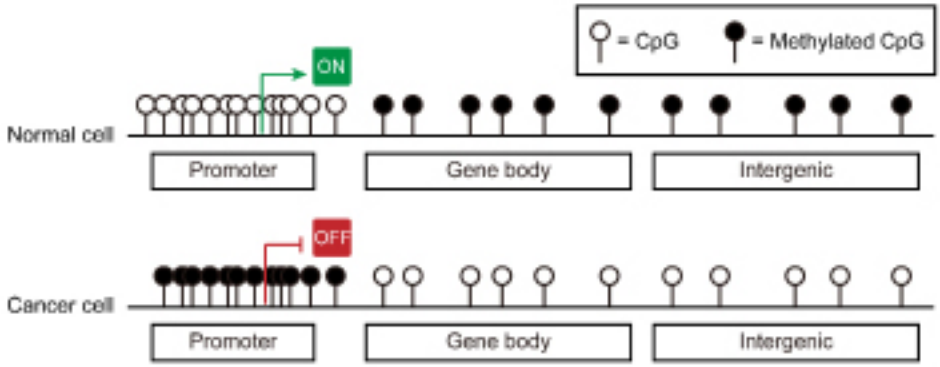

Figure 1: Normal can cancer cells exhibit distinct DNA methylation profiles.

There are significant differences in DNA methylation pattern between normal (top) and cancer (bottom) cells across the entire genome. The cancer epigenome is characterized by simultaneous global losses in DNA methylation (commonly observed in gene body and intergenic regions), interspersed with abnormal hypermethylation in promoter CGI regions.

2. Overview of DNA Methylation

In the mammalian genome, DNA methylation is introduced by the action of the DNA methyltransferase enzymes (DNMTs) that transfer a methyl group from S-adenosylmethionine (SAM) to the fifth carbon of cytosine ring to form 5-methylcytosine (5mC) and typically occurs in the context of cytosine-guanine (CpG) dinucleotides (**Figure 2**) [4]. There are about 28 million CpG sites scattering throughout the human genome, and most (approximately 70%) of them are normally methylated. Indeed, these CpG nucleotides present at a lower-than-expected abundance [5, 6]. This has been explained by the spontaneous deamination of the methylcytosine in the CpG dinucleotides into a thymine, making methylated cytosines usually susceptible to mutation and consequent depletion [7]. However, these CpG dinucleotides tend to cluster in short regions of the genome, known as "CpG islands (CGIs)", usually near the 5' transcription start sites (TSSs) of genes [8,9]. Based on the data released by the UCSC Genome Bioinformatics Site (http://genome.ucsc.edu), about 27,800 CGIs identified in the human genome. These CGIs can be found at the 5' promoter region of approximately 70% of human genes (10). Differing from the bulk of the genome, most CpG loci located within promoter CGIs generally lack DNA methylation in normal somatic cells (Figure 2) [11]. DNA methylation within gene promoter CGIs has been associated with permanent expression silencing such as that noted in the inactive X chromosome in women [12]. It should be noted that although significant proportion of CGIs is located in promoter region, a large class of CGIs can be found within gene bodies (Intragenic), or between genes (Intergenic), however, CGIs in these atypical regions (Intragenic and intergenic CGIs are defined as "orphan" CGIs) show evidence for promoter function (**Figure 3**) [13,14]. These findings support a strong relationship between

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CGIs and transcription initiation.

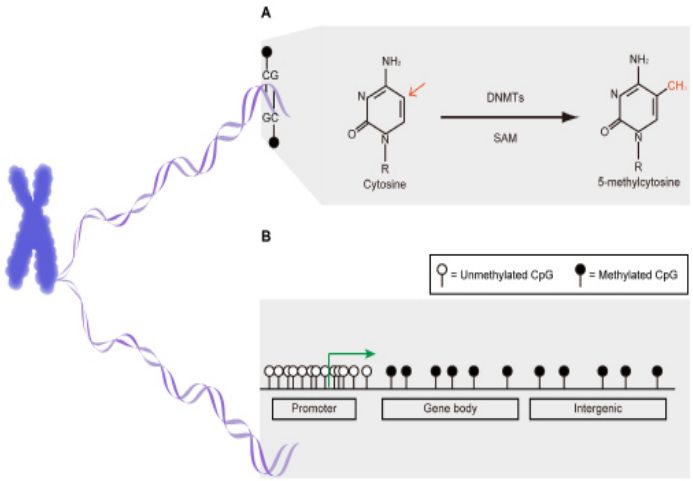

Figure 2: Overview of DNA methylation pattern.

(A) Cells utilize DNA methyltransferase (DNMT) to catalyze the addition of a methyl group to the fifth carbon position of cytosines primarily within CpG dinucleotide contexts (5-methyl-cytosine; 5mC). This process has various effects on transcription, genome instability, and DNA packaging within cells. (B) CpG dinucleotides tend to enrich (CpG islands) in the gene promoter regions. CpG islands at promoters the genes are normally unmethylated, allowing transcription.

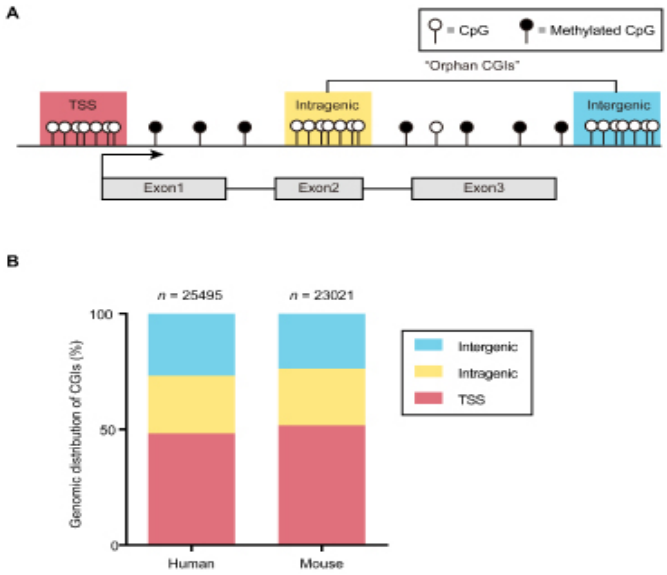

Figure 3: Classification and distribution of CGIs in human and mouse genome.

(A) Schematic illustration of CGIs located at annotated TSSs, within gene bodies (Intragenic), or between genes (Intergenic). CGIs located in intragenic and intergenic regions are termed as “orphan” CGIs. (B) Classification of CGIs with respect to different genomic locations in the human and mouse genome as determined by Illingworth and colleagues (14). The total number of CGIs is shown at the top of each bar.

3. Aberrant DNA Methylation Changes in Gastric Cancer

Hypermethylation of CpG-rich promoters in tumor-suppressor genes, which would lead

to transcriptional silencing, is well-studied in various malignancies [15-18]. Accumulating evidence suggests aberrant hypermethylation of tumor-suppressor genes is also involved in gastric carcinogenesis [19-21].

As a strong candidate for tumor-suppressor gene originally identified in GC, runt-related transcription factor 3 (*RUNX3*) has been extensively studied in gastric carcinogenesis, particularly the methylation status of its promoter. Qing-Lin Li *et al.* [22] proposed that *RUNX3* inactivation through hypermethylation of promoter CGI is a critical event in the pathogenesis of GC. Similarly, subsequent studies [23] study of 1113 subjects with different gastric lesions, methylation status of *RUNX3* determined by methylation-specific PCR showed significantly increased in cases with advanced gastric lesion during the process of carcinogenesis [24]. Moreover, the frequency of *RUNX3* methylation was increased in sequential steps during gastric carcinogenesis, as Tai Young Kim and colleagues reported that 8.1% of chronic gastritis (n = 99), 28.1% of intestinal metaplasia (n = 32), 27.3% of gastric adenomas (n = 77), and 64% of primary gastric carcinomas (n = 75) were methylated in the CGI of *RUNX3* gene [25]. Similarly, Xiao-Xiao Lu and colleagues have also observed that GC tissues showed the highest methylation proportion (75.2%, n = 202) compared with premalignant gastric lesions, including chronic atrophic gastritis (15.9%, n = 220), intestinal metaplasia (36.7%, n = 196), gastric adenoma (41.8%, n = 134), and dysplasia (54.9%, n = 102) [26]. Moreover, *RUNX3* methylation is corelated with depth of tumor invasion, lymph node and distant metastasis, as well as lymphatic vessel and venous invasion in GC (26). Another study demonstrated that *RUNX3*-knockout mice developed hyperplastic gastric epithelia due to promotion of proliferation and suppressed apoptosis in gastric epithelial cells [22], whereas the enforced restoration of RUNX3 expression activates apoptotic pathway in GC [27]. It has been also reported that *RUNX3*-deficiency leads to premalignant changes in the gastric epithelia including intestinal metaplasia in animal model [28].

Ras association domain family member 1A (*RASSF1A*) is another tumor-suppressor gene that has been reported to be frequently silenced and inactivated by aberrant hypermethylation of its promoter region in GC [29]. Ka-Fai To and colleagues examined the presence of gene promoter hypermethylation in different gastric lesions and found that GC tissues had a higher frequency of hypermethylation in *RASSF1A* gene (26%, n = 31) compared with intestinal metaplasia lesion with GC (14%, n = 21) and without GC (7%, n = 15) [30]. Likewise, a pyrosequencing-based quantitative analysis of DNA methylation showed that 35.2% of GC tissues (98 GC cases and 64 controls) exhibit hypermethylation in *RASSF1A* gene [31]. Demao Yao et. al evaluated the promoter methylation status of a panel of cancer-associated genes using quantitative methylation-specific PCR and found a significantly higher methylation level of *RASSF1A* gene in GC tissues (n = 141) than normal gastric tissues (n = 36; $P < 0.0001$) [32]. Moreover, hypermethylation of *RASSF1A* correlated with TNM stage and poor prognosis of

GC patients [33]. Therefore, *RASSF1A* is a promising diagnostic and therapeutic target in GC.

Promoter hypermethylation of O^6-methylguanine-DNA methyltransferase (*MGMT*) gene resulting in gene silencing and loss of function was commonly found in GC (34). Located on 10q26, *MGMT* encodes a DNA repair protein that removes a methyl group from mutagenic O^6-methylguanine, which is produced by alkylating agents and can make a mismatched pair with thymine, causing transition mutation through DNA replication [35]. N Oue and colleagues first reported that transcriptional suppression of *MGMT* by aberrant methylation of the promoter region may play an important role in gastric carcinogenesis [36]. Subsequently, many studies have shown that promoter hypermethylation of the *MGMT* gene occurs more frequently in GC tissues than in normal gastric tissues [37-39]. Moreover, one study with 119 patients reported that GC patients with methylation had shorter survival time than the patients without methylation in *MGMT* gene (29.9 years vs. 55.7 years on average, $P = 0.03$) [40].

Indeed, many other genes are also found to be aberrantly hypermethylated in GC. For example, adenomatous polyposis coli (*APC*) [41], Ras association domain family member 2 (*RASSF2*) [33, 40], cadherin 1 (*CDH1*) (42), protocadherin 10 (*PCDH10*) [43], protocadherin 17 (*PCDH17*) (44), mutL homolog 1 (*hMLH1*) [45], cyclin dependent kinase inhibitor 2A (*CDKN2A*) (46, 47), death associated protein kinase 1 (*DAPK1*) [32,48], homeobox A1 (*HOXA1*) [49], homeobox D10 (*HOXD10*) [50], and so on. Taken together, these findings suggest that promoter hypermethylation of specific genes, especially the tumor-suppressor genes, is an important hallmark of GC, which plays a key role in the initiation and progression of GC. Although methylated tumor-suppressor genes are being intensively investigated in GC, the underlying functions and mechanisms need to be carefully examined.

As mentioned above, a number of tumor-suppressor genes are silenced by aberrant promoter hypermethylation has been preferentially investigated in GC, on the other hand, global DNA hypomethylation have been frequently observed in GC [51-53]. Global DNA hypomethylation has been associated with cancer development through effects on genomic instability [54, 55]. Another potential consequence of DNA hypomethylation is the reactivation of normally silenced genes [56]. In fact, global DNA methylation has been associated with early stages of GC [57].

4. Contribution of DNA Methylation Changes to Gastric Intestinal Metaplasia

Intestinal metaplasia (IM) of the stomach is characterized by the replacement of normal gastric epithelium by intestinal phenotype (goblet cells and enterocytes) and confer an increased risk of GC [58,59]. It should be mentioned that gastric IMs also universally had altered DNA methylation [60]. With the advent of high-throughput sequencing and array-based DNA methylation profiling methods, increasing studies have reported that aberrant DNA methylation usually present in IM lesions [52]. Recently, our group reported that aberrant

DNA methylation occurs in gastric cardiac IM even in cancer-free subjects [61]. Interestingly, abnormal DNA methylation patterns have been identified in Barrett's esophagus (BE: IM lesions in the distal esophagus) using Illumina HumanMethylation27 array [62], Illumina GoldenGate methylation array [63], or Illumina MethylationEPIC array [64]. Indeed, some of these methylation changes are indicative of cancer initiation. Collectively, these observations provide insights on the molecular events governing gastric IM development.

5. Conclusions and Future Perspectives

Epigenetics has undoubtedly emerged as a new frontier in gastric cancer research. Herein, we propose that more attention should be paid to decoding the DNA methylome in gastric premalignant lesions including IM and intraepithelial neoplasia in future studies. Understanding the earliest molecular events associated with GC initiation remains a key bottleneck to transform our approach to cancer prevention and early detection. Although TCGA has provided unprecedented insights into the molecular events associated with cancers, there are few studies concerning DNA methylation profiling precancerous lesions. Thus, some scientists proposed the development of a "Pre-Cancer Genome Atlas (PCGA)" to characterize the molecular alterations in precancerous lesions [65, 66]. As GCs are usually evolve from ben premalignant lesions and have a natural history of progression that provides a window of opportunity for intervention. In summary, DNA methylation alterations during the gastric carcinogenesis could help us understanding the biological characteristics and can be used for risk prediction, early detection, diagnosis, targets for early intervention, and prognosis for GC (**Figure 4**).

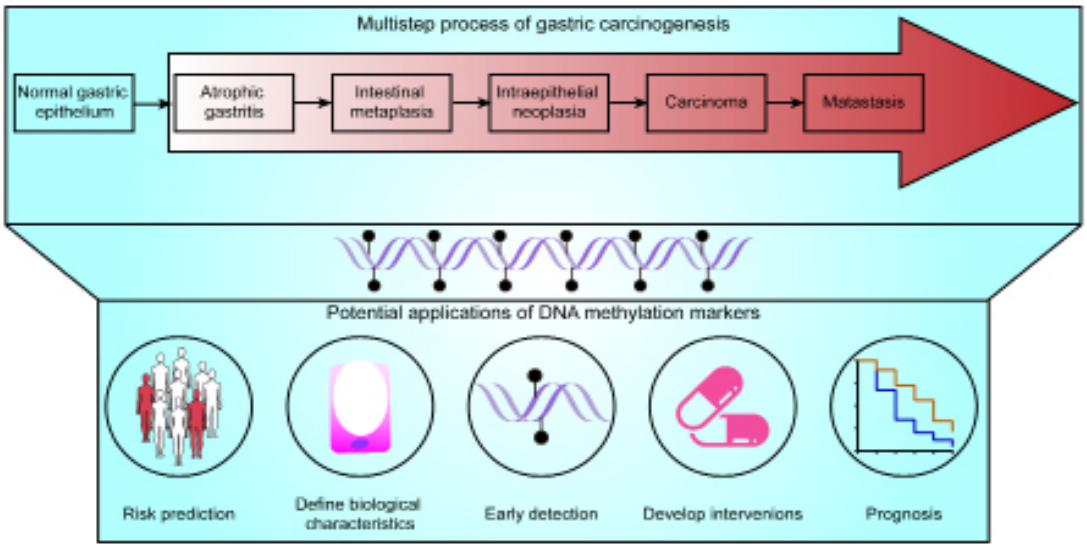

Figure 4: Major benefits of DNA methylation profiling in different stages of gastric carcinogenesis.

The detailed information regarding DNA methylation profiling in different stages of gastric carcinogenesis will generate a greater understanding of the biological underpinnings of how gastric precancerous lesions transform into GC and provide rationale for designing strategies for risk prediction, early detection, intervention, and prognosis evaluation of GC.

6. References

1. Peterson AJ, Menheniott TR, O'Connor L, Walduck AK, Fox JG, Kawakami K, et al. Helicobacter pylori infection promotes methylation and silencing of trefoil factor 2, leading to gastric tumor development in mice and humans. Gastroenterology. 2010;139(6):2005-17.

2. Schmid CA, Müller A. FoxD3 is a novel, epigenetically regulated tumor suppressor in gastric carcinogenesis. Gastroenterology. 2013;144(1):22-5.

3. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res. 1998;72:141-96.

4. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004;4(2):143-53.

5. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. Science. 2001;291(5507):1304-51.

6. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature. 2001;409(6822):860-921.

7. Glass JL, Thompson RF, Khulan B, Figueroa ME, Olivier EN, Oakley EJ, et al. CG dinucleotide clustering is a species-specific property of the genome. Nucleic Acids Res. 2007;35(20):6798-807.

8. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. J Mol Biol. 1987;196(2):261-82.

9. Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. Proc Natl Acad Sci U S A. 2002;99(6):3740-5.

10. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. Proc Natl Acad Sci U S A. 2006;103(5):1412-7.

11. Illingworth RS, Bird AP. CpG islands--'a rough guide'. FEBS Lett. 2009;583(11):1713-20.

12. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci. 2006;31(2):89-97.

13. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010;466(7303):253-7.

14. Illingworth RS, Gruenewald-Schneider U, Webb S, Kerr AR, James KD, Turner DJ, et al. Orphan CpG islands identify numerous conserved promoters in the mammalian genome. PLoS Genet. 2010;6(9):e1001134.

15. Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum Mol Genet. 2007;16 Spec No 1:R50-9.

16. Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. J Pathol. 2002;196(1):1-7.

17. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128(4):683-92.

18. Toyota M, Issa JP. Epigenetic changes in solid and hematopoietic tumors. Semin Oncol. 2005;32(5):521-30.

19. Sapari NS, Loh M, Vaithilingam A, Soong R. Clinical potential of DNA methylation in gastric cancer: a meta-analysis. PLoS One. 2012;7(4):e36275.

20. Gigek CO, Chen ES, Calcagno DQ, Wisnieski F, Burbano RR, Smith MA. Epigenetic mechanisms in gastric cancer. Epigenomics. 2012;4(3):279-94.

21. Oh JH, Jung SH, Hong SJ, Rhyu MG. DNA Methylation as Surrogate Marker For Gastric Cancer. J Cancer Prev.

2015;20(3):172-8.

22. Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, et al. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell. 2002;109(1):113-24.

23. Fan XY, Hu XL, Han TM, Wang NN, Zhu YM, Hu W, et al. Association between RUNX3 promoter methylation and gastric cancer: a meta-analysis. BMC Gastroenterol. 2011;11:92.

24. Li WQ, Pan KF, Zhang Y, Dong CX, Zhang L, Ma JL, et al. RUNX3 methylation and expression associated with advanced precancerous gastric lesions in a Chinese population. Carcinogenesis. 2011;32(3):406-10.

25. Kim TY, Lee HJ, Hwang KS, Lee M, Kim JW, Bang YJ, et al. Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma. Lab Invest. 2004;84(4):479-84.

26. Lu XX, Yu JL, Ying LS, Han J, Wang S, Yu QM, et al. Stepwise cumulation of RUNX3 methylation mediated by Helicobacter pylori infection contributes to gastric carcinoma progression. Cancer. 2012;118(22):5507-17.

27. Sakakura C, Hagiwara A, Miyagawa K, Nakashima S, Yoshikawa T, Kin S, et al. Frequent downregulation of the runt domain transcription factors RUNX1, RUNX3 and their cofactor CBFB in gastric cancer. Int J Cancer. 2005;113(2):221-8.

28. Ito K, Chuang LS, Ito T, Chang TL, Fukamachi H, Salto-Tellez M, et al. Loss of Runx3 is a key event in inducing precancerous state of the stomach. Gastroenterology. 2011;140(5):1536-46.e8.

29. Byun DS, Lee MG, Chae KS, Ryu BG, Chi SG. Frequent epigenetic inactivation of RASSF1A by aberrant promoter hypermethylation in human gastric adenocarcinoma. Cancer Res. 2001;61(19):7034-8.

30. To KF, Leung WK, Lee TL, Yu J, Tong JH, Chan MW, et al. Promoter hypermethylation of tumor-related genes in gastric intestinal metaplasia of patients with and without gastric cancer. Int J Cancer. 2002;102(6):623-8.

31. Balassiano K, Lima S, Jenab M, Overvad K, Tjonneland A, Boutron-Ruault MC, et al. Aberrant DNA methylation of cancer-associated genes in gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). Cancer Lett. 2011;311(1):85-95.

32. Yao D, Shi J, Shi B, Wang N, Liu W, Zhang G, et al. Quantitative assessment of gene methylation and their impact on clinical outcome in gastric cancer. Clin Chim Acta. 2012;413(7-8):787-94.

33. Ben Ayed-Guerfali D, Benhaj K, Khabir A, Abid M, Bayrouti MI, Sellami-Boudawara T, et al. Hypermethylation of tumor-related genes in Tunisian patients with gastric carcinoma: clinical and biological significance. J Surg Oncol. 2011;103(7):687-94.

34. Bhat AA, Wani HA, Waza AA, Malik RA, Masood A, Jeelani S, et al. Diminished expression of MGMT & RASSF1A genes in gastric cancer in ethnic population of Kashmir. J Gastrointest Oncol. 2016;7(6):989-95.

35. Sharma S, Salehi F, Scheithauer BW, Rotondo F, Syro LV, Kovacs K. Role of MGMT in tumor development, progression, diagnosis, treatment and prognosis. Anticancer Res. 2009;29(10):3759-68.

36. Oue N, Shigeishi H, Kuniyasu H, Yokozaki H, Kuraoka K, Ito R, et al. Promoter hypermethylation of MGMT is associated with protein loss in gastric carcinoma. Int J Cancer. 2001;93(6):805-9.

37. Yousuf A, Bhat MY, Pandith AA, Afroze D, Khan NP, Alam K, et al. MGMT gene silencing by promoter hypermethylation in gastric cancer in a high incidence area. Cell Oncol (Dordr). 2014;37(4):245-52.

38. Wang M, Li Y, Gao J, Li Y, Zhou J, Gu L, et al. p16 Methylation is associated with chemosensitivity to fluorouracil in patients with advanced gastric cancer. Med Oncol. 2014;31(6):988.

39. Jin J, Xie L, Xie CH, Zhou YF. Aberrant DNA methylation of MGMT and hMLH1 genes in prediction of gastric cancer. Genet Mol Res. 2014;13(2):4140-5.

40. Shi J, Zhang G, Yao D, Liu W, Wang N, Ji M, et al. Prognostic significance of aberrant gene methylation in gastric cancer. Am J Cancer Res. 2012;2(1):116-29.

41. Zhou X, Jiao D, Dou M, Zhang W, Hua H, Chen J, et al. Association of APC gene promoter methylation and the risk of gastric cancer: A meta-analysis and bioinformatics study. Medicine (Baltimore). 2020;99(16):e19828.

42. Grady WM, Willis J, Guilford PJ, Dunbier AK, Toro TT, Lynch H, et al. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet. 2000;26(1):16-7.

43. Yu J, Cheng YY, Tao Q, Cheung KF, Lam CN, Geng H, et al. Methylation of protocadherin 10, a novel tumor suppressor, is associated with poor prognosis in patients with gastric cancer. Gastroenterology. 2009;136(2):640-51.e1.

44. Hu X, Sui X, Li L, Huang X, Rong R, Su X, et al. Protocadherin 17 acts as a tumour suppressor inducing tumour cell apoptosis and autophagy, and is frequently methylated in gastric and colorectal cancers. J Pathol. 2013;229(1):62-73.

45. Ling ZQ, Tanaka A, Li P, Nakayama T, Fujiyama Y, Hattori T, et al. Microsatellite instability with promoter methylation and silencing of hMLH1 can regionally occur during progression of gastric carcinoma. Cancer Lett. 2010;297(2):244-51.

46. Ficorella C, Cannita K, Ricevuto E, Toniato E, Fusco C, Sinopoli NT, et al. P16 hypermethylation contributes to the characterization of gene inactivation profiles in primary gastric cancer. Oncol Rep. 2003;10(1):169-73.

47. Ksiaa F, Ziadi S, Amara K, Korbi S, Trimeche M. Biological significance of promoter hypermethylation of tumor-related genes in patients with gastric carcinoma. Clin Chim Acta. 2009;404(2):128-33.

48. Yuan W, Chen J, Shu Y, Liu S, Wu L, Ji J, et al. Correlation of DAPK1 methylation and the risk of gastrointestinal cancer: A systematic review and meta-analysis. PLoS One. 2017;12(9):e0184959.

49. Kang GH, Lee S, Cho NY, Gandamihardja T, Long TI, Weisenberger DJ, et al. DNA methylation profiles of gastric carcinoma characterized by quantitative DNA methylation analysis. Lab Invest. 2008;88(2):161-70.

50. Wang L, Chen S, Xue M, Zhong J, Wang X, Gan L, et al. Homeobox D10 gene, a candidate tumor suppressor, is downregulated through promoter hypermethylation and associated with gastric carcinogenesis. Mol Med. 2012;18(1):389-400.

51. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202-9.

52. Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, et al. Genomic and Epigenomic Profiling of High-Risk Intestinal Metaplasia Reveals Molecular Determinants of Progression to Gastric Cancer. Cancer Cell. 2018;33(1):137-50.e5.

53. Baba Y, Murata A, Watanabe M, Baba H. Clinical implications of the LINE-1 methylation levels in patients with gastrointestinal cancer. Surg Today. 2014;44(10):1807-16.

54. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature. 1998;395(6697):89-93.

55. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003;300(5618):455.

56. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene. 2002;21(35):5400-13.

57. Cravo M, Pinto R, Fidalgo P, Chaves P, Glória L, Nobre-Leitão C, et al. Global DNA hypomethylation occurs in the early stages of intestinal type gastric carcinoma. Gut. 1996;39(3):434-8.

58. den Hoed CM, Holster IL, Capelle LG, de Vries AC, den Hartog B, Ter Borg F, et al. Follow-up of premalignant lesions in patients at risk for progression to gastric cancer. Endoscopy. 2013;45(4):249-56.

59. Kim N, Park RY, Cho SI, Lim SH, Lee KH, Lee W, et al. Helicobacter pylori infection and development of gastric cancer in Korea: long-term follow-up. J Clin Gastroenterol. 2008;42(5):448-54.

60. Sugimoto R, Habano W, Yanagawa N, Akasaka R, Toya Y, Sasaki A, et al. Molecular alterations in gastric cancer and the surrounding intestinal metaplastic mucosa: an analysis of isolated glands. Gastric Cancer. 2021;24(2):382-91.

61. Lin R, Li C, Liu Z, Wu R, Lu J. Genome-wide DNA methylation profiling identifies epigenetic signatures of gastric cardiac intestinal metaplasia. J Transl Med. 2020;18(1):292.

62. Xu E, Gu J, Hawk ET, Wang KK, Lai M, Huang M, et al. Genome-wide methylation analysis shows similar patterns in Barrett's esophagus and esophageal adenocarcinoma. Carcinogenesis. 2013;34(12):2750-6.

63. Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, et al. DNA methylation profiling in Barrett's esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics. 2011;6(12):1403-12.

64. Jammula S, Katz-Summercorn AC, Li X, Linossi C, Smyth E, Killcoyne S, et al. Identification of Subtypes of Barrett's Esophagus and Esophageal Adenocarcinoma Based on DNA Methylation Profiles and Integration of Transcriptome and Genome Data. Gastroenterology. 2020;158(6):1682-97.e1.

65. Campbell JD, Mazzilli SA, Reid ME, Dhillon SS, Platero S, Beane J, et al. The Case for a Pre-Cancer Genome Atlas (PCGA). Cancer Prev Res (Phila). 2016;9(2):119-24.

66. Srivastava S, Ghosh S, Kagan J, Mazurchuk R. The PreCancer Atlas (PCA). Trends Cancer. 2018;4(8):513-4.