

DNA Methylation in Cancer Tissues

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Abstract

Cancer linked DNA hypo-methylation and hyper-methylation are present throughout the human genome. The hyper-methylation facilitates cancer progress by repressing the tumor suppressor gene. Hypo-methylation contribution towards cancer has not yet been clear. Recent studies of tissue specific methylation have suggested that DNA hypo-methylation aid tumor formation by many pathways. Loss of DNA methylation associated with cancer may alter transcription. In addition, DNA hypo-methylation might affect promoter usage production of intra-genic non-coding RNA transcripts, co-transcriptional splicing and initiation and elongation of transcription. Studies of hemi methylation of DNA in cancerous cells as well as normal tissues suggest that active de-methylation can explain cancer associated DNA hypo-methylation. New studies that genomic 5-hydroxymethylcytosine is intermediate in DNA de-methylation exhibits cancer associated losses. It suggests that both decreased hydroxyl-methylation and methylation of DNA play important role in carcinogenesis.

Keywords: Methylation; Gene expression; Tumor cell; Genome consortium

Introduction

Almost all cancer types depict both hyper-methylation and hypo-methylation. Hypo-methylation accounts for tissue specificity of DNA methylation [1]. Hypo-methylation and hyper-methylation of genome associated with cancer are usually independent of each other [2,3]. But recent studies show that cancer specific in DNA methylation are hyper-methylation of unique regions of gene and hypo-methylation of DNA repeats albeit with many notable exceptions [4-9] (Figure 1).

Deep sequencing of genome and recent whole genome analysis of the cancer methylome shows that there is much more role of DNA hypo-methylation and hyper-methylation in cancer. Although there is difference of frequency with which sequence undergo hypo or hyper-methylation [10-15]. This article reviews genome and chromatin epigenetics in normal as well as cancerous tissues [16-26]. Recent studies pay focus to role of epigenetic marks in the genes as well as intergenic transcription and promoters. These evidences are likely to be linked with cancer associated hypo-methylation. cancer-associated DNA hypo-methylation probably favors oncogenesis as well as effect normal gene expression like

- Alteration of intra nuclear positioning of chromatin
- Modulating the sequestration transcription factors at tandem DNA repeats
- Activating a small number of endogenous retroviral elements [27,28].

In addition, the little-studied area of DNA hemi-methylation in cancer is discussed in this review.

DNA methylation subgroups according to tumor types.

Each tumor type has specific DNA methylation patten. For example, characteristic pattern of high methylation CGIs was discovered in

colorectal cancer. It was defined as CpG island methylator phenotype (Table 1).

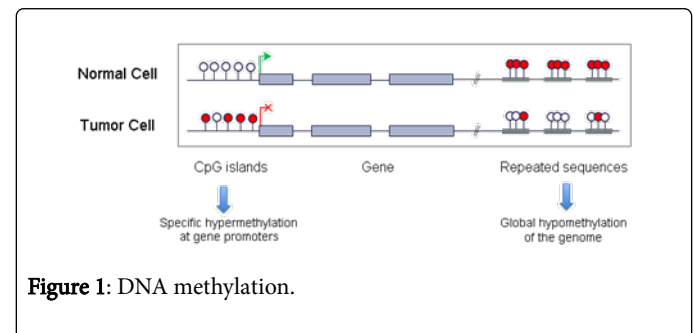


Figure 1: DNA methylation.

Genomic Hypo-Methylation Profiles in Cancer

Recent modern genome analyses of DNA methylation suggested that cancer specific portions methylomes consist of hypo-methylated DNA repeats and hyper-methylated gene regions [1,5,29]. DNA repeats are used as surrogate for methylation changes (usually losses of 5 mC) that are associated with certain tumor types [4,15,28,30,31]. Global DNA hypo-methylation analyses in human cancer by HPLC of enzymatic DNA digest depicted these cancer DNA fractions had almost same ratios of mole % 5 mC to those normal tissues of human body. It was concluded that hypo-methylation was not confused to DNA repeats. As cancer linked hypo-methylation of DNA occurs in somewhat unique sequences in and around genes. It includes metastasis associated genes [4,32,33].

Recent studies of DNA methylation in various normal and cancer cells suggested much tissue specificity in genome of normal samples and cancer linked DNA methylation [10,12,13,34-38]. Regions of cancer linked changes in DNA methylation are found in clustered short interspersed as well as in long blocks [5,35,37,39,40]. Recent studies also proved cause and effect relationship between normal tissues DNA hypo-methylation and increased transcription as well as

cancer linked hypo-methylation and cancer associated increase in gene expression [13,14,16,18,21,41-48].

Tumor type	Project and country	Number of methylomes
Breast	BRCA-US	971
Ovary	OV-US	572
Kidney	KIRC-US	491
Head and neck	THCA-US	488
Uterus	UCEC-US	481
Lung	LUAD-US	460
Colorectal	COAD-US	414
Lung	LUSC-US	410
Head and neck	HNCS-US	407
Brain	GBM-US	393
Skin	SKCM-US	338
Stomach	STAD-US	328
Brain	LGG-US	293
Bladder	BLCA-US	198
Prostate	PRAD-US	196
Blood	LAML-US	194
Pancreas	PACA-AU	167
Blood	CLLE-ES	159
Colorectal	READ-US	150
Liver	LIHC-US	149
Kidney	KIRP-US	142
Cervix	CESC-US	127
Brain	PBCA-DE	115
Ovary	OV-AU	93
Pancreas	PAAD-US	72
Pancreas	PAEN-AU	23

Table 1: International cancer genome consortium projects with methylomes generated by Infinium bead chips.

A small % of annotated gene promoters overlap tissue specific T-DMR or cancer specific C-DMR methylated DNA regions [42,49]. However most of T-DMR are not major type of vertebrate DNA promoter which are part of CpG island. Among the genes with T-DMR promoters some become activated upon self-induced demethylation with 5-deoxyazacytidine [42].

Enhancers sometimes show relation between upregulation of associated gene and demethylation in normal cells for example the binding of FoxA1/FOXA1 [50]. This transcription regulatory factor can open up DNA of inactivated enhancers [51-53].

In embryonic stem cells, local DNA methylation is associated with pioneer factor to certain tissue specific non-CGI promoters. Pioneer factors are implicated in different types of cancer [54]. Enhancers regions are likely to face demethylation in tumors. However, loss of DNA methylation from transcription regulatory region don't cause change in expression but it might facilitate [55].

DNA region enriched in hypo-methylation can increase expression of some of the affected genes [10,35,56]. Such broad hypo-methylation reflect high order chromatin structure.

Genomic Hypo-Methylation in Cancer within Gene Bodies

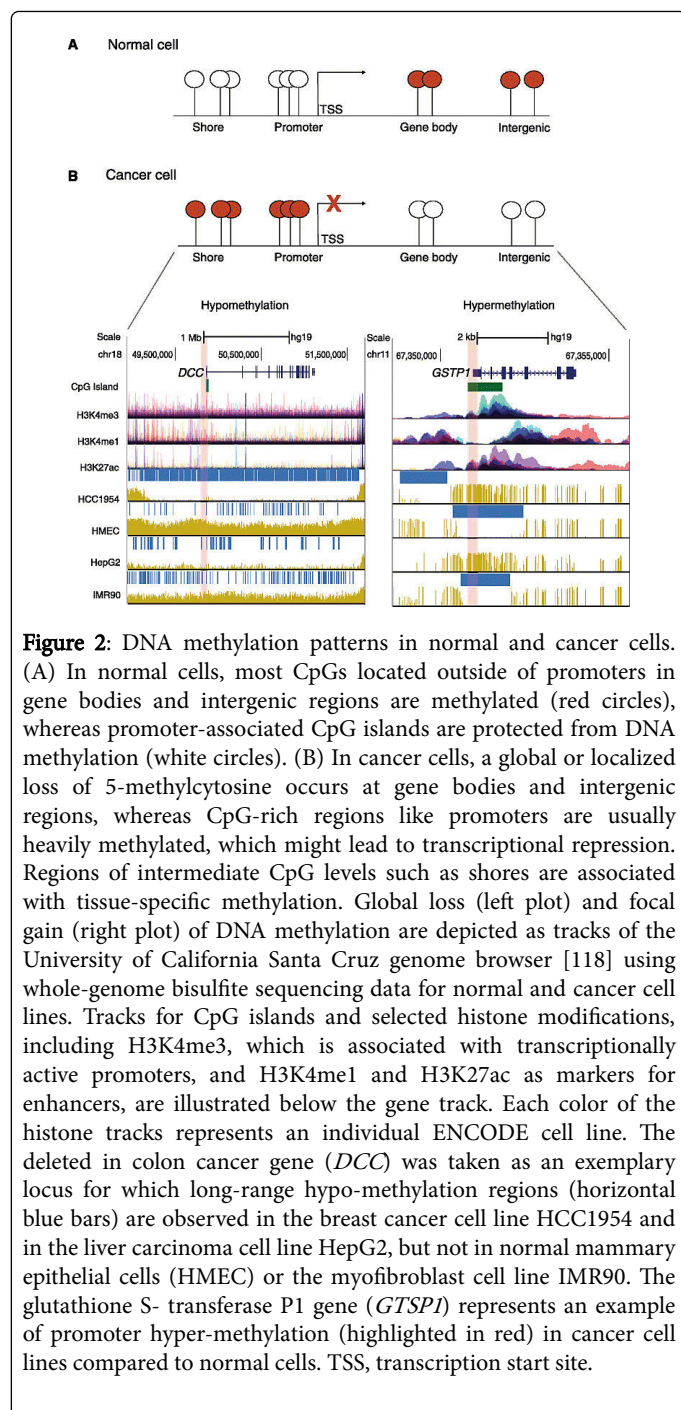
Recent studies Shows involvement of intragenic epigenetic marks in the regulation of normal gene expression. T-DMRS have been found in many genes. Increased methylation in gene body or promoter flanking region of certain genes with increased transcription [55,57,58]. In addition to this, there is non-randomness between position of CpG methylation within gene and external bodies i.e. exon intron [59,60]. These findings are consistent with relationship of DNA and chromatin epigenetics [57,58,61,62]. The average DNA methylation is linked with higher levels of transcription specially by its relation to nucleosome position [63]. For example, in downstream of CpG poor promoters it was observed that methylation of DNA antagonizes binding of Polycomb repressor complex. But in some genes lower expression was related with increase in gene body methylation [64]. Recently, the presence of 5-hydroxymethylcytosine (5 hmC) as the sixth naturally programmed base in vertebrate DNA has been established [65]. DNA methylation patterns in normal and cancer cells are shown in Figure 2.

Hypo-Methylation of DNA Repeats in Cancer

Global losses of DNA methylation with less increase in methylation in portions of genome are of cancer [3,4]. In most cancers, major type of methylation observed is that of tandem repeats [4,66,67]. Most type of hypo-methylation is results of demethylation in cancer stem cells. Hypo-methylation of minor portion of tandem repeat may cause cancer by induction of retro viral element transcription [28]. In addition, it might affect the transcription of nearby genes [68,69]. LINE-1 is highly repeated gene sequence, hypo-methylation of LINE-1 and alu repeats have been observed in many types of cancer [70-74]. Similar types of repeats that are hypo-methylated has been observed in Wilms tumor, ovarian cancer and adenocarcinoma [1,75]. Additional types of tandem repeats are also involved in malignancies [7,15,31,36,75-77]. Satellite DNA repeats sometimes show strongest DNA hypo-methylation for all type of sequence analyzed [15,56]. Cancer associated DNA hypo-methylation depends on stage, grade and tumor specimen [39,78]. This hypo-methylation has also been observed in non-tumor cells present adjacent to tumor cells [10,44,66,79-82]. Repeat DNA hypo-methylation serves as highly informative marker [39,75,83,84].

DNA Hypo-Methylation and Germ Cells

Most of genes that are expressed in testis have very low ratio of methylation in testis although same genes are highly methylated in other somatic tissues [85]. In sperms DNA repeats show very low methylation level compared with normal postnatal somatic tissues [70,81,86-88].



In Seminomatous testicular germ cell tumors another interface between germ line epigenome and cancer has been seen. Strong global DNA hypo-methylation was observed. Seminomas show none of the CGI hyper-methylation but it depicts DNA hypo-methylation [89]. Therefore, cancers can develop without gene region hyper-methylation but with extreme overall genomic hypo-methylation.

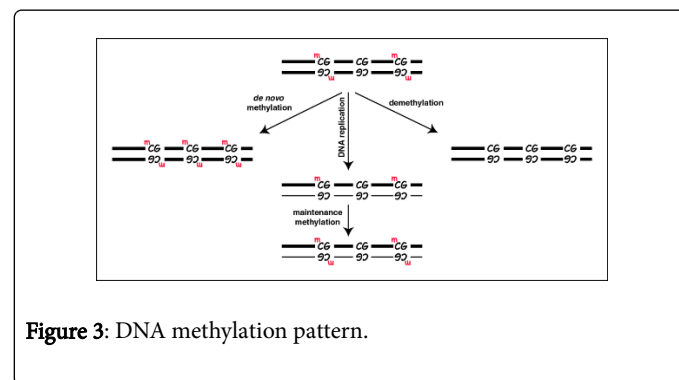
Opposite Cancer-Linked Changes in DNA Methylation in DNA Repeats: Hypo- and Hyper-Methylation

Opposite types of cancer linked methylation changes sometimes occur in same DNA sequence as in case of NBL2 (A Sequence repeat near centromeres of acrocentric chromosome) [31]. NBL2 was hypo-methylated at HhaI sites in 17% of ovarian carcinomas and hyper-methylated in 70% of ovarian carcinomas [31]. Various postnatal somatic tissues depict methylation at HhaI sites [76,77]. Few cancer DNAs digest with HhaI showed two fractions of NBL2 sequence one with hyper-methylation and one with hypo-methylation. There is evidence that hypo-methylation at NBL2 and hyper-methylation at NBL2 predominates in cancerous cells that suggest site specificity of methylation stats of CpG sites [6,90]. Thus, DNA can be made unstable during carcinogenesis so that CpG sites that are close to each other undergoes opposite changes in methylation

D4Z4 (a macro satellite located at sub telomeric region) also exhibit strong hypo-methylation and hyper-methylation in the bulk of array [7].

Maintenance of DNA Methylation Patterns Through Hemi Methylated Intermediates

Methylation at each site is assumed to be governed by de novo methylation (Figure 3) and maintenance methylation, these are independent of each other. The maintenance of methylation has been attributed to methyltransferase Dnm1. Different mechanism of *de novo* and maintenance methylation has led to stochastic models for methylation inheritance.



Insights into Cancer-Associated DNA Demethylation from Studies of DNA Hemi Methylation

Introduction of hairpin sequencing has enabled the methylation status more clearly site by site [90]. This sequencing allows analysis at every CG pair in a given region on DNA strands. A caveat about this method is that it can't made a difference between 5 hmC and 5 mC. As 5 hmc is predominantly in gene regions while cancer cell lines have low levels of 5 hmc [65,91,92] (Figure 4).

By sodium bisulfite based whole-methylome analysis using next-generation sequencing NGS, it was observed that 90% of cytosine in human H1 embryonic stem cells and IMR 90 fetal lung fibroblasts [93]. While nearly all of the methyl cytosine detected in IMR90 were in the CG context while considerable methylation was observed in non-CG context in H1 stem cells. Methylation at mCHG sites in H1 ES was also highly asymmetrical, with 98% of such sites observed to be methylated

on only one strand. Non-CpG methylation was also found to be significantly higher on the antisense strand of gene bodies, suggesting a nonrandom bias in the observed asymmetry. Non-CpG methylation disappeared upon differentiation of the H1 stem cells, but was restored in differentiated cells induced to form pluripotent stem cells. These findings suggest that asymmetrical methylation at non-CG dinucleotide sites may contribute to maintenance of the pluripotent state. They are reminiscent of the less frequent, hemi-methylated CG dinucleotide sites that we and Laird et al. have seen in various DNA repeats [6,94] or single-copy sequences [90] in normal or cancer tissues.

Hemi Methylated CpG Dyads in Cancer

Our studies of hemi-methylated DNA in cancer suggest the involvement of active demethylation in generating cancer linked hypo-methylation. DNA methylation changes at NBL2 and Sat2 in ovarian and Wilms tumor were studied [6,94]. In study of 13 CpGs by hairpin genome sequencing it was revealed that there is greater variability in methylation pattern in the cancer [94-149]. In the same way Analysis of 14 CpG in NBL2 repeats revealed high degree of variation in methylation pattern within each sample [6].

In a simulation study analyzing Sat2 and NBL2 it was found that methylation patterns in carcinomas were best explained by a mechanism that accounts for site to site correlation.

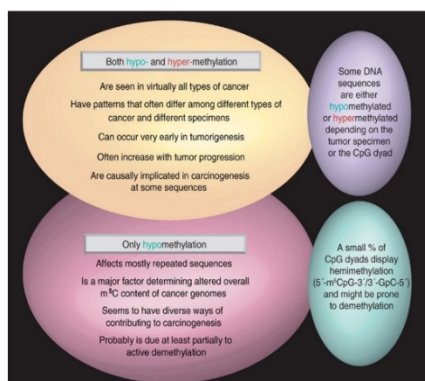


Figure 4: Similarities and differences in cancer-associated hypo- and hyper-methylation of DNA.

Conclusions

We conclude that during carcinogenesis highly methylate DNA sequence become partially de-methylated by active demethylation. Active demethylation might start cancer associated demethylation and a failure of maintenance methylation. The result could explain that tumor progression is frequently linked to a progressive decline in methylation.

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