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The Effect of DNA Methylation on Gene Regulation and Human Cancer Development

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Abstract **Review Article**

DNA methylation is the prominent chemical process in regulating gene expression, which is strongly associated with normal development and cell functions. DNA methyltransferases (*DNMTs*) serve both functions of establishing and maintenance of the original pattern DNA methylation. Epigenetic modifications are resulted from alterations of DNA methylation patterns occurring in coding strands, thus increase DNA adduct formation, somatic mutations, and oncogene activation. Promoter hypermethylation silences tumor-suppressor, and regulation and expression of gene due to DNA methylation have been mostly focused in human cancer research. But, global DNA hypomethylation contributing to genomic instability and cell transformation has been also shown as a cause of oncogenesis. DNA methylation of the promoter region for genes associated to cancer is raising as a potential marker for early detection, prognosis and real-time follow-up of tumor dynamics. This paper aims to review the crucial role of DNA methylation in gene regulation and the effect of the aberrations in DNA methylation in human cancer progression and development. The elucidation of aberrant DNA methylation deemed as a cancer-inducing mechanism may help the discovering of prognostic DNA methylation markers useful in cancer therapy.

Keywords: DNA methylation, epigenetics, hypermethylation, hypomethylation, gene expression.

INTRODUCTION

Identification of cancer-specific epigenetic alterations was shown as one the fundamental element which may help for cancer diagnosis. Epigenetics have been described as occurrence of stably heritable phenotype in gene, which does not depend on the changes in the DNA sequence [1]. The major epigenetic mechanisms in the human genome include modifications of histones, which are the main protein components of chromatin, and methylation of the cytosine nucleotide in DNA [2]. DNA methylation is an epigenetic mechanism utilized by the cell to control gene expression, and is occurred by the taransfering of a methyl group to the cacbon-5 position of the cytosine ring of DNA [3] (Figure-1). It represents a relatively stable and conserved mark, which make it an attractive choice for epigenetic studies. In mammalians, DNA methylation occurs in the context of cytosine-phosphate-guanosine (CpG) dinucleotides regions of DNA, and guanine is preceded by a cytosine nucleotide [3]. The estimation of methylated CpGs in mammals was found between 70 to 80 percent [4]. CpG islands as genomic regions with high frequency of CpG sites are typically associated with active transcription, but also contain largely unmethylated CpGs [5]. Approximately 70 % of annotated genes are estimated to be associated with a CpG island in their promoter regions [6]. Currently, the researchers have been discovered up to four DNA methyltransferases (*DNMTs)* describe as key enzymes responsible for catalysis of DNA methylation mechanism including *DNMT1*, *DNMT3a*, *DNMT3b* and *DNMT3L* (Figure-1) [7]. *DNMT1* is responsible for maintenance of methyl groups that are already present on one of the DNA strands and reproduces also DNA methylation patterns from hemi-methylated DNA [7]. The *DNMT3* consists of *DNMT3a* and *DNMT3b* facilitates the methylation patterns early in development and carcinogenesis [8]. Furthermore, it has been reported that interaction between *DNMT3L* and *Dnmt3a or Dnmt3b* causes stimulation of DNA activity of those two *DNMTs*; *DNMT3a* and *DNMT3b* [9].

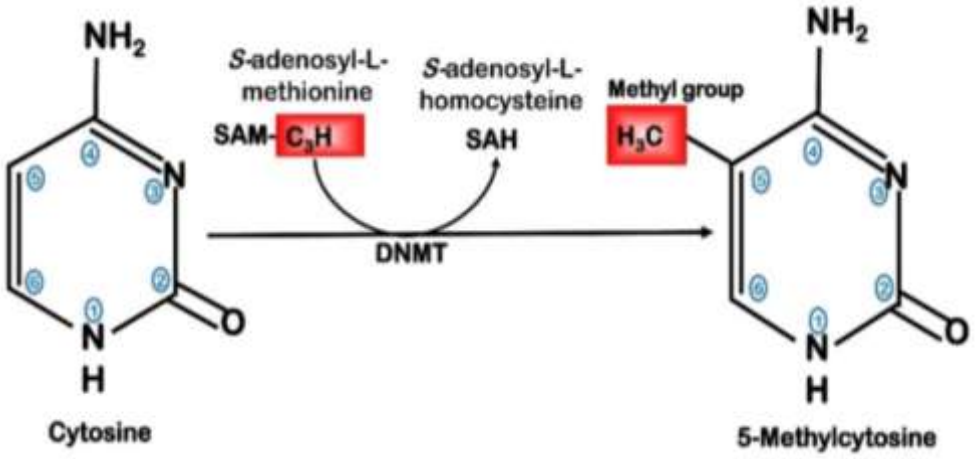

Fig-1: DNA methyltransferase (DNMT) catalyzes the methylation reaction (Modified, based on Bruce Richardson, 2007)

DNA methylation is a crucial process in human genome involving in regulation of gene expression and maintaining genome stability through chromatin structure modeling [10]. DNA methylation can either physically impede the binding of transcription factors, or mediate transcriptional repression by attracting proteins that compact chromatin, which suppresses gene expression [11]. DNA methylation has different effects depending on genomic regions; in gene bodies it is associated with transcription activity, while in promoter it is correlated with gene silencing [12] (Figure-2).

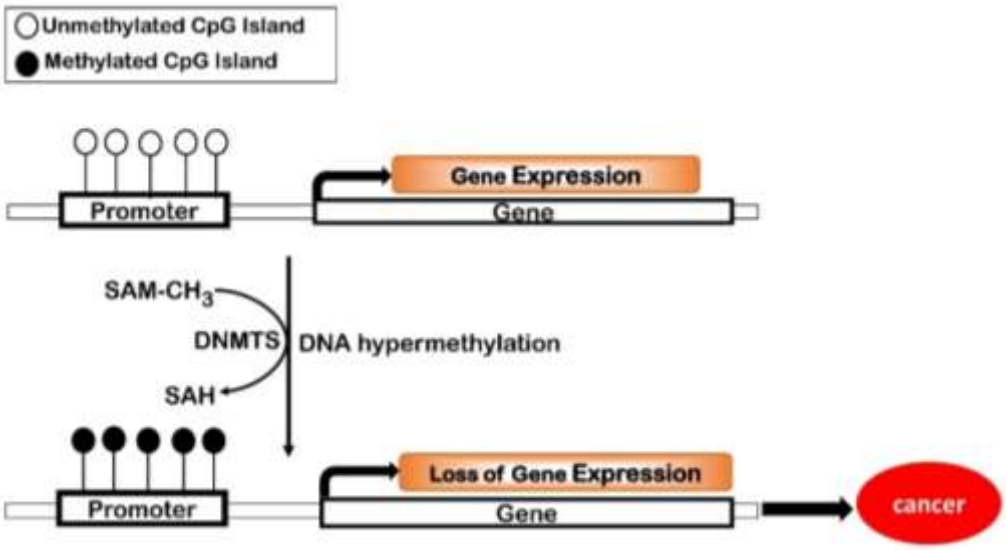

Fig-2: The typical CpG Island of a tumor suppressor gene is represented in a normal and a tumor cell (Modified, based on Thuy et al.; 2017)

For its implication in genes regulating developmental process, DNA methylation has important roles for proper biological development and functioning. It is essential for genomic imprinting [13], X-chromosome inactivation [14] and differentiation, and maintenance of cellular identity [15]. Furthermore, DNA methylation alterations have been indicated as promising targets in cancer treatment through the development of powerful diagnostic, prognostic, and predictive biomarkers, that can be used for the treatment of cancer patients to a new level [16]. DNA methylation markers are more advantages than other molecular markers depending on their chemically and biologically stability which are high compared to RNA or most proteins [17]. Alterations in DNA methylation was identified to affect regulation of gene expression, thus plays a crucial role for changes in cellular growth and division leading to serious diseases including cancer. Particularly, it causes tumor suppressor genes to contribute to tumor initiation and progression. Cancer researchers have difficulties of getting required information on the association between altered DNA methylation and gene regulation. This paper identifies some effect of altered DNA methylation on gene expression and cancer development, which may help for developing more effective cancer therapies.

Hypomethylation in the regulation of gene expression

Hypomethylation is one of the DNA methylation process caused by the loss of a methyl group or the unmethylated state of the most CpG sites in a specific sequence that is normally methylated in somatic tissues [18]. In general, hypomethylation of the genome and of specific genes was reported in human tumors [19]. Interestingly, enough evidence was provided by research study showing the association between hypomethylation of specific genes and transcriptional activity [20]. Transcription activation of repeated sequences was reported to be influenced by hypomethylation of repeated DNA sequences [21]. Besides, hypomethylation is also associated with gene expression. DNA hypomethylation was found to play important role in B cell differentiation and gene overexpression. Moreover, it was suggested that DNA hypomethylation correlated with gene expression, may influence plasma cell division and differentiation [22]. Interestingly, global hypomethylation was shown as crucial factor involved not only in regulation of programmed death-ligand 1 (*PD-L1*), but also causes its constitutive expression [23]. In addition, overexpression of the *ER-α* gene correlated with aberrant DNA hypomethylation in its promoter region in uterine leiomyoma, which might be caused by the reduced level of *DNMT*-3 [24]. Hypomethylation at later site of 5'-region of the calcitonin was also reported to associate with over-expression of the calcitonin gene in medullary thyroid carcinoma [25]. The research showed the hypomethylation of *ST6GALNAC1* gene at 2 base pair upstream of the transcription start site in *ER*−/*PR*− breast cancer, and that might induce gene expression by activating transcription due to the location of the methylation site near the promoter sequence [26]. Body-hypomethylated genes occupying a unique epigenetic niche within the human genome not only strongly influence expression, but also cause disruption on regulatory function [27]. Currently, researchers reported the association between DNA hypomethylation with over-expression tumor-related genes, such as maspin [28] and synuclein γ [29] and cancer/testis antigens including melanoma [30], and that was found in various human cancer.

Hypermethylation in the regulation of gene expression

Many studies have been described DNA hypermethylation as one of the key factor influencing gene expression. CpG island hypermethylation is a common mechanism occurred in the tumor suppressor gene, and essentially involved in the inactivation of those genes in human cancers [31]. Interestingly, hypermethylation was firstly discovered in a promoter region of calcitonin gene [25]. Aberrant hypermethylation occurring in promoter region causes the silencing of tumor suppressor genes and represent an alternative inactivating mechanism to mutations (Figure-2). Aberrant hypermethylation in the promoter region has been described for several tumor suppressor genes in breast cancer including *CDH1*, *RASSF1A*, and *BRCA1*. Promoter hypermethylation of *BRCA1* was shown to cause inactivation *BRCA1* expression, and that resulted breast tumorigenesis, and it is proposed to be a potential biomarker utilized for prognostic assessment [32, 33]. In addition, *de novo* methylation of CpG islands in gene promoter or enhancer regions has been reported as an important factor which can influence loss of gene expression [34]. However, hypermethylation of CpG-rich regions within gene body regions may involve in silencing of one of two or more alternative promoters of a gene altering expression of particular transcript gene isoforms [35, 36]. Hypermethylation of gene body or transcribed regions was also reported to be associated with higher gene expression levels [12], and transcription running across the CpG island [37]. Interestingly, hypermethylation of the CpG island-promoter is associated with genes, which are essential in various cellular pathways such as cell cycle, DNA repair, carcinogen metabolism, cell adherence, apoptosis, cell growth, etc [38].

DNA hypermethylation represses transcription activity of gene through several mechanisms such as inhibition of transcription factors like *AP-2*, *c-Myc/Myn*, *E2F*, *NF-κB* to their binding sites within promoter regions. The other mechanism consists of the binding of proteins specific for m5CpG dinucleotides to methylated DNA. For instance, some essential binding proteins such as methyl-CpG binding proteins (*MeCP1* and *MeCP2*), and methyl-CpG binding domain *MBD* proteins (*MBD1-4*) were reported to be recruited during the process [39]. As a common mechanism for large number genes, methylation in the promoter region leading to inactivation of estrogen receptor gene alpha (*ER α*) is associated with aging in some tissues of the cardiovascular system, and that is essential in atherogenesis [40]. Moreover, higher levels of DNA methylation in promoter region of some identified genes have been shown to be associated with hormone receptor positive status of breast tumors [41]. On the other hand, aberrant hypermethylation is associated with inactivation of both estrogen (*ER α*) gene and progesterone receptor (*PR*) gene [40, 42]. Currently, aberrant promoter hypermethylation is considered as the core mechanism leading transcriptional inactivation.

DNA methylation and cancer

DNA methylation was reported in many studies as epigenetic marks essential in cancer genome [43]. DNA methylation was shown to involve significantly in cancer development due to the reason that methylation causes silencing of tumor suppressor genes within the promoter regions (Figure-2), and can also cause mutation in the gene itself [44]. Abnormal DNA methylation of imprinted loci was reported in various types of human cancer, including colon, breast, liver, bladder, Wilms, ovarian, esophageal, prostate, and

bone cancers. In addition, current studies on the applications of omics technologies have shown that there are numerous differential DNA methylations associated to cancers, including hepatocellular carcinoma, glioblastoma, breast cancer, squamous cell lung cancer, thyroid carcinoma, and leukemia [45-54]. Several types of aberration were shown in both DNA methylation and the proteins involving in DNA methylation during cancer development, and those include not only hypermethylation of tumor suppressor genes and abnormal expression of DNA methyltransferases, but also DNA hypomethylation of unique genes and repetitive sequences was found in carcinogenesis [55-57]. The loss of DNA methylation was reported in 1983, as the first-described epigenetic changes linked to human cancer, and also genes of cancer cell showed the significant hypomethylation than normal tissue [58]. In addition, DNA hypomethylation was found to contribute to genomic instability and the initiation of intestinal cancer [59]. It was shown that the global DNA hypomethylation in breast cancer was linked to repressive chromatin domains formation and silencing of tumor suppressor genes [60].

Expression of imprinted genes are another type of genes reported to be influence by abnormal DNA methylation, as the loss of imprinting insulin-like growth factor-2 (*IGF2*) gene, and the tightly-linked *H19* were found to favor tumorigenesis in various cancer types due to the overexpression and global chromatin instability [61]. Interestingly, detecting a loss of imprinting in *IGF2* gene was suggested to be a powerful tool for the diagnosis of human cancer. DNA hypermethylation was identified as the most promising biomarker that can be used as an effective diagnostic tool for human cancers detection, especially in lung cancer treatment [62]. It was reported that DNA methylation can lead to inactivation of X-chromosome (*XCI*) [63], and that has been shown to occur in breast and ovarian cancer patients, in *BRCA1* and possibly *BRCA2* mutation carriers in comparison to control subjects. Therefore, it is correlated with a significant increase in the age of diagnosis of those women's cancer; breast and ovarian cancer [64]. In addition, DNA methylation might provide a potential, tumor-specific marker as showed to plays a key role in *PR* gene silencing in leukemia [42]. Promoter hypermethylation causes silencing of expression of very important transcription factors and the associated component such as TGF-β signaling and human runt-related transcription factor 3 (Runx3), involving in various roles in control of cell proliferation and differentiation therefore, leads to the development numerous human cancer, including gastric cancer [65], cholangiocarcinoma [66], pancreatic cancer [67], and esophageal squamous cell carcinoma [68]. Moreover, suppression of expression TGF-β and its receptors due to aberrant DNA hypermethylation was also reported in renal carcinoma [69], lung cancer and prostate cancer [70]. DNA hypermethylation significantly occurs in many genes involving in biochemical pathways associated with tumor development or progression. These genes play important role in function of numerous cellular processes such as cell cycle, DNA repair, apoptosis, metastasis, detoxification, hormone response, Ras signaling, and Wnt signaling [71].

It was reported that aberrant promotor hypermethylation of tumor suppressor genes and cancer related genes occurs at the early stage of ovarian cancer development, and that was found for numerous genes include *OPCML*, *BRCA1*, *p16 and TMS1* [72, 73]. DNA methylation of tumor suppressor gene specific to cancer cells provides opportunities for novel, noninvasive early detection strategies for various human cancers. For instance, detection of methylated tumor suppressor genes in sputum may be utilized to detect lung cancer, and in urine for bladder cancer [74, 75]. Interestingly, it was suggested that high-density CG islands and CpG island shores are associated with differential methylation in cancers. Shores correlated with hypomethylation and gene overexpression in cancer have been found for genes involving in the cell cycle. That suggests an important role for shores region for involving in the unregulated growth, which is a characteristic for cancer development [76]. Numerous studies have been focused on DNA methylation of tumor suppressor genes for the purpose of identifying DNA methylation biomarkers of cancer. However, hypomethylation is also essential, because critical genes for cancer growth and metastasis are associated with hypomethylation in cancer [77-79]. DNA demethylation is essential in cancer through activation of several pro-metastatic genes, including the heparanase gene [77], *MMP2* encoding matrix metalloproteinase-2 [78], and *uPA* which activates urokinase plasminogen activator [79]. Utilizing functional biocomputational analysis, the hypomethylated genes were hypothesized to be correlated with cell growth, invasion, and metastasis functions, which are mainly associated with cancer development and metastasis [80]. In addition, dissimilarity in epigenetic reprogramming was identified between primary tumor and distant metastases in the identical patient [81], however, driver mutations were not identified among the metastases [82]. Therefore, epigenetic dysregulation was suggested to play an important role in tumor development and metastasis, and also indicate its potential application in cancer diagnosis [83, 84], prognosis [85] and treatment [86, 87]. Furthermore, the discovery of novel epigenetically inactivated tumor suppressor genes can provide knowledge on tumorigenesis in depth, and give a basis for further research for the discovery and development of new targeted therapies like demethylating agents.

CONCLUSION AND PERSPECTIVES

DNA methylation is involved in normal development of mammals in different process, including proper growth, cell adhesion, and genetic

transmission, but defects in DNA methylation cause diseases. Dysregulation of the DNA methyltransferases leads to aberrant methylation as shown in various type of human cancers. Furthermore, altered DNA methylation involved in inactivation of tumor suppressor genes and that plays crucial role in the control of cell proliferation and transformation, therefore, may initiate or cause progression of cancer. Given the prominent roles recognized for DNA methylation in clinical studies, increasing efforts have been devoted to targeting oncogenic DNA methyltransferase genes and proteins. Moreover, the genetic and epigenetic may have synergistic effect contributing to cancer development. Aberrant DNA methylation changes, that are stable and inherited through multiple cell division, occur early in carcinogenesis, thus it could be utilized as a noninvasive biomarker for cancer early detection and prognosis. In addition, methylation biomarkers can be utilized for predicting response or resistance to chemotherapy. Reversibility of DNA methylation is another feature which plays a key role in discovering epigenetic drugs currently in use for the treatment of patients with hematological malignancies. However, the utilization of methylation markers in the treatment of different types of human cancer is still inadequate due to certain factors such as our incomplete knowledge about patterns of DNA methylation, various detection methods, specimens type (tissue, stool, and blood), and cancer heterogeneity. Therefore, there is still a pressing need for further randomized clinical trials and large-scale investigations, especially in different populations in order to identify specific, sensitive, and cost-effective methylation biomarkers for human cancers. Better understanding the effect of DNA methylation on gene regulation and human cancer initiation and progression has certainly helped in the discovery and development of promising tools useful for cancer treatment.

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REFERENCES

1. Nebbioso A, Tambaro FP, Dell'Aversana C, Altucci L. Cancer epigenetics: Moving forward. PLoS genetics. 2018; 14(6): e1007362.
2. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circulation. 2011; 123(19): 2145-56.
3. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2013; 38(1): 23-38.
4. Gruber DR, Toner JJ, Miears HL, Shernyukov AV, Kiryutin AS, Lomzov AA, Endutkin AV, Grin IR, Petrova DV, Kupryushkin MS, Yurkovskaya AV, Johnson EC, Okon M, Bagryanskaya EG, Zharkov DO, Smirnov SL. Oxidative damage to epigenetically methylated sites affects DNA stability, dynamics and enzymatic demethylation. Nucleic Acids Research. 2018; gky893-gky.
5. Jeziorska DM, Murray RJS, De Gobbi M, Gaentzsch R, Garrick D, Ayyub H, Chen T, Li E, Telenius J, Lynch M, Graham B, Smith AJH, Lund JN, Hughes JR, Higgs DR, Tufarelli C. DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. Proceedings of the National Academy of Sciences. 2017; 114(36): E7526.
6. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(5): 1412-7.
7. Bestor TH. The DNA methyltransferases of mammals. Human molecular genetics. 2000; 9(16): 2395-402.
8. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999; 99(3): 247-57.
9. Suetake I, Shinozaki F, Miyagawa J, Takeshima H, Tajima S. DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. The Journal of biological chemistry. 2004; 279(26): 27816-23.
10. Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y, Cao L. DNA Methylation, Its Mediators and Genome Integrity. International Journal of Biological Sciences. 2015; 11(5): 604-17.
11. Booij L, Wang D, Lévesque ML, Tremblay RE, Szyf M. Looking beyond the DNA sequence: the relevance of DNA methylation processes for the stress–diathesis model of depression. Philosophical Transactions of the Royal Society B: Biological Sciences. 2013; 368(1615):
12. Yang X, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G. Gene body methylation can alter gene expression and is a therapeutic target in cancer. Cancer cell. 2014; 26(4): 577-90.
13. SanMiguel JM, Bartolomei MS. DNA methylation dynamics of genomic imprinting in mouse development. Biology of reproduction. 2018; 99(1): 252-62.
14. Sado T, Okano M, Li E, Sasaki H. De novo DNA methylation is dispensable for the initiation and propagation of X chromosome inactivation. Development (Cambridge, England). 2004; 131(5): 975-82.
15. Suelves M, Carrio E, Nunez-Alvarez Y, Peinado MA. DNA methylation dynamics in cellular commitment and differentiation. Briefings in functional genomics. 2016; 15(6): 443-53.
16. Koch A, Joosten SC, Feng Z, de Ruijter TC, Draht MX, Melotte V, Smits KM, Veeck J, Herman JG, Van Neste L, Van Criekinge W, De Meyer T, van

Engeland M. Analysis of DNA methylation in cancer: location revisited. Nature Reviews Clinical Oncology. 2018; 15(7): 459-66.
17. Laird PW. The power and the promise of DNA methylation markers. Nature reviews Cancer. 2003; 3(4): 253-66.
18. Feinberg AP, Tycko B. The history of cancer epigenetics. Nature Reviews Cancer. 2004; 4(143.
19. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics. 2009; 1(2): 239-59.
20. Mendizabal I, Zeng J, Keller TE, Yi SV. Body-hypomethylated human genes harbor extensive intragenic transcriptional activity and are prone to cancer-associated dysregulation. Nucleic Acids Res. 2017; 45(8): 4390-400.
21. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. Biochimica et biophysica acta. 2007; 1775(1): 138-62.
22. Barwick BG, Scharer CD, Bally APR, Boss JM. Plasma cell differentiation is coupled to division-dependent DNA hypomethylation and gene regulation. Nature immunology. 2016; 17(10): 1216-25.
23. Chatterjee A, Rodger EJ, Ahn A, Stockwell PA, Parry M, Motwani J, Gallagher SJ, Shklovskaya E, Tiffen J, Eccles MR, Hersey P. Marked global DNA Hypomethylation Is Associated with Constitutive PD-L1 Expression in Melanoma. iScience. 2018; 4(312-25.
24. Asada H, Yamagata Y, Taketani T, Matsuoka A, Tamura H, Hattori N, Ohgane J, Hattori N, Shiota K, Sugino N. Potential link between estrogen receptor-alpha gene hypomethylation and uterine fibroid formation. Molecular human reproduction. 2008; 14(9): 539-45.
25. Baylin SB, Hoppener JW, de Bustros A, Steenbergh PH, Lips CJ, Nelkin BD. DNA methylation patterns of the calcitonin gene in human lung cancers and lymphomas. Cancer Res. 1986; 46(6): 2917-22.
26. Li L, Lee K-M, Han W, Choi J-Y, Lee J-Y, Kang GH, Park SK, Noh D-Y, Yoo K-Y, Kang D. Estrogen and progesterone receptor status affect genome-wide DNA methylation profile in breast cancer. Human Molecular Genetics. 2010; 19(21): 4273-7.
27. Mendizabal I, Zeng J, Keller TE, Yi SV. Body-hypomethylated human genes harbor extensive intragenic transcriptional activity and are prone to cancer-associated dysregulation. Nucleic acids research. 2017; 45(8): 4390-400.
28. Akiyama Y, Maesawa C, Ogasawara S, Terashima M, Masuda T. Cell-type-specific repression of the maspin gene is disrupted frequently by demethylation at the promoter region in gastric intestinal metaplasia and cancer cells. The American journal of pathology. 2003; 163(5): 1911-9.
29. Gupta A, Godwin AK, Vanderveer L, Lu A, Liu J. Hypomethylation of the synuclein gamma gene CpG island promotes its aberrant expression in breast carcinoma and ovarian carcinoma. Cancer Res. 2003; 63(3): 664-73.
30. Jang SJ, Soria JC, Wang L, Hassan KA, Morice RC, Walsh GL, Hong WK, Mao L. Activation of melanoma antigen tumor antigens occurs early in lung carcinogenesis. Cancer Res. 2001; 61(21): 7959-63.
31. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene. 2002; 21(5427.
32. Truong PK, Lao TD, Doan TP, Le TA. BRCA1 promoter hypermethylation signature for early detection of breast cancer in the Vietnamese population. Asian Pacific journal of cancer prevention : APJCP. 2014; 15(22): 9607-10.
33. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst. 2000; 92(7): 564-9.
34. Pfeifer GP. Defining Driver DNA Methylation Changes in Human Cancer. International journal of molecular sciences. 2018; 19(4): 1166.
35. Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP. A human B cell methylome at 100-base pair resolution. Proc Natl Acad Sci U S A. 2009; 106(3): 671-8.
36. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJM, Haussler D, Marra MA, Hirst M, Wang T, Costello JF. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010; 466(7303): 253-7.
37. Jeziorska DM, Murray RJS, De Gobbi M, Gaentzsch R, Garrick D, Ayyub H, Chen T, Li E, Telenius J, Lynch M, Graham B, Smith AJH, Lund JN, Hughes JR, Higgs DR, Tufarelli C. DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114(36): E7526-E35.
38. Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum Mol Genet. 2007; 16 Spec No 1(R50-9.
39. Luczak MW, Jagodzinski PP. The role of DNA methylation in cancer development. Folia histochemica et cytobiologica. 2006; 44(3): 143-54.
40. Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, Milliken EE, Issa JP. Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. Cardiovascular research. 1999; 43(4): 985-91.

41. Benevolenskaya EV, Islam ABMMK, Ahsan H, Kibriya MG, Jasmine F, Wolff B, Al-Alem U, Wiley E, Kajdacsy-Balla A, Macias V, Rauscher GH. DNA methylation and hormone receptor status in breast cancer. Clinical Epigenetics. 2016; 8(1): 17.
42. Liu ZJ, Zhang XB, Zhang Y, Yang X. Progesterone receptor gene inactivation and CpG island hypermethylation in human leukemia cancer cells. FEBS letters. 2004; 567(2-3): 327-32.
43. Sun W, Bunn P, Jin C, Little P, Zhabotynsky V, Perou CM, Hayes DN, Chen M, Lin D-Y. The association between copy number aberration, DNA methylation and gene expression in tumor samples. Nucleic Acids Research. 2018; 46(6): 3009-18.
44. Wajed SA, Laird PW, DeMeester TR. DNA methylation: an alternative pathway to cancer. Annals of surgery. 2001; 234(1): 10-20.
45. McCann AH, Miller N, O'Meara A, Pedersen I, Keogh K, Gorey T, Dervan PA. Biallelic expression of the IGF2 gene in human breast disease. Hum Mol Genet. 1996; 5(8): 1123-7.
46. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. Cancer Res. 2002; 62(22): 6442-6.
47. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR, Feinberg AP. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science. 2003; 299(5613): 1753-5.
48. Ulaner GA, Vu TH, Li T, Hu JF, Yao XM, Yang Y, Gorlick R, Meyers P, Healey J, Ladanyi M, Hoffman AR. Loss of imprinting of IGF2 and H19 in osteosarcoma is accompanied by reciprocal methylation changes of a CTCF-binding site. Hum Mol Genet. 2003; 12(5): 535-49.
49. Murphy SK, Huang Z, Wen Y, Spillman MA, Whitaker RS, Simel LR, Nichols TD, Marks JR, Berchuck A. Frequent IGF2/H19 domain epigenetic alterations and elevated IGF2 expression in epithelial ovarian cancer. Molecular cancer research : MCR. 2006; 4(4): 283-92.
50. Byun HM, Wong HL, Birnstein EA, Wolff EM, Liang G, Yang AS. Examination of IGF2 and H19 loss of imprinting in bladder cancer. Cancer Res. 2007; 67(22): 10753-8.
51. Jelinic P, Shaw P. Loss of imprinting and cancer. J Pathol. 2007; 211(3): 261-8.
52. Vu TH, Nguyen AH, Hoffman AR. Loss of IGF2 imprinting is associated with abrogation of long-range intrachromosomal interactions in human cancer cells. Hum Mol Genet. 2010; 19(5): 901-19.
53. Bhusari S, Yang B, Kueck J, Huang W, Jarrard DF. Insulin-like growth factor-2 (IGF2) loss of imprinting marks a field defect within human prostates containing cancer. The Prostate. 2011; 71(15): 1621-30.
54. Leick MB, Shoff CJ, Wang EC, Congress JL, Gallicano GI. Loss of imprinting of IGF2 and the epigenetic progenitor model of cancer. Am J Stem Cells. 2012; 1(1): 59-74.
55. Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet. 2001; 10(7): 687-92.
56. Issa JP, Vertino PM, Wu J, Sazawal S, Celano P, Nelkin BD, Hamilton SR, Baylin SB. Increased cytosine DNA-methyltransferase activity during colon cancer progression. J Natl Cancer Inst. 1993; 85(15): 1235-40.
57. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene. 2002; 21(35): 5400-13.
58. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature. 1983; 301(5895): 89-92.
59. Sheaffer KL, Elliott EN, Kaestner KH. DNA Hypomethylation Contributes to Genomic Instability and Intestinal Cancer Initiation. Cancer prevention research (Philadelphia, Pa). 2016; 9(7): 534-46.
60. Hon GC, Hawkins RD, Caballero OL, Lo C, Lister R, Pelizzola M, Valsesia A, Ye Z, Kuan S, Edsall LE, Camargo AA, Stevenson BJ, Ecker JR, Bafna V, Strausberg RL, Simpson AJ, Ren B. Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer. Genome research. 2012; 22(2): 246-58.
61. Leick MB, Shoff CJ, Wang EC, Congress JL, Gallicano GI. Loss of imprinting of IGF2 and the epigenetic progenitor model of cancer. American journal of stem cells. 2011; 1(1): 59-74.
62. Balgkouranidou I, Liloglou T, Lianidou ES. Lung cancer epigenetics: emerging biomarkers. Biomarkers in medicine. 2013; 7(1): 49-58.
63. Duncan CG, Grimm SA, Morgan DL, Bushel PR, Bennett BD, Barnabas BB, Bouffard GG, Brooks SY, Coleman H, Dekhtyar L, Guan X, Han J, Ho S-l, Legaspi R, Maduro QL, Masiello CA, McDowell JC, Montemayor C, Mullikin JC, Park M, Riebow NL, Schandler K, Schmidt B, Sison C, Smith R, Stantripop S, Thomas JW, Thomas PJ, Vemulapalli M, Young AC, Roberts JD, Tyson FL, Merrick BA, Wade PA, Program NCS. Dosage compensation and DNA methylation landscape of the X chromosome in mouse liver. Scientific Reports. 2018; 8(1): 10138.
64. Lose F, Duffy DL, Kay GF, Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer AOCSMG, Kedda MA, Spurdle AB. Skewed X Chromosome Inactivation and Breast and Ovarian Cancer Status: Evidence for X-Linked Modifiers of BRCA1. JNCI: Journal of the National Cancer Institute. 2008; 100(21): 1519-29.
65. Chen W, Gao N, Shen Y, Cen JN. Hypermethylation downregulates Runx3 gene

expression and its restoration suppresses gastric epithelial cell growth by inducing p27 and caspase3 in human gastric cancer. Journal of gastroenterology and hepatology. 2010; 25(4): 823-31.
66. Dachrut S, Banthaisong S, Sripa M, Paeyao A, Ho C, Lee SA, Kosinski C, Patil MA, Zhang J, Chen X, Sripa B, Pairojkul C. DNA copy-number loss on 1p36.1 harboring RUNX3 with promoter hypermethylation and associated loss of RUNX3 expression in liver fluke-associated intrahepatic cholangiocarcinoma. Asian Pacific journal of cancer prevention : APJCP. 2009; 10(4): 575-82.
67. Nomoto S, Kinoshita T, Mori T, Kato K, Sugimoto H, Kanazumi N, Takeda S, Nakao A. Adverse prognosis of epigenetic inactivation in RUNX3 gene at 1p36 in human pancreatic cancer. British journal of cancer. 2008; 98(10): 1690-5.
68. Long C, Yin B, Lu Q, Zhou X, Hu J, Yang Y, Yu F, Yuan Y. Promoter hypermethylation of the RUNX3 gene in esophageal squamous cell carcinoma. Cancer Invest. 2007; 25(8): 685-90.
69. Cooper SJ, Zou H, Legrand SN, Marlow LA, von Roemeling CA, Radisky DC, Wu KJ, Hempel N, Margulis V, Tun HW, Blobe GC, Wood CG, Copland JA. Loss of type III transforming growth factor-beta receptor expression is due to methylation silencing of the transcription factor GATA3 in renal cell carcinoma. Oncogene. 2010; 29(20): 2905-15.
70. Shah JN, Shao G, Hei TK, Zhao Y. Methylation screening of the TGFBI promoter in human lung and prostate cancer by methylation-specific PCR. BMC Cancer. 2008; 8(284.
71. Cheung H-H, Lee T-L, Rennert OM, Chan W-Y. DNA methylation of cancer genome. Birth defects research Part C, Embryo today : reviews. 2009; 87(4): 335-50.
72. Makarla PB, Saboorian MH, Ashfaq R, Toyooka KO, Toyooka S, Minna JD, Gazdar AF, Schorge JO. Promoter hypermethylation profile of ovarian epithelial neoplasms. Clin Cancer Res. 2005; 11(15): 5365-9.
73. Teodoridis JM, Hall J, Marsh S, Kannall HD, Smyth C, Curto J, Siddiqui N, Gabra H, McLeod HL, Strathdee G, Brown R. CpG island methylation of DNA damage response genes in advanced ovarian cancer. Cancer Res. 2005; 65(19): 8961-7.
74. Carvalho AL, Jeronimo C, Kim MM, Henrique R, Zhang Z, Hoque MO, Chang S, Brait M, Nayak CS, Jiang WW, Claybourne Q, Tokumaru Y, Lee J, Goldenberg D, Garrett-Mayer E, Goodman S, Moon CS, Koch W, Westra WH, Sidransky D, Califano JA. Evaluation of promoter hypermethylation detection in body fluids as a screening/diagnosis tool for head and neck squamous cell carcinoma. Clin Cancer Res. 2008; 14(1): 97-107.
75. Hoque MO, Begum S, Topaloglu O, Chatterjee A, Rosenbaum E, Van Criekinge W, Westra WH, Schoenberg M, Zahurak M, Goodman SN, Sidransky D. Quantitation of promoter methylation of multiple genes in urine DNA and bladder cancer detection. J Natl Cancer Inst. 2006; 98(14): 996-1004.
76. Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, Wen B, Wu H, Liu Y, Diep D, Briem E, Zhang K, Irizarry RA, Feinberg AP. Increased methylation variation in epigenetic domains across cancer types. Nature genetics. 2011; 43(768.
77. Shteper PJ, Zcharia E, Ashhab Y, Peretz T, Vlodavsky I, Ben-Yehuda D. Role of promoter methylation in regulation of the mammalian heparanase gene (*HPSE*). Oncogene. 2003; 22(49): 7737-49.
78. Shukeir N, Pakneshan P, Chen G, Szyf M, Rabbani SA. Alteration of the methylation status of tumor-promoting genes decreases prostate cancer cell invasiveness and tumorigenesis in vitro and in vivo. Cancer Res. 2006; 66(18): 9202-10.
79. Pakneshan P, Szyf M, Farias-Eisner R, Rabbani SA. Reversal of the hypomethylation status of urokinase (uPA) promoter blocks breast cancer growth and metastasis. The Journal of biological chemistry. 2004; 279(30): 31735-44.
80. Stefanska B, Huang J, Bhattacharyya B, Suderman M, Hallett M, Han ZG, Szyf M. Definition of the landscape of promoter DNA hypomethylation in liver cancer. Cancer Res. 2011; 71(17): 5891-903.
81. McDonald OG, Li X, Saunders T, Tryggvadottir R, Mentch SJ, Warmoes MO, Word AE, Carrer A, Salz TH, Natsume S, Stauffer KM, Makohon-Moore A, Zhong Y, Wu H, Wellen KE, Locasale JW, Iacobuzio-Donahue CA, Feinberg AP. Epigenomic reprogramming during pancreatic cancer progression links anabolic glucose metabolism to distant metastasis. Nature genetics. 2017; 49(3): 367-76.
82. Makohon-Moore AP, Zhang M, Reiter JG, Bozic I, Allen B, Kundu D, Chatterjee K, Wong F, Jiao Y, Kohutek ZA, Hong J, Attiyeh M, Javier B, Wood LD, Hruban RH, Nowak MA, Papadopoulos N, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Limited heterogeneity of known driver gene mutations among the metastases of individual patients with pancreatic cancer. Nature genetics. 2017; 49(3): 358-66.
83. Guo S, Diep D, Plongthongkum N, Fung HL, Zhang K, Zhang K. Identification of methylation haplotype blocks aids in deconvolution of heterogeneous tissue samples and tumor tissue-of-origin mapping from plasma DNA. Nature genetics. 2017; 49(4): 635-42.
84. Li W, Zhang X, Lu X, You L, Song Y, Luo Z, Zhang J, Nie J, Zheng W, Xu D, Wang Y, Dong Y, Yu S, Hong J, Shi J, Hao H, Luo F, Hua L, Wang P, Qian X, Yuan F, Wei L, Cui M, Zhang T, Liao

Q, Dai M, Liu Z, Chen G, Meckel K, Adhikari S, Jia G, Bissonnette MB, Zhang X, Zhao Y, Zhang W, He C, Liu J. 5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers. Cell research. 2017; 27(10): 1243-57.

85. Lapinska K, Faria G, McGonagle S, Macumber KM, Heerboth S, Sarkar S. Cancer Progenitor Cells: The Result of an Epigenetic Event? Anticancer research. 2018; 38(1): 1-6.
86. Choi SJ, Jung SW, Huh S, Chung YS, Cho H, Kang H. Alteration of DNA Methylation in Gastric Cancer with Chemotherapy. Journal of microbiology and biotechnology. 2017; 27(8): 1367-78.
87. Byler S, Goldgar S, Heerboth S, Leary M, Housman G, Moulton K, Sarkar S. Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer research. 2014; 34(3): 1071-7.