Review Article:

Deoxyribonucleic acid (DNA) methylation and its impact in generation of cancer

J. Rajeswari, G. P. Chakravarthi

Department of Biochemistry, Acharya Nagarjuna University, Guntur

ABSTRACT

The arrangement of genes in the chromosome is dependent on histone modifications, deoxyribonucleic acid (DNA) binding proteins and methylation of cytosines within 5^1–cytosine-phosphate-Guanine–3^1(CpG) dinucleotides. DNA methylation can modify the gene activity without changing the gene sequence. Aberrant hypomethylation and hypermethylations, causal or heritable gene expressions play an important role in tumour initiation and progression. Global hypomethylation at some part of genome and hypermethylation at the promoter regions of the tumour suppressor genes could generate mutations in several types of cancers. Reversal or inhibition of DNA methylation mechanism provides a promising improvement in the treatment of cancer along with chemotherapy. A combined approach utilising epigenetic treatment along with standard chemotherapy appears to hold promise as a future therapy.

Key words: *Epigenetics, Deoxyribonucleic acid methylation, Methyl transferases, Cancer*

INTRODUCTION

Deoxyribonucleic acid (DNA) methylation is one of several epigenetic mechanisms that cells use to control gene expression. It occurs in both prokaryotes, eukaryotes, to a lesser extent in drosophila and is absent in *Coenorhabditis elegans*.[1,2] DNA methylation occurs at adenine and cytosine residues in prokaryotes but predominantly at cytosine residues in eukaryotes and is the only covalent modification known to be present in higher eukaryotes. It involves in transfer of methyl group from S-adenosyl methionine to fifth position of cytosine in the presence of DNA methyl transferase (DNMT) enzyme and thus formed 5-methyl cytosine is considered to be the fifth base.[3]

DNA methylation generally occurs at cytosine in a dinucleotide and is distributed across the genome unevenly. As DNA is made up of 4 bases, the probability of having any dinucleotide in genome is approximately 6%, while the observed frequency of CpG dinucleotide is not more than one tenth of this 6%. A sequence with more than 60% G+C content and having at least 0.6% of CpG is considered to be a CpG island.[4] CpG sequence is gradually lost from the genome during evolution due to potential mutagenic ability to deaminate 5-methyl cytosine to thymine. Majority of this dinucleotide methylated at cytosine is a part in transcriptionally inactive DNA or heterochromatin.

In contrast, some of non-methylated CpG dinucleotides available in the promoters or coding regions of the genes are known as CpG islands. These islands are smaller regions of CpG di nucleotides that range from 0.5 kb to 5 kb in their size and known to be present with an expected or even higher CpG dinucleotide frequency. Methylation in CpG is observed in some of the genes located in inactive X-chromosome, such as insulin like growth factor-

Corresponding author: Dr J. Rajeswari, Assistant Professor, Department of Biochemistry, Acharya Nagarjuna University, Guntur, India. **e-mail:** rjinka@yahoo.com

2 (IGF2), repetitive sequences like the DNA sequence first characterised by *Arthobacter luteus* restriction enzyme (Alu) and retroposons like Long interspersed nuclear element 1 (L1) etc.[5,6,7] Complex changes of demethylation and methylations occur during normal development of a cell. Several housekeeping genes could escape this methylation, but tissue specific genes undergo one more round of demethylation for their specifications.[8-11] The irregularities in the process of methylation are considered to be one of the two hits mentioned in Knudson's hypothesis in the generation of cancer. In the current review we discuss briefly some of relevant aspects of DNA methylation strategies and later summarizing their mutational patterns in cancer progression.

DNA methyl transferases

The machinery of DNA methylation in mammalian cells includes a group of enzymes known as DNMTs and hemi-methylated CpG dinucleotides as substrate. DNMT1 is highly conserved among eukaryotes when compared to other two (DNMT2 and DNMT3) and is involved in maintenance and regulation of methylation. These enzymes share a common catalytic domain containing 10 conserved amino acid motifs; however a regulatory domain towards their N-terminus is observed only in DNMT1 and DNMT 2.[12-14]

An active DNMT1, is a monomeric, 1616 amino acid lengthy protein, contains a large regulatory domain connected to a small c-terminal catalytic domain by lys-gly repeat hinge region[15] and is specific to hemi-methylated substrates over the unmethylated substrates.[18-21] A yeast two hybrid system revealed that DNMT1 functions by associating with proliferating cell nuclear antigen (PCNA), a cell cycle regulator cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1 (P21/WAF1),[20] and also with several other transcription repressors like histone methylases, histone deacetylases and methyl CpG binding

proteins like methyl CpG binding domain protein 2 (MBD2) etc to repress their transcription.[14, 21, 22]

Other DNMTs are DNMT 2, a small protein with 415 amino acids, lacking a N-terminal domain[23] and DNMT3 that, resembles DNMT1 with a plant homeodomain (PHD) and proline-tryptophan– tryptophan- proline (PWWP) domain at N-terminal end. A variant DNMT3a2 is generated from DNMT 3a and DNMT 3b1, DNMT 3b 2, DNMT 3b3, DNMT 3b4, DNMT 3b5 from DNMT 3b by alternate splicing.[15, 24,25] Like DNMT1, DNMT 3a and 3b are also interacting with different proteins like trimethylation of lysine 9 of histone 3 (H3K9) methyl transferases and histone deacetyl transferases.[26-28]

Mechanism of DNA methylation

Repression of transcription by DNA methylation

Two different mechanisms were proposed to explain the transcriptional repression mediated by DNA methylation. First mechanism is direct inhibition of binding of sequence specific transcriptional factors to their promoters and there by repressing the transcription. These transcriptional factors could bind to the promoters of their genes only when the CpG islands are unmethylated. This mechanism of repression is found in a variety of transcription factors like activating protein 2 (AP-2), nuclear factor Kappa–light chain enhancer of activated B cells (NF-kB), phospho protein p53, (p53) signal transducer and activator of transcription 3 (STAT 3), and heterochromatin protein 1 (HP1).[29, 30]

Other mechanism includes, binding of methyl cytosine binding proteins (MeCP) or methyl binding domain (MBD) proteins to methylated DNA and there by not allowing transcription factors to bind. (MeCP) family contain MeCP1 and MeCP2, could recognize and bind to 5' methyl cytosine. MeCP2 can bind to a single

symmetrical m5C in a CpG dinucleotide where as MeCP1 inhibits transcription of specific genes that contain more than ten 5-methyl cytosines. The MDB proteins after binding to CpG islands forms complex with other transcriptional repressing proteins like histone modifiers histone de acetylase 1 (HDAC1), histone de acetylase 2 (HDAC2), and ligands containing death associated protein 6 (DAXX). The whole complex of proteins induces a closed conformation of chromatin by preventing the access to transcriptional factors in generation of gene silencing.[31-34]

DNA methylation in altering chromatin conformation

The process of DNA methylations in chromatin conformation has a strong correlation with each other in transcriptional regulation. Along with DNA methylation post-transcriptional modifications of histones are also implicated in the organisation of chromatin structure and in the process of transcriptional regulation.[37] In general, transcriptionally inactive genes are hyper methylated, packed with hypo acetylated histones and form late replicating hetero-chromatin. Both these epigenetic changes function in association with one another.

Both acetylation and methylations are a part of post transcriptional modifications of histones. All acetylations on lysines of histones 3 and 4 make chromatin transcriptionally active. Acetylation of lysine (K) 9,14,18,23 and 8 of histone 3 (H3), H3K9, H3K14, H3K18, H3K23 and lysine 5,8,12 and 16 of histone 4(H4), H4K5, H4K8, H4K12 and H4K16 are observed among several transcriptionally active conformations.[38] Methylation on lysine residues can activate or inhibit the transcription depending on the extent to which methylation is carried out. Methylation of H3K4, H3K36 and H3K79 are known to be associated with transcriptional activation of the associated genes where as methylation of H3K9, H3K27 and H4K20 are associated with the repression.[36-38]
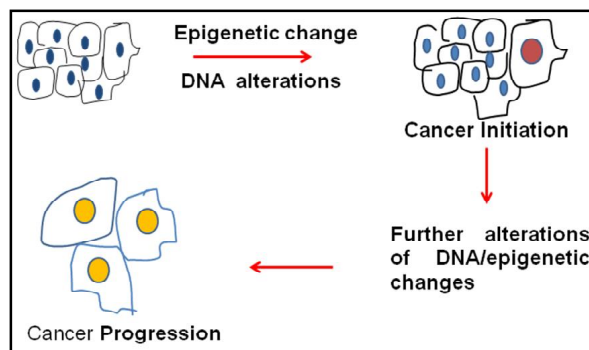
DNA methylation and cancer

Reasons for aberrant methylation in tumour cells

Changes in DNA methylation are accomplished as first epigenetic alterations in cancer initiation and progression (Figure 1). Aberrations in methylation pattern of cancerous cells leads to both acquisition and loss of methyl groups at various places in a genome of the cell. It became very difficult to explain the reason beyond this differential methylation pattern in cancerous cells when compared to normal cells. However certain factors or mechanisms attributed to this abnormal methylation pattern is due to their direct or indirect association with DNA methyl transferases. The reason for hypomethylation in the genome of many tumour cells is not explained, even though DNMT1 activity is increased. Alterations in DNMT enzyme activity is due to mutations in the post transcriptional modifications of the associated proteins in cancer cells. Several mutations are seen in p23, PCNA, and Annexin V, which associate with DNMT1 in tumour cells.[39, 40]

DNA hypomethylation

As reported earlier, methylation patterns in normal and cancerous cells are vice versa.[41,42] In normal cells hypermethylation is seen in bulk of the genome and hypo methylation in CpG islands surrounding the promoters. However, in cancerous cells a decrease in DNA

Figure 1: The schematic diagram showing progression of cancer by genetic alterations by DNA methylation DNA = deoxyribonucleic acid

methylation coupling with hypermethylation of CpG islands surrounding the promoters of genes is observed, further involving the regulation of tumour growth.[43, 44]

Loss of methylation (hypomethylation) can occur due to failure in maintaining the methylation and acquisition of DNA methylation at a previously non-methylated CpG islands by methylation activity. DNA hypomethylation leads to aberrant activation of genes in non-coding regions through a variety of mechanisms that contribute to cancer development and progression. Many reports show a strong correlation between tumour transformation and progression in the process of hypomethylation. Global DNA hypomethylation is observed at various genomic sequences including repetitive elements, retrotransposons, CpG poor promoters, introns and gene deserts.[45] Hypomethylation at these sites lead to their activation and further integrate the induced insertional mutagenesis in the genome to transforms the cells. L1 retroposon insertions were shown to inactivate the expression of *apc* and *C-myc* genes in colon and breast cancers.[46] Several studies showed that, the changes in methylation pattern of exogenously introduced retroviral sequence can induce aneuploidy.[47] DNA hypomethylation in the pericentric heterochromatin regions of chromosomes 1 and 16 are found in breast cancer, Wilms' tumour and in immunodeficiency states centromeric region instability and facial anomalies (ICF).[48-50]

Reduced levels of global DNA methylation have been reported for a variety of malignancies in the past decade. Hypomethylation of proto-oncogenes has been observed particularly in liver tumours and leukaemias. A variety of proto-oncogenes, such as, the *c-fos, c-myc,* Harvey rat sarcoma viral oncogene homologue *(Ha-ras),* Kirsten rat sarcoma viral oncogene homolog *(Ki-ras)* genes in liver tumours,

erythroblastic leukaemia gene *(Erb-A1)* and B-cell lymphoma 2 *(bcl-2)* genes in various leukaemia are known for their reduced levels of DNA methylation.[51,52] In addition, DNA hypomethylation can lead to the activation of growth-promoting genes, such as, related-rat sarcoma *(R-Ras)* oncogene, mammary serine protease inhibitor (MASPIN) in gastric cancer, calcium binding protein *(S-100)* in colon cancer, melanoma-associated antigen (MAGE) in melanoma and a loss of imprinting (LOI) in many tumours.[45]

DNA hypermethylation

In contrast to hypomethylation, site specific hypermethylation of CpG island contributes to tumourigenesis by silencing tumour suppressor genes and genes involved in the regulation of cell cycle and transcription.[7] Hypermethylation of tumour suppressor genes like retinoblastoma protein (Rb), p16, Mut L homologue (MLH) and breast cancer 1 early onset (BRCA1), involved in various cellular processes such as DNA repair, cell cycle, cell adhesion, apoptosis and angiogenesis are found to be integral in cancer development and progression by serving as a secondary hit in Knudson's two hit model.[53]

Indirectly, hypermethylation can silence gene transcription factors and DNA repair genes. Promoter hypermethylation induces silencing of transcription factors like runt related transcription factor 3 (RUNX3) in oesophageal cancer, GATA binding protein-4 (GATA-4) and GATA binding protein-5 (GATA-5) in colorectal and gastric cancers leading to inactivation of their downstream targets. Silencing of DNA repair genes (MLH1, BRCA1) lead to rapid cancer progression by accumulating genetic lesions. Loss of gene glutathione s-transferase pi 1 (GSTP1), involved in detoxification of DNA damaging electrophiles is observed in prostate cancer[54] and mismatch repair gene hMLH1 in colorectal cancer.[55]

Therapy for methylation in reversal of cancer

The reversible nature of epigenetic changes has led to the possibility of 'epigenetic therapy' in treatment of cancer in the restoration of a 'normal epigenome' by reversing the causal epigenetic aberrations. In this process several epigenetic drugs have been employed in chemotherapy that can effectively reverse DNA methylation and histone modification aberrations.[56] They comprise DNA methyl transferase inhibitors and histone deacetylase inhibitors.

A remarkable cytotoxic drug, 5-azacytidine (5-Aza-CR) and 5-aza-2'-deoxycytidine (5-Aza-CdR), lead to the inhibition of DNA methylation by causing differentiation. A cytidine analog, 5-azacytidine, when incorporated into DNA of rapidly growing cancer cells, forms a covalent complex with DNA methyl transferases and inhibits their activity.[57] This drug induces reduction in DNA methylation, causing growth inhibition in cancer cells by activating tumour suppressor genes which are aberrantly silenced in cancer.[56] The other (FDA) approved drugs 5-Aza-CR, 5-aza-CdR and Zebularine have been used in the treatment of myelodysplastic syndromes for promising results.[55,58,59] Histone deacetylase inhibitors like trichostatin A and hydroxamic acid are also being frequently used in epigenetic therapy. DNA methylation is targeted by using relatively new approaches like anti sense ribonucleic acid (RNA) and small interfering RNA. Development of several small molecule inhibitors such as aminomethyl pyrimidinys amino phenys quinolinys bezamide (SGI-1027), N – phthalyl L tryptophan (RG108) and (MG98) show the inhibitory effects by either blocking catalytic/cofactor-binding sites of DNMTs or by targeting their regulatory messenger RNA sequences.[60]

Future prospects and challenges

Epigenetic changes by DNA methylations have led the importance of genome in regulating the essential cellular processes in normal and diseased status like cancer. Better understandings of global DNA methylation patterns in cancer have enabled to design better treatment strategies. A combinatorial approach by utilizing epigenetic treatment along with standard chemotherapy holds significant promise for successful treatment of cancer in future. Such approaches might also help in sensitizing cancer cells at the early stage of transformation for better treatment.

REFERENCES

1. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21.

2. Gowher H, Leismann O, Jeltsch A. DNA of Drosophila melanogaster contains 5-methylcytosine. EMBO J 2000;19:6918-23.

3. Bestor TH, Verdine GL. DNA methyl transferases. Curr Opin Cell Biol 1994;6:380-9.

4. Bird A. CpG rich islands and the function of DNA methylation. Nature 1986;321:209-13.

5. Lyle R, Watanabe D, Te Vruchte D, Lerchner W, Smrzka OW, Wutz A, et al. The imprinted antisense RNA at the Igf2r locus overlaps but does not imprint Mas1. Nat Genet 2000;25:19-21.

6. Liu WM, Maraia RJ, Rubin CM, Schmid CW. Alu transcripts: Cytoplasmic localisation and regulation by DNA methylation. Nucleic Acids Res 1994;22:1087-95.

7. Baylin SB, Herman JG. DNA hypermethylation in tumourigenesis: Epigenetics joins genetics. Trends Genet 2000;16:168-74.

8. Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development 1987;99:371-82.

9. Kafri T, Ariel M, Brandeis M, Shemer R, Urven L, Mc Carrey J, et al. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes & Dev 1992;6:705-14.

10. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science 2001;293:1089-93.

11. Tada M, Tada T, Lefebvre L, Barton SC, Surani MA. Embryonic germ cells induce epigenetic

reprogramming of somatic nucleus in hybrid cells. EMBO J 1997;16:6510-20.

12. Brown R, Strathdee G. Epigenomics and epigenetic therapy of cancer. Trends Mol Med 2002;8:S43-S48.

13. Rhee I, Jair KW, Yen RW, Lengauer C, Herman JG, Kinzler KW, et al. CpG methylation is maintained in human cancer cells lacking DNMT1. Nature 2000;404:1003-7.

14. Okano M, Bell DW, Haber DA, Li E. DNA methyl transferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99:247-57.

15. Michał W, Łuczak P, Jagodzinski P. The role of DNA methylation in cancer development. Folia Histochemica Et Cytobiologica 2006;44:143-54.

16. Fatemi M, Hermann A, Pradhan S, Jeltsch A. The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. J Mol Biol 2001;309:1189-99.

17. Zucker KE, Riggs AD, Smith SS. Purification of human DNA (cytosine-5-)-methyltransferase. J Cell Biochem 1985;29:337-49.

18. Flynn J, Glickman JF, Reich NO. Murine DNA cytosine-C5 methyltransferase: pre-steady- and steady-state kinetic analysis with regulatory DNA sequences. Biochemistry 1996;35:7308-15.

19. Mertineit C, Yoder JA, Taketo T, Laird DW, Trasler JM, Bestor TH, et al. Sex-specific exons control DNA methyltransferase in mammalian germ cells. Development 1998;125:889-97.

20. Chuang LS, Ian HI, Koh TW, Ng HH, Xu G, Li BF, et al. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science 1997;277:1996-2000.

21. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 2003;31:2305-12.

22. Tatematsu KI, Yamazaki T, Ishikawa F. MBD2-MBD3 complex binds to hemi-methylated DNA and forms a complex containing DNMT1 at the replication foci in late S phase. Genes Cells 2000;5:677-88.

23. Dong A, Yoder JA, Zhang X, Zhou L, Bestor TH, Cheng X, et al. Structure of human DNMT2, an enigmatic DNA methyltransferase homolog that displays denaturant-resistant binding to DNA. Nucleic Acids Res 2001;29:439-48.

24. Chen T, Ueda Y, Xie S, Li E. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. J Biol Chem 2002;277:38746-54.

25. Weisenberger DJ, Velicescu M, Preciado-Lopez MA, Gonzales FA, Tsai YC, Liang G, et al. Identification and characterization of alternatively spliced variants of DNA methyltransferase 3a in mammalian cells. Gene 2002;298:91-9.

26. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 2003;31:2305-12.

27. Fuks F, Burgers WA, Godin N, Kasai M, Kouzarides T. Dnmt3a binds deacetylases and is recruited by a sequence specific repressor to silence transcription. EMBO J 2001;20:2536-44.

28. Hermann A, Gowher H, Jeltsch A. Biochemistry and biology of mammalian DNA Methyltransferases,CMLS. Cell Mol Life Sci 2004;61:2571-87.

29. Singal R, Ginder GD. DNA methylation. Blood 1999;93:4059-70.

30. Tate PH, Bird AP. Effects of DNA methylation on DNA-binding proteins and gene expression. Curr Opin Genet Dev 1993;3:226-31.

31. Singal R, Ferris R, Little JA. Methylation of the minimal promoter of an embryonic globin gene silences transcription in primary erythroid cells. Proc Natl Acad Sci USA 1997;94:13724-9.

32. Singal R, van Wert J, Bashambu M. Cytosinemethylation represses glutathione S-transferase P1 (GSTP1) gene expression in human prostate cancer cells. Cancer Res 2001;61:4820-26.

33. Prokhortchouk E, Hendrich B. Methyl CpG binding proteins and cancer: Are MeCpGs more important than MBDs? Oncogene 2002;21:5394-9.

34. Tamaru H, Selker EU. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 2001;414:277-83.

35. Fahrner JA, Eguchi S, Herman JG. Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. Cancer Res 2002;62:7213-8.

36. Hong L, Schroth GP, Matthews HR, Yau P, Bradbury EM. Studies of the DNA binding properties of histone H4 amino terminus. Thermal

denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 "tail" to DNA. J Biol Chem 1993;268:305-14.

37. Sims RJ III, Nishioka K, Reinberg D. Histone lysine methylation: a signature for chromatin function. Trends Genet 2003;19:629-39.

38. Pogo BG, Allfrey VG, Mirsky AE. RNA synthesis and histone acetylation during the course of gene activation in lymphocytes. Proc Natl Acad Sci USA 1966;55:805-12.

39. Issa JP, Vertino PM, Wu J, Sazawal S, Celano P, Nelkin BD, et al. Increased cytosine DNA-methyltransferase activity during colon cancer progression. J Natl Cancer Inst 1993;85:1235-40.

40. Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, et al. The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumours. Nucleic Acids Res. 1999;27:2291-8.

41. Feinberg AP, Gehrke CW, Kuo KC, Ehrlich M. Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res 1988;48:1159-61.

42. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. Science 1985;228:187-90.

43. Baylin SB, Herman JG. DNA hypermethylation in tumourigenesis: epigenetics joins genetics. TIG 2000;16:168-74.

44. Jones PA, Laird PW. Cancer epigenetics comes of age. Nature Genet 1999;21:163-6.

45. Shama S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010;31:27-36.

46. Jurgens B, Schmitz-Drager BJ, Schulz WA. Hypomethylation of L1 LINE sequence prevailing in human urothelial carcinoma. Cancer Res 1996;56:5698-703.

47. Rhee I, Jair KW, Yen RWC, Lengauer C, Herman JG, Kinzler KW, et al. CpG methylation is maintained in human cancer cells lacking DNMT1. Nature. 2000;404:1003-7.

48. Okano M, Bell DW, Haber DA, Li W. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999; 99:247-57.

49. Xu GL, Bestor TH, Bourc'his D, Hsieh CL, Tommerup N, Bugge M, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 1999;402:187-91.

50. Hansen RS, Wijmenga C, Luo P, Stanek AM, Caneld TK, Weemaes CMR, et al. The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc Natl Acad Sci USA 1999;96:14412-7.

51. Vachtenheim J, Horakova I, Novotna H. Hypomethylation of CCGG sites in the 3' region of H-ras proto-oncogene is frequent and is associated with H-ras allele loss in non-small cell lung cancer. Cancer Res 1994;54:1145-8.

52. Cheah MS, Wallace CD, Hoffman RM. Hypomethylation of DNA in human cancer cells: a site-specific change in the c-myc oncogene. J Natl Cancer Inst 1984;73:1057-65.

53. Stirzaker C, Millar DS, Paul CL, Warnecke PM, Harrison J, Vincent PC, et al. Extensive DNA methylation spanning the Rb promoter in retinoblastoma tumours. Cancer Res 1997;57:2229-37.

54. Singal R, van Wert J, Bashambu M. Cytosine methylation represses glutathione S-transferase P1 (GSTP1) gene expression in human prostate cancer cells. Cancer Res 2001;61:4820-6.

55. Herman JG, Umar A, Polyak K, Gra JR, Ahuja N, Issa J-PJ, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 1998;98:6870-5.

56. Yoo CB. Epigenetic therapy of cancer; past, present and future. Nat Rev Drug Discov 2006;5:37-50.

57. Taylor SM, Jones PA. Mechanism of action of eukaryotic DNA methyl transferase. Use of 5-azacytosine-containing DNA. J Mol Biol 1982;162:679-92.

58. Jones PA. Altering gene expression with 5-azacytidine. Cell 1985;40:485-6.

59. Cheng JC, Yoo CB, Weisenberger DJ. Preferential Response of Cancer Cells to Zebularine. Cancer Cell 2004;6:151-8.

60. Palakurthy RK, Wajapeyee N, Santra MK, Gazin C, Lin Gobeil S, Green MR, et al. Epigenetic silencing of the RASSF1A tumour suppressor gene through HOXB3-mediated induction of DNMT3B expression. Mol Cell 2009;36:219-30.