Animal Cells and Systems 12: 117-125, 2008

Animal Cells
and
Systems

http://www.AnimalCells.or.kr

Cancer and Epigenetics

Jae-Bum Bae and Young-Joon Kim*

Department of Biochemistry, Genome Regulation Center, Yonsei University, Seoul 120-749, Korea

One of the interesting trends in genome research is the study about epigenetic modification above single gene level. Epigenetics refers study about heritable change in the genome, which accompany modification in DNA or Chromatin besides DNA sequence alteration. We used to have the idea that the coding potential of the genome lies within the arrangement of the four bases A, T, G, C; However, additional information that affects phenotype is stored in the distribution of the modified base 5-methylcytosine. This form of information storage is flexible enough to be adapted for different somatic cell types, yet is stable enough to be retained during mitosis and/or meiosis. Epigenetic modification is a modification of the genome, as opposed to being part of the genome, so is known as "epigenetics" (Greek for "upon" genetics). This modification could be methylation on Cytosine base or post translational modification on histone protein (methylation, acetylation, phosphorylation, Sumoylation)(DimitrijeviÊ et al 2005). In this review, we would like to focus on the relationship of DNA methylation and cancer.

Epigenetic Modification Machinery

There are 3 families of "methyl" protein related with DNA methylation, DNA methyltransferase(DNMT), DNA Demethylase (DMTase), and Methyl Binding Protein (MBDs, MECP2).

DNA methyltransferase

DNA methylation is mediated by 3 DNA methyl transferases (DNMT1, DNMT3a, DNMT3b) (Fig. 1). DNMT3a, 3b is responsible for de novo DNA methylation whereasDNMT1 is known as maintenance function. In detail, DNMT1 has a preference on hemimethylated DNA, suggesting that it has maintenance role and is responsible for copying the DNA methylation pattern on newly synthesized DNA. Mutations in the murine *Dnmt1* gene result in global hypomethylation and lethality in *Dnmt1* knockout mice. DNMT3a and 3b are thought to function as *de novo* DNMTs and the murine enzymes are required for *de novo* methylation after embryonic implantation as well as the *de novo* methylation of newly integrated retroviral sequences. These enzymes were shown to have equal preferences *in vitro* for unmethylated and hemimethylated DNA. Similar to *Dnmt1*-/- mice, mouse knockouts of *Dnmt3a* and *3b* are also lethal. DNMT3a also methylates non-CpG sequences and can function as a transcriptional corepressor. DNMT3b is required for the methylation of centromeric satellite repetitive elements and transcriptional repression. Mutations in human *DNMT3b* have been shown to cause immunodeficiency, chromosomal instabilities, and facial abnormalities (ICF) syndrome. Recent studies have shown that DNMTs function in cooperation with each other to facilitate DNA methylation in both human and mouse systems.

All DNMT proteins contain highly conserved C-terminal catalytic domains while their N-terminal regions are quite variable. The N-terminal regulatory domain of each DNMT is thought to direct nuclear localization and mediate interactions with other proteins. The C-terminal catalytic domains of DNMT contain several highly conserved motifs important for their enzymatic catalysis (motifs IV and VI), DNA binding (motif IX), and *S*-adenosylmethionine cofactor binding (motifs I and X). Studies of prokaryotic (cytosine-5) DNMT have shown motif VIII to be also highly conserved and a part of the core catalytic active site together with motifs IV and VI. The nonconserved region between motifs VIII and IX represents the target recognition domain (TRD) that may be responsible for sequence specificity.

Unlike DNMT1 and 3a, DNMT3b is the only DNMT protein that is expressed as alternatively spliced variants that affect the integrity of the catalytic domain. Among these, DNMT3b1 and 3b2 both contain all of the highly conserved motifs (I, IV, VI, VIII, IX, and X) as well as the TRD in the catalytic domain, but the DNMT3b2 variant

*To whom correspondence should be addressed.
Tel: +82-2-2123-3632; Fax: +82-2-312-8834
E-mail: yjkim@yonsei.ac.kr

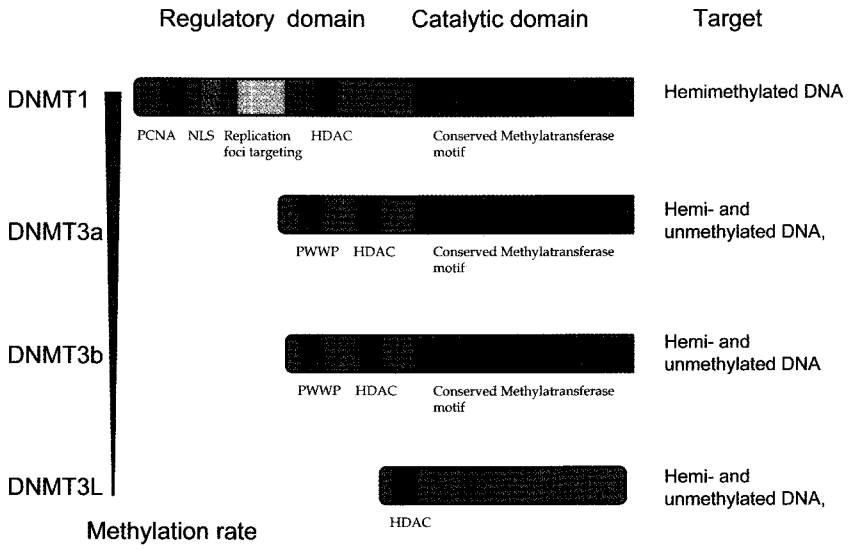

Fig. 1. Comparison of DNMT proteins. N terminal regulatory domain is described with interaction partner. PCNA:proliferating cell nuclear antigen, NLS: Nuclear Localizing signal, HDAC: Histone Deacetylase, PWWP:conserved Pro-Trp-Trp-Pro motif.

lacks exons 10 and 11. Human and murine DNMT3b3 and murine Dnmt3b6 lack the less conserved motif VII, the more conserved motif VIII, the TRD, and the nine amino acids of motif IX. DNMT3b3 and Dnmt3b6 only differ in that exons 10 and 11 are absent in DNMT3b3 while they are present in Dnmt3b6. Recently, it has been suggested that while murine Dnmt3b3 is catalytically inactive both *in vitro* and *in vivo*, the human DNMT3b3 isoform was capable of methyl transfer. The specific roles of individual DNMT3b splice variants are not fully understood; however, DNMT3b isoforms are overexpressed in a variety of human cancers. More recently, DNMT3b4 has been suggested to function as a negative regulator of DNA methylation in hepatocellular carcinoma cells despite its lack of catalytic activity. Gene targeting experiments have shown that DNMT3b plays an important role in the hypermethylation of CpG islands in human cancers as well as in the maintenance methylation of repetitive elements in mouse cells (Weisenberger et al., 2004).

DNA Demethylase (DMTase)

Removal of methyl group (or demethylation) is indispensable for active transcription of many genes, which could be obtained following three possible paths: Direct active process - demethylation, or indirect active process - mismatch/excision repair, or passive loss of the methyl group due to failure of maintenance. MBD2 is the only member of a family of methyl-CpG-binding proteins that has been reported to be both a transcriptional repressor and a DNA demethylase (dMTase) (Li 1999; Detich et al., 2002).

MBD2 itself does not demethylate DNA *in vitro*. In some *in vivo* systems MBD2 represses or activates transcriptions of genes by binding to -CpG- sequences. Indirect removal of $-CH_3$ could occur by removal of whole cytosine base.

There could be a possibility that the demethylation machinery is a huge protein complex, while MBD2 only function as a chaperon-component. Although it is apparent from the works of others that MBD2 may not be a demethylase, the inertness of some fractions rich in MBD2 to demethylation reaction can be argued as there might be different sets of MBD2 inhibitor in cells (Patra et al., 2008).

MBDs (Methyl CpG Binding protein)

Another group of methyl related proteins is MBDs (Methyl CpG binding Protein) (Fig. 2). Repressor complexes are recruited to methylated DNA through the binding of methyl-CpG binding domain proteins (MBDs). These complexes contain proteins that have histone deacetylase and chromatin remodeling activities, leading to the formation of a more compact and transcriptionally inactive chromatin. The earliest discovered MBD, MeCP2, has been found to associate with the Sin3a co-repressor complex and can also repress transcription in a histone deacetylase-independent manner (Table 1). MBD1, MBD2, and MBD3 were later discovered and were also shown to be involved in transcriptional repression.

MeCP2, MBD1, MBD2, MBD3 and MBD4 comprise a family of vertebrate proteins that share the methyl-CpG-binding domain (MBD) (Fig. 2, Table 1). The MBD, consisting of about 70 residues, possesses a unique á/â-sandwich structure with characteristic loops, and is able to bind single methylated CpG pairs as a monomer. All MBDs except MBD4, an endonuclease that forms a complex with the DNA mismatch-repair protein MLH1, form complexes

Table 1. Function and interaction partner of MBD protein family

MBD Family	Function	Interaction partner
MBD1	preferentially represses transcription of methylated templates in a HDAC-dependent manner, efficiently binds to densely methylated DNAs and that MBD1 localizes to the hypermethylated region of chromosome, likely to repress transcription by the promoter occupation via MBD-methylated DNA contact	SETDB, Suv39h1-HP1*alpha*, p150-CAF-HP1
MBD2	preferentially represses methylated templates, MeCP2, MBD2, and MBD3 are embedded in the histone deacetylase complexes and are involved in packing the genomic DNA into the inactive chromatin, leading to transcriptional repression	part of the MeCP1/NURD complex, MBD3L1
MBD3	a core component of the Mi-2/NuRD transcriptional co-repressor complex	Mi-2/NuRD, MBD2, Dnmt3a, Brg1,
MBD4	homology with bacterial DNA repair enzymes and is a glycosylase that can efficiently remove thymine or uracil from a mismatched CpG in vitro, MBD4 suppresses CpG mutability and tumorigenesis in vivo	component of the Sin3a/HDAC (histone deacetylase) chromatin-modifying complex, Sin3A-HDAC1, FADD
MECP2	bind preferentially to a single methyl-CpG pair, distributed throughout the nucleus in human cells	Sin3A-HDAC1, CEBP(Coactivator)

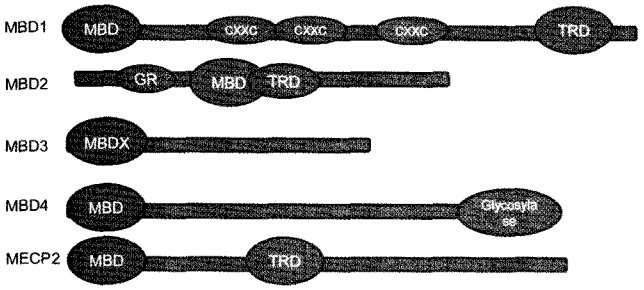

Fig. 2. Comparison of MBD proteins MBD: bind to methylated DNA sequence, CxxC: bind to nonmethylated CpG dinucleotide, TRD: C-terminal Transcriptional Repression domain, GR: Gly-Arg rich motif. MBD X: well conserved MBD domain that doesnot recognize methylated DNA owing to crucial amino acid change, NuRD:Nucleosome Remodeling and Histone Deacetylation, SETDB: Histone metyhltransferase, C/EBP: CAAT enhancer Binding protein, Sin3a: Repressor, Brg1: Bromodomain containing protein, HDAC: Histone Deacetylase, CAF: chromatin associated factor, FADD: Fas associated Death Domain, play a role in mediating apoptosis of cells in response to DNA damaging agents (modified from Klose et al., 2006).

with histone deacetylase. It has been established that MeCP2, MBD1 and MBD2 are involved in histone deacetylase-dependent repression and it is likely that this is also the case for MBD3. The current model proposes that MBD proteins are involved in recruiting histone deacetylases to methyl CpG-enriched regions in the genome to repress transcription. The lack of selectivity for MBD association with particular DNA sequences indicates that other mechanisms account for their recruitment to particular regions in the genome.

A database search for sequence homologous to the MBD led to the identification of a protein containing an MBD-like motif located at its N-terminus. This protein, originally called protein containing MBD (PCM1), was renamed MBD1. MBD1 was shown to bind methylated DNA and to repress transcription from a methylated promoter *in vitro*. It was initially believed to be a component of the MeCP1 complex. An additional search of an EST database found three more genes in mammalian cells that encode proteins containing putative MBDs, namely Mbd2, Mbd3 and Mbd4. Alignment of the MBD-like regions from the murine MBD1 to MBD4 and MeCP2 proteins showed that two subgroups could be established. The MBD of MBD4 is most similar to that of MeCP2 in primary sequence, while the MBDs of MBD1, MBD2 and MBD3 are more similar to each other than to those of either MBD4 or MeCP2. The presence of an intron located on a conserved position in all five genes indicates that the MBDs within each protein are evolutionarily related.

MBD1, a 70-kDa protein and the biggest member of the MBD family, has its MBD on the N-terminus and two or three cystein-rich regions (CxxC motifs) that are related to those in DNA methyltransferase protein 1 and the mammalian trithorax-like protein HRX. The exact number of these motifs present in MBD1 depends on alternative splicing. There is also another alternative splicing event, detected in cDNA derived from mouse brain, where the third exon (encoding the C-terminal half of the MBD) is removed. Furthermore, there is a third alternative splicing event resulting in the replacement of the terminal 20 amino acids with an alternative 44 amino-acid terminus. The physiological relevance of these variants remains to be explored.

There are two isoforms of MBD2, corresponding to initiation of translation at either the first (MBD2a, 43.5 kDa) or second (MBD2b, 29.1 kDa) methionine codons. The *mbd2* gene is also subjected to alternative splicing events that can produce nonsense transcripts. The shortest form of MBD2, MBD2b, has been proposed to be a DNA demethylase.

MBD3 also has isoforms produced by alternative splicing. The most abundant is a 32-kDa protein which shares high homology to MBD2b (80% similar, 72% identical). The second variant contains an insertion of a small exon (20 amino acids) in the MBD, with the rest of its sequence being identical with that of the short form of

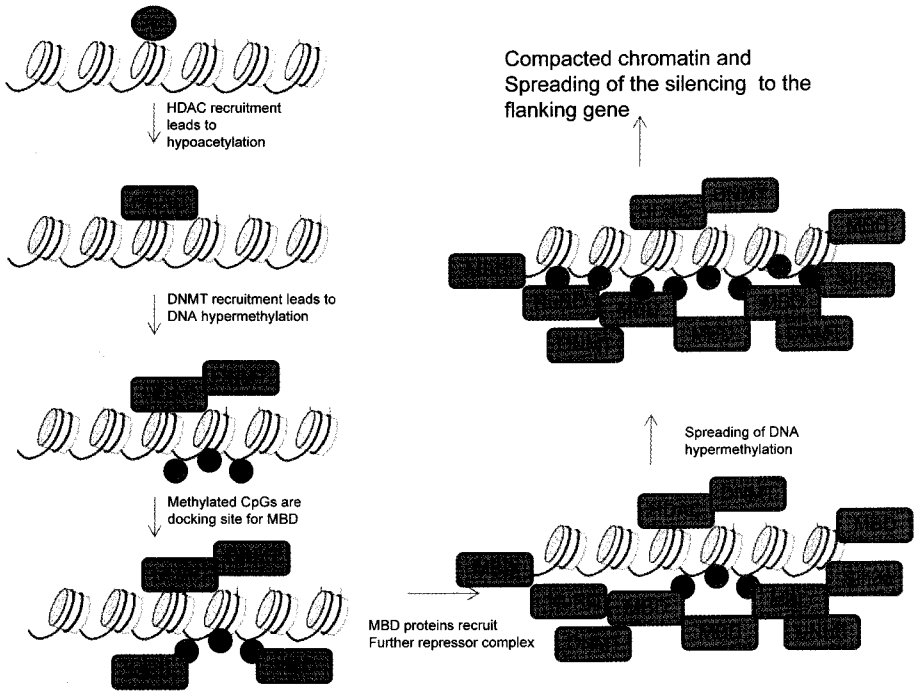

Fig. 3. Mechanism of DNA methylation mediated gene regulation.

MBD3. These two MBD3 variants have been detected in human, mouse and *Xenopus* systems. Considering its high similarity to MBD2b, demethylase activity has also been tested for MBD3. No demethylase activity was detected.

MBD4 is a 62-kDa protein with the MBD resided close to its N-terminus. There is also evidence of alternative splicing events in MBD4, even if none of them affect the MBD region. Database searches of the C-terminal region of MBD4 showed homology with bacterial DNA-repair enzymes. Among the related proteins are the 8-oxoGA mispair-specific adenine glycosylase MutY of *Escherichia coli*, the GT mismatch-specific glycosylase Mig of *Methanobacterium thermoautotrophicum*, the thymine glycol glycosylase EndoIII of *E. coli*, and the photodimer-specific UV-endonuclease of *Micrococcus luteus*. In fact, it has recently been proved that MBD4 can efficiently remove thymine or uracil from a mismatched CpG site *in vitro*, and the combined specificities of binding and catalysis indicate that this enzyme may function to minimize mutation at methyl-CpG (Ballestar et al., 2001).

Cancer and DNA Methylation

DNA methylation and gene expression regulation mechanism

DNA methylation is known to be related with gene expression regulation by several mechanisms (Fig. 2). There could be two explanation models for this regulation mechanism. The first model proposes that this modification causes transcriptional repression by directly interfering with the binding of transcriptional factors to the DNA. This hypothesis has been confirmed by the identification of a number of transcriptional regulators that cannot bind methylated recognition elements (Eden et al., 1994).

But, the existence of transcription factors irrelevant to DNA methylation status and the recent report that these modication is often possible in repressing transcription only after chromatin has been assembled, suggest that this direct effect is not the main mechanism by which modied DNA Inhibits gene expression (Buschausen et al., 1987; Kass et al., 1993; Kass et al., 1997).

A second model proposes that methylation attracts proteins that specically bind to modied DNA, thus preventing access to other factors required for gene induction. Indeed, several proteins containing a methylated-DNA binding domain (MBD) have recently been described, and several of them (MBD1, MBD2, MBD3, MBD4, MeCP2) have been involved in transcriptional repression. The biochemical characterization of the identied MBD proteins showed that most of them are interacted with histone deacetylases, suggesting that methylated CpGs (mCpGs) suppress transcription by establishing a repressive chromatin environment. In accordance with this model, histones assembled on methylated DNA are hypoacetylated than histones assembled on nonmethylated DNA, and also methylated transfected genes can be reactivated by treatment with trichostatin A (one of best characterized Histone Deacetylase [HDAC] inhibitor). Furthermore, a

distinctive chromatin structure, insensitive to nuclease digestion, is formed on methylated DNA.

Some reports proposed that CpG methylation and histone deacetylation act synergistically to silence gene expression. But the other reports proposed that histone 2A Lys27 trimethylation is not directly related with DNA methylation in gene silencing, which means these two modifications could comprise independent mechanism in gene expression regulation (Curradi et al., 2002; Kondo et al., 2008).

Brief history of aberrant methylation study in Cancer

When a general role for DNA methylation in gene silencing was established several decades ago, it was proposed that aberrant patterns of DNA methylation might play a role in tumorigenesis. As compared with normal cells, the cancer cells show major changes in their DNA methylation pattern. Some studies found evidence for a significant decrease in the total 5-methylcytosine content in cancer cells (Hypomethylation), and the occurrence of global hypomethylation in cancer was confirmed in later studies. Hypomethylation occurs mainly at DNA repetitive elements and might contribute to the genome instability frequently seen in cancer. Hypomethylation might also contribute to overexpression of oncogenic proteins and was shown to be associated with loss of imprinting of IGF2 (insulin growth factor 2), leading to aberrant activation of the normally silent maternally inherited allele. This was found to be associated with an increased risk for colon cancer. But the precise mechanisms of global hypomethylation patterns are currently unknown.

In contrast, aberrant hypermethylation at normally unmethylated CpG islands occurs. The CpG island promoter of the Rb (Retinoblastoma) gene, found to be hypermethylated in retinoblastoma, was the rst tumor suppressor shown to harbor such a modication. This discovery was soon followed by studies showing promoter hypermethylation and silencing of other tumor suppressor genes such as VHL (von Hippel–Lindau) in renal cancer, the cell cycle regulator CDKN2 A/p16 in bladder cancer, the mismatch repair gene hMLH1 in colon cancer, and many others (Gal-Yam et al., 2008).

Hypermethylation in Cancer

Reports of hypermethylation in cancer far outnumber the reports of hypomethylation in cancer. And there are several protective mechanisms that prevent the hypermethylation of the CpG islands. These include active transcription, spontaneous demethylation, replication timing, and local chromatin structure preventing access to the DNA methyltransferase. These are the genes involved in cell cycle regulation ($p16^{INK4a}$, $p15^{INK4a}$, *Rb*, p^{14ARF}), genes associated with DNA repair (*BRCA1, MGMT*), apoptosis (*DAPK, TMS1*), drug resistance, detoxification, differentiation, angiogenesis, and metastasis. Although certain genes such as *RASSF1A* and *p16* are commonly methylated in a variety of cancers, other genes are methylated in specific cancers. One example is the *GSTP1* (Glutathione S transferase pi 1) gene, which is hypermethylated in more than 90% of prostate cancers but is largely unmethylated in acute myeloid leukemia.

Many tumors show some kind of hypermethylation of one or more genes. One of the most detailed studies was conducted on lung cancer, and more than 40 genes were found to have some degree of alteration in DNA methylation patterns. Of the various genes studied, the commonly hypermethylated ones include *RARß*, *RASSF1A*, *CDNK2A*, *CHD13*, and *APC*.

Hypermethylation results in loss of expression of a variety of genes important in the development of breast cancer. These include steroid receptor genes, cell adhesion genes, and inhibitors of matrix metalloproteinases. Among the genes commonly hypermethylated in breast cancer are the $p16^{NK4A}$, estrogen receptor (*ER*) alpha, the progesterone receptor (*PR*), *BRCA1*, *GSTP1*, *TIMP-3*, and E-cadherin. The steroid receptor genes, *ER* and *PR*, have long been associated with breast cancer. Methylation studies of these have shown that the *ER* gene has a CpG island in its promoter and first exon areas. The *ER* gene is unmethylated in normal cells and in *ER*-positive cell lines but shows a high degree of methylation in more than half of primary cancers. The *BRCA1* gene, located at chromosome 17q21, is one of the more commonly associated genes in breast cancer, and the protein product is reduced or absent. DNA methylation has been proposed as one of the causes of its inactivation.

In addition to hypermethylation on CpG island in promoter region, a lot of hypermethylation exists on gene body region as well as intergenic region which mainly consist of repetitive element, revealed in genome scale methylation analysis by next generation sequencing platform such as Solexa technology (Lister et al., 2008).

Putative Mechanism of Hyperrmethylation

Global DNA hyperrmethylation can be evoked by overexpression of DNA methyltransferase (DNMTs). Although overexpression of DNA methyltransferases (Dnmt's) could be a mechanism for aberrant genome methylation, it does not explain the specific regional hypermethylation in cancer cells. The mechanisms involved in targeting of methylation to specific genes in cancer is not well understood. In one report, the leukemia-promoting PML-RAR fusion protein induced gene hypermethylation and silencing by recruiting DNA methyltransferases to target promoters. (PML-RAR is an oncogenic transcription factor found in acute promyelocytic leukemias (APLs) (Di Croce et al., 2002).

This result suggests a scenario in which oncogenic transcription factors aberrantly recruit Dnmt's to target promoters. Newly methylated CpGs then become docking sites for methyl-binding proteins, which in turn interact with both HDAC complexes and Dnmt's. The assembled complexes could be further stabilized by interactions between Dnmt's and HDAC1. If the initial recruitment step is not prevented, it may eventually lead to spreading of hypermethylation to the neighboring regions, locking these into a stably silenced chromatin state (Di Croce et al., 2002).

Hypomethylation in Cancer

Hypomethylation is another aberrant DNA methylation observed in a wide variety of cancers. It is common in solid cancers such as metastatic hepatocellular cancer, in cervical cancer, prostate cancers, and also in hematologic malignancies such as B-cell chronic lymphocytic leukemia. The global hypomethylation seen in a number of cancers, such as breast, cervical, and brain, show a progressive increase with the grade of malignancy. The pericentric heterochromatin regions on chromosomes 1 and 16 are heavily hypomethylated in patients with immunodeficiency, centromeric instability, and facial abnormalities.

A mutation of *DNMT3b* has been found in patients with immunodeficiency, centromeric instability, and facial abnormalities, which causes the instability of the chromatin. Hypomethylation has been hypothesized to contribute to oncogenesis by activation of oncogenes such as *cMYC* and *H-RAS* or by activation of latent retrotransposons or by chromosome instability.

Long interspersed nuclear elements (LINE) are the most common mobile DNAs or retro-transposons in the human genome. Hypomethylation of these mobile DNAs causes transcriptional activation and has been found in many types of cancer, such as urinary bladder cancer. Hypomethylation of the mobile DNA can also cause disruption of expression of the adjacent gene as well (Di Croce et al., 2002).

Putative Mechanism of Hypomethylation

Decreased dietary folate (Vitamin B) intake markedly perturbs global DNA methylation . The resultant low levels of *S*-adenosyl methionine (SAM) result in DNA global hypomethylation. DNA demethylase MBD2 also might have an important role in DNA hypomethylation.

And site specific hypomethylation event is well explained in MAGE-A1 gene as a one possible mechanism. The site-specific hypomethylation of *MAGE-A1* in tumor cells relies on a transient process of demethylation followed by a persistent local inhibition of remethylation due to the presence of transcription factors. (De Smet et al., 2002; Partha et al., 2004)

Methylation and Tumorigenesis

Relation of methylation level and cancer incidence is well documented, but how changed methylation level leads to tumorigenesis is less well known. In vivo the question of causality has only been addressed by loss-of-function studies. Dnmt3b overexpression study shows that Dnmt3b1 enhanced the number of colon tumors in $Apc^{Min/+}$ mice approximately twofold and increased the average size of colonic microadenomas, whereas Dnmt3a1 had no effect. The overexpression of Dnmt3b1 caused loss of imprinting and increased expression of *Igf2* as well as methylation and transcriptional silencing of the tumor suppressor genes *Sfrp2*, *Sfrp4*, and *Sfrp5*. Dnmt3b1 but not Dnmt3a1 efficiently methylates the same set of genes in tumors and in nontumor tissues, demonstrating that de novo methyltransferases can initiate methylation and silencing of specific genes in phenotypically normal cells. This suggests that DNA methylation patterns in cancer are the result of specific targeting of at least some tumor suppressor genes rather than of random, stochastic methylation followed by clonal selection due to a proliferative advantage caused by tumor suppressor gene silencing (Linhart et al., 2007).

Methylation Biomarker in Cancer

A large amount of publications has been published in the recent years about altered methylation patterns in human cancers. Tumor-specific methylation changes in different genes have been identified. The potential clinical application platform from information is under developing in cancer diagnosis, prognosis, and therapeutics.

Cancer Methylation Biomarker discovery Technique

The sodium bisulfite conversion method is ideal method for mapping the normal and aberrant patterns of methylation. Bisulfite treatment converts unmethylated cytosines to uracil (which finally converted to thymine), leaving methylated cytosines unchanged. After bisulfite modification, there are a number of methods available to study CpG island methylation. These include sequencing, methylation-specific polymerase chain reaction (MSP), combined bisulfite restriction analyses (COBRA), methylation-sensitive single nucleotide primer extension, and methylation-sensitive single-strand conformational polymorphism, and MIRA-Seq. Among these various techniques available, MIRA-Seq by Solexa technology seems to be most powerful to analyze methylation status as genome wide level. Furthermore, direct Bisulfite-Sequencing by Solexa technology was successfully introduced in Arabidopsis genome methylation sequencing experiment which makes methylation sequencing more vulnerable in genome scale.

Recent advances in the techniques for detection of methylation include powerful tools such as sodium bisulfite conversion sequencing, cDNA microarray, restriction landmark genomic scanning [using methylation sensitive restriction enzyme], CpG island microarrays, MedIP (methylation dependent Immunoprecipitation)-chip [using antibody against methylcytosine containing DNA fragment], MIRA (Methylated CpG Island Recovery Assay)-chip [using Methylated CpG binding protein, MBD2b and MBD3L1 which pulldown methylated CpG DNA fragment], and MIRA-Seq technology [Using Solexa system from Illumina]. For genome wide screening, CpG island microarray, MedIP-chip, and MIRA-seq methods are popular, but MIRA technique showed most consistent recovery rate and sensitivity than others (Lister et al., 2008).

DNA Methylation as a Prognostic Marker

Because DNA methylation is closely related to the development of cancer, it would be interesting to know whether its presence or absence affects the prognosis as well. This would help in modifying initial treatment options, monitoring patient response to therapy, and predicting survival. Recently, many studies have shown several methylated genes to be closely related to the prognosis.

The methylation profile may also help in predicting response to a chemotherapeutic agent. Methylation of the promoter region of the DNA repair gene *MGMT* increased the sensitivity of gliomas to alkylating agents. In another study, it was found that methylation of the *hMLH1* gene in colorectal cell lines was associated with increased resistance to the drug fluorouracil (Partha et al., 2004).

To diagnose cancer by DNA methylation status, various biomarkers were investigated and tested both academic institutions and bioventure industries.

To mining, Cottrel and colleagues used methylation sensitive arbitrarily primed PCR and methylated CpG island amplification from pooled frozen sample prostate cancer patients. A total of 441 sequences were obtained that mapped to unique locations in the genome, which finally narrowed down to 3 novel DNA methylation markers (GPR7, ABHD9 and an expressed sequence tag on chromosome 3 (Chr3-EST)) (Cottrell et al., 2007).

Colectal Cancer

In colorectal cancers, the promoter region of the *CDKN2A* gene was found to be hypermethylated in 61.1% of tumor samples. and p16 promoter methylation was detected in 42% of tumors. In another study, *TMEFF2*, *NGFR*, and *SEPT9*, were tested with plasma samples. *TMEFF2* methylation was detected in 65% of plasma samples from colorectal cancer patients and not detected in 69% of the controls. The corresponding results for *NGFR* were 51% and 84%; for *SEPT9*, the values were 69% and 86% (Lofton-Day et al., 2008).

Prostate cancer

A total of 26 novel biomarkers were successfully validated, a number of which including PITX2 specifically discriminate prostate cancer from benign prostate conditions such as BPH (benign prostatic hyperplasia). These biomarkers have the potential to augment diagnostic specificity of the best-characterized prostate cancer methylation biomarker, GSTP1. The discrimination of prostate cancer from benign prostate conditions is one of the major shortfalls of non methylation based testing such as PSA (prostate specific antigen) testing, the current standard in prostate cancer screening (Epigenomics Inc, 2008).

Lung Cancer

Several genes methylated in lung cancer show promise as a prognostic marker. These include the *RASSF1A* gene, which when methylated is seen to be associated with poor prognosis in stage I adenocarcinomas of the lung, and was seen more commonly in poorly differentiated lung cancer than well-differentiated ones.[105] In another study, hyper-methylated *p16* was found to be associated with higher-stage disease and reduced disease-free survival in non-small-cell lung cancer. In a recent study investigated the frequency of methylation of eight different genes (*RARβ*, tissue inhibitor of metalloproteinase-3 [*TIMP-3*], *p16, MGMT, DAPK, CDH1, p14*^{ARF} [*p14*], and glutathione S-transferase P1 [*GSTP1*]) in a large number of resected primary NSCLCs and also in the corresponding nonmalignant lung tissues. While methylation of *RARβ, TIMP-3, p16, MGMT, DAPK*, and *CDH1* occurred frequently in the tumors, it was not seen in the vast majority of corresponding nonmalignant lung tissues. A total of 82% of the NSCLCs showed methylation of at least one of these genes. These results demonstrate that methylation is a major mechanism for the inactivation of certain TSGs in lung cancers (Zöchbauer-Müller et al., 2002).

Breast Cancer

Widschwendter et al. have used a moderate-throughput, fluorescence-based, semiautomated quantitative technique called MethyLight to screen a panel of 35 methylation markers in 148 cases of breast cancer. Interestingly, they found that among these 35 markers, the best predictor of ER status was methylation of the PR gene (*PGR*). Conversely, the best predictor of PR status was methylation of the ER gene (*ESR1*) (Widschwendter et al., 2004).

Epigenetic Therapy against DNA Methylation

Owing to their dynamic nature and potential reversibility, epigenetic modications are promising therapeutic targets in

cancer. Various chemicals that alter DNA methylation and histone modication patterns are currently being examined as single agents or in combination with other drugs in clinical settings. There is developing method using epigenetics therapy to overcome cancerous epigenetic modification. One of these is re-expression of abnormally silenced tumor suppressor genes. DNA methyltransferase inhibitors such as azacitidine and decitabine, can reverse gene methylation, allowing normal transcription. Most DNA methylation inhibitors (DNMTi) that have been clinically tested, belong to the nucleoside analog family. These drugs are converted into deoxynucleotide triphosphates intracellularly and are incorporated into replicating DNA in place of cytosine. Their main mechanism of action is probably through trapping of the methyl transferases at sites of nucleoside incorporation, which depletes the cells of enzymatic activity, resulting in heritable demethylated DNA. Because incorporation occurs during DNA synthesis, only replicating cells are demethylated by DNMTi, which may confer the preference for highly proliferating cancer cells. The hypomethylation that ensues over the following cell division (Gal-Yam et al., 2008)

A second approach to gene re-expression is by inhibition of histone deacetylase (HDAC). Removal of acetyl groups from lysine group of histones leads to a transcriptionally repressive conformation of chromatin (heterochromatin), preventing transcription of genes packaged within that region of heterochromatin. Inhibiting HDAC by Tricostatin A or sodium phenylbutyrate may lead to remodeling of chromatin to a transcriptionally active conformation (euchromatin), resulting in normal transcription. 2,3 In detail, methyltransferase inhibitors and HDAC inhibitors have been studied in myelodysplastic syndromes (MDS), hematologic disorders in which DNA methylation and gene silencing have been demonstrated (Partha et al., 2004).

CONCLUDING REMARKS

Methylation change in cancer is most important both in the field of cancer prognosis and gene expression regulation. And gene silencing and activation in cancer is key regulatory mechanism. Understanding the mechanism underlying methylation regulation in cancer will shed light on the field of cancer therapy and gene expression regulation.

REFERENCES

Ballestar E and Wolffe AP (2001) Methyl-CpG-binding proteins. Targeting specific gene repression *Eur J Biochem* 268(1): 1-6.

Buschausen G, Wittig B, Graessmann M, and Graessmann A (1987) Chromatin structure is required to block transcription of the methylated herpes simplex virus thymidine kinasegene. *Proc Natl Acad Sci USA* 84: 1177-1181.

Cottrell S, Jung K, Kristiansen G, Eltze E, Semjonow A, Ittmann M, Hartmann A, Stamey T, Haefliger C, and Weiss G (2007) Discovery and validation of 3 novel DNA methylation markers of prostate cancer prognosis. *J Urol* 177(5): 1753-8.

Curradi M, Izzo A,Badaracco G, and Landsberge N (2002) Molecular Mechanisms of Gene Silencing Mediated by DNA Methylation. *Mol Cell Biol* 3157-3173.

Detich N, Theberge Y, and Szyf M (2002) Promoter-specific Activation and Demethylation by MBD2/Demethylase. *J Biol Chem*, 277(39): 35791-35794,

De Smet C, Loriot A, and Boon T (2004) Promoter-Dependent Mechanism Leading to Selective Hypomethylation within the 5' Region of Gene *MAGE-A1* in Tumor Cells. *Mol Cell Biol*,24(11): 4781-4790.

Di Croce L, Raker VA, and Corsaro M (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 295: 1079-1082.

DimitrijeviÊ D (2005) Cancer epigenome: A review *Arch Oncol* 13(3-4): 108-14.

Eden S and Cedar H (1994) Role of DNA methylation in the regulation of transcription. *Curr Opin Genet Dev* 4: 255-259.

Epigenomics Inc, www.epigenomics.com

Gal-Yam EN, Saito Y, Egger G, and Jones PA (2008) Cancer epigenetics: modifications, screening, and therapy. *Annu Rev Med* 59: 267-80.

Kass SU, Goddard JP, and Adams RL (1993) Inactive chromatin spreads from a focus of methylation. *Mol Cell Biol* 13: 7372–7379.

Kass SU, Landsberger N, and Wolffe PA (1997) DNA methylation directs a time-dependent repression of transcription initiation. *Curr Biol* 7: 157-165.

Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, Yamochi T, Urano T, Furukawa K, Kwabi-Addo B, Gold DL, Sekido Y,Huang TH, and Issa JP (2008) Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat Genet* 40: 741-750.

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

Li E (1999) The mojo of methylation *Nat Gen* 23: 5-6

Linhart HG, Lin H, Yamada Y, Moran E, Steine EJ, Gokhale S, Lo G, Cantu E, Ehrich M, He T, Meissner A, and Jaenisch R (2007) Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing. *Gen Dev* 21: 3110-3122.

Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, and Ecker JR (2008) Highly integrated single-base resolution maps of the epigenome in Arabidopsis. *Cell* 133(3): 523-36.

Lofton-Day C, Model F, DeVos T, Tetzner R, Distler J, Schuster M, Song X, Lesche R, Liebenberg V, Ebert M, Molnar B, Grutzmann R, Pilarsky C, and Sledziewski A (2008) Diagnostics and Genetics. DNA methylation Biomarkers for Blood based Colorectal Cancer screening.*Clin Chem* 54: 414-423.

Partha DM and Singal R (2004) DNA Methylation and Cancer *Jour Clin Onco* 22(22): 4632-4642.

Patra SK, Patra A, Rizzi F, Ghosh TC, and Bettuzzi S (2008) Demethylation of (Cytosine-5-C-methyl) DNA and regulation

of transcription in the epigenetic pathways of cancer *Dev* 27(2): 315-34.

Weisenberger DJ, Velicescu M, Cheng JC, Gonzales FA, Liang G, and Jones PA (2004) Role of the DNA Methyltransferase Variant DNMT3b3 in DNA Methylation *Mol Can Res* 2: 62-72.

Widschwendter M, Siegmund KD, Müller HM, Fiegl H, Marth C, Müller-Holzner E, Jones PA, and Laird PW (2004) Association of Breast Cancer DNA Methylation Profiles with Hormone Receptor Status and Response to Tamoxifen. *Cancer Research* 64: 3807-381.

Zöchbauer-Müller S, Minna JD, and Gazdar AF (2002) Aberrant DNA Methylation in Lung Cancer: Biological and Clinical Implications. *The Oncologist*, 7(5): 451-457.

[Received May 16, 2008; accepted August 26, 2008]