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# Mechanisms of abnormal gene expression in tumor cells

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*Abstract*. Epigenetic mechanisms are involved in critical nuclear processes such as transcriptional control, genome stability, replication and repair. Recent evidence suggests that changes in the epigenetic repertoire can drive tumorigenesis. This review examines the latest experimental evidence that questions the mechanisms underlying the consequence of epigenetic changes in gene regulation and cancer development.

*Key words*: Cancer, chromatin, DNA methyltransferase, methylation, transcriptional silencing, tumor suppressor gene.

## Introduction

There are many ways in which genes are regulated, and the field of epigenetics has seen a recent surge of interest in the study of modifications of the genome and histone tails to explain transcriptional competence. The term epigenetics refers to heritable changes in gene expression that are not the result of changes in the DNA code. DNA methylation is the best studied of these mechanisms with CpG methylation recognized as a major component of gene silencing in cancer [1]. Microinjection experiments using methylated gene constructs indicate that transcriptional repression occurs once chromatin is assembled [2]. Nuclease resistance in mammalian nuclei is due to CpG methylation, and this correlated with transcriptional repression mediated by methyl-CpG binding (MBD) proteins [3, 4]. It is not coincidental then that MeCP2, a global transcriptional repressor, silences gene activity and binds to chromatin in a methylation-dependent manner [5]. Before focusing on the impact of DNA methylation in tumorigenesis, the relevance of epigenetic mechanisms and transcriptional control is discussed.

### *DNA methylation influences chromatin function*

Recent studies are beginning to provide a molecular explanation as to how chromatin assembly on methylated DNA can repress transcription. It is well

established that the capacity of DNA methylation to silence gene activity is strengthened when operating within a chromatin environment [6]. Methyl-CpG binding proteins, MeCP1 and MeCP2 repress transcription by binding to the methyl-CpG moieties within a promoter, thereby occluding regulatory factors from the transcriptional complex. These results led to the demonstration that transcriptional silencing is inversely correlated to methylation density [7]. How these observations fit in with gene silencing and chromatin was unclear at the time. Microinjection experiments showed that methylated and unmethylated DNA have the capacity to form active transcription complexes. It was only once chromatin was assembled several hours later on methylated DNA that an eventual loss of DNase I hypersensitivity and inhibition of transcriptional activity was realized [6].

Considerable evidence has now accumulated demonstrating that DNA methylation represents a major epigenetic mark. DNA demethylation results in gene activation, whereas methylation of promoter sequences represses gene activity [2, 8]. Either site-specific CpG methylation interferes with transcription factors that would normally bind to the consensus sequence (direct model of repression), or the methyl-CpG moiety attracts methylation-dependent transcriptional repressors (indirect) to silence gene activity. For example, methylation of the E box sequence site directly inhibits *c-myc* [9] and *Sp1* binding to the (m)Cp(m)CpG binding site [10]. The capacity to silence gene transcription would presumably inhibit the assembly of basal transcriptional proteins to core promoters. However, this silencing mechanism would be limited to a fraction of sequences within the genome and would not account for transcriptional regulation at a global level [11].

The methylation-specific repressor MeCP2 has the capacity to repress transcription from methylated promoters [5]. The transcriptional repressor domain (TRD) binds the co-repressors mSin3A and histone deacetylases. The recruitment of histone deacetylases to methylated DNA provides a means to explain the silencing phenomenon mediated by CpG methylation, and this is supported by observations that repression can be overcome using deacetylase inhibitors such as trichostatin A (TSA) [12]. In another set of experiments involving the microinjection of methylated and unmethylated gene constructs, Jones and colleagues [13] definitively demonstrated that CpG methylation could specifically alter chromatin remodeling and gene transcription. Silencing conferred by MeCP2 could be reversed by inhibition of histone deacetylase, facilitating the remodeling of chromatin and transcriptional activation [14].

There are a number of key features that set each MBD protein apart; for example, MBD1 can repress transcription in a methylation-dependent manner and this mechanism of repression is sensitive to TSA. However, HDAC1 antibodies do not deplete MBD1 protein, suggesting that the mechanism of repression is likely to be different when compared to that of MBD2 and MeCP2. The MBD proteins have a high binding affinity to densely methylated DNA and are dynamically linked with histone deacetylases [15]. It is plausible that histone

deacetylases other than HDAC1 may be involved in repression. MBD2 and MBD3 appear to be part of a larger co-repressor network that includes the nucleosome remodeling histone deacetylase (NuRD) complex, along with Mi-2, a member of the SWI2/SNF2 family [16–18]. Although we are beginning to understand how methylation and co-repressors regulate transcription, we still do not know the molecular components that localize methylation-specific determinants during gene repression. Recent experimental evidence challenges the notion that DNA methyltransferases function solely in DNA methylation to reveal remarkable molecular functionality [19]. In this next section I discuss the capacity of the DNMTs in transcriptional repression and what seems to be a common theme in tumorigenesis.

*DNMTs, methylation and cancer*

In mammals, four members of the DNA methyltransferase family have been identified, three (DNMT1 [20], DNMT3a and DNMT3b [21]) have functional methylation activity. All except DNMT2 (no regulatory domain) have a catalytic methyltransferase domain at the C terminus responsible for methyl-group transfer and an N-terminal region with a putative regulatory domain [22, 23]. Both N- and C-terminal regions are required for DNMT1 catalysis, while the C-terminal region is sufficient for DNMT3a and DNMT3b [24, 25]. The notion that DNMT enzymes other than DNMT1 could be responsible for methylation was confirmed in DNMT1 knockout ES cells which retained *de novo* methylation activity [26]. Furthermore, colorectal carcinoma cells lacking DNMT1 had decreased DNA methyltransferase activity, although they displayed only a 20% decrease in overall genomic methylation [27]. Accumulating evidence reveals that the biological function of DNA methylases extends to cooperation with chromatin remodelling determinants involved in critical functions, such as transcriptional control, DNA replication, chromosome segregation and genome stability (summarized in Tab. 1). These studies are starting to provide some molecular clues to how changes in genomic methylation precipitate in cancer, and perhaps the mistargeting of DNMTs explain changes in cancer. DNMT3a and DNMT3b are also transcriptional repressors in a methylation-independent manner [28, 29]. For example RP58 associates with DNMT3a and is typically found on transcriptionally repressed heterochromatin [29]. In addition, repression by the RP58-DNMT complex is not methylation dependent, thus expanding the functional role of DNMTs beyond that of methyltransferase activity. To what extent DNMT3a/3b are involved in the initiation of gene silencing is not yet clear, although it is interesting to note there are distinct localization properties between DNMT1 and DNMT3 enzymes. Unlike DNMT1, which is localized to replication foci throughout S phase, DNMT3a and DNMT3b target heterochromatic foci in late S phase and proposed to establish transcriptionally silent heterochromatin independent of replication [28]. Recent observations

Table 1. DNMT associated binding partners that modify chromatin

| Binding partner | Proposed function | Refs |
|---|---|---|
| DNMT1 | | |
| HDAC1 | Chromatin remodeling, transcriptional silencing | [54] |
| HDAC2 | Chromatin remodeling, transcriptional silencing | [55] |
| DMAP1 | Histone deacetylation following DNA replication, transcriptional silencing | [55] |
| pRB | Chromatin remodeling, transcriptional silencing | [56] |
| MBD3 | Binds hemi-methylated DNA, transcriptional silencing | [57] |
| PCNA | Targeting to replication foci, maintain DNA methylation | [58–60] |
| RUNX1/MTG8 | Targeted recruitment and silencing in acute myeloid leukemia | [61] |
| p53 | Transcriptional silencing | [62] |
| RGS6 | Cooperates with DMAP1 complex, transcriptional silencing | [63] |
| SuV39H1 | Histone tail modification at H3K9 | [64] |
| p33ING1 | Cooperates with DMAP1 and co-repressor complex, histone modification | [65] |
| DNMT3a/DNMT3b | | |
| RP58 | Maintain transcriptionally repressive chromatin in late S-phase | [28, 29] |
| Condensin | Mitotic chromosome condensation | [66] |
| hSNF2H | Epigenetic regulation | [67] |
| DNMT3L | | |
| HDAC1 | Transcriptional silencing | [68, 69] |

reveal that the DNMT3L protein can mediate transcriptional repression by its biochemical interaction with histone deacetylase. These observations suggest the methylation machinery are connected with chromatin remodeling; however, the biggest challenge in the area is to determine the mechanisms by which the determinants are localized and segregated on target genes. In the next section, I discuss possible mechanisms that could explain aberrant DNA methylation patterns in cancer.

### *Mistargeting of the DNMT co-repressor complex*

A question that has long caused confusion in the cancer-epigenetics field is the specificity of genomic methylation patterns. Recent studies in the area have revealed interesting exceptions to the belief that hypermethylation of tumor suppressor genes is the primary mechanism of cancer development [30]. Indeed, hypomethylation events have been described and attributed to genom-

ic instability in cancer [31, 32]. Almost two decades ago, studies demonstrated that reductions in genomic methylation are associated with cancer progression [33, 34]. One of the best-studied models of cancer development is tumor suppressor gene silencing and has been studied in different contexts and diseases. For example, the retinoblastoma tumor suppressor gene is silenced by CpG methylation [35]. Alternatively, demethylating agents such as azacytidine have been used to induce promoter sequence hypomethylation and derepress gene silencing [36]. Clearly, experimental evidence suggests that hypomethylation and hypermethylation events can be associated with tumor development. However, hypermethylation of tumor suppressor genes and transcriptional repression do not explain how determinants could be mistargeted in cancer when hypomethylation is believed to be the primary cause of tumorigenesis. In this section I consider recent advances to our knowledge of methylation-mediated mechanisms in cancer and examine both hyper- and hypo- methylation events in cancer.

*Gene silencing, DNMT recruitment and chromatin disruption*

DNA hypermethylation has been described in a number of cancer types including retinoblastoma, breast cancer, colorectal carcinoma, melanoma, leukemia and renal carcinoma [37–43]. Histone deactylase inhibitors such as TSA are not effective to derepress hypermethylated promoters [12]. The mechanism of repression is believed to involve the recruitment of a co-repressor complex that belong to the MBD protein family. Epigenetic modifiers such as 5adC and TSA reactivate gene activity by promoting DNA demethylation and increased in histone tail acetylation (see Fig. 1) [44, 45].

Disruption of the DNMT1 gene in colorectal carcinoma cells ($DNMT1^{-/-}$) significantly decreases methylatransferase activity, and is correlated with changes in DNA methylation [27]. By contrast, the tumor suppressor gene *p16INK4A* and *Alu* repeats retained characteristic hypermethylation pattern and remained transcriptionally repressed. However, when $DNMT1^{-/-}$ cells were exposed to the demethylating agent 5adC, *p16INK4A* showed demethylation and derepression, suggesting other methyltransferase activities could cooperate with silencing. Recent studies have brought to light additional enzymes that participate with cancer progression in carcinoma. Depletion of DNMT1 and DNMT3b show marked reductions in methylation content at repetitive sequences and derepression of tumor suppressor genes *p16INK4a* and *TIMP3* (see Tab. 2) [46]. The findings of these experiments suggest that DNMT cooperativity, transcriptional silencing and methylation could contribute to tumorigenesis. The results do not explain how the DNMT methyltransferases and associated co-repressors are specified on focal areas of promoters to silence transcription while the genome experiences global hypomethylation events. To understand changes in methylation regulating transcription, it is often useful to examine different cancer models. PML-RAR is a

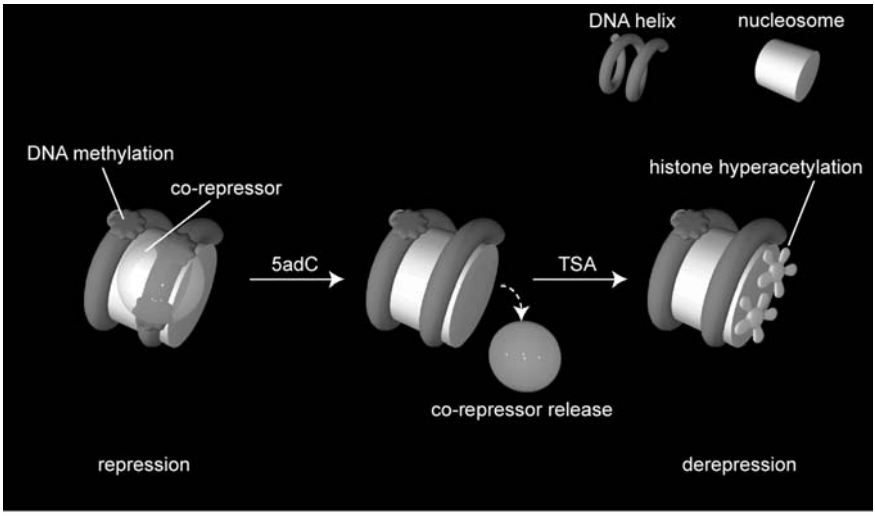


Figure 1. Model of methylation-mediated transcriptional regulation. Hypermethylation of the promoter sequence is dominant in silencing gene transcription. Methylated CpG sequences become recruitment sites for methyl-CpG-specific proteins and are associated with HDAC and Sin3 co-repressors. Demethylation by 5adC reduces the silencing potential mediated by methylation and the robust release of the co-repressor complex. Hyperacetylation of histone tails can be induced using HDAC inhibitors such as TSA, thereby decondensing chromatin and allowing assembly of activator complexes that drive gene expression.

mutant oncogenic transcription factor caused by translocation between promyelocytic leukaemia (PML) and retinoic acid receptor (RAR). This fusion protein recruits histone deacetylase and is thought to remodel chromatin and regulate transcription [47]. Evidence suggests that PML-RAR can recruit DNMTs to RA target genes with consequential promoter hypermethylation and transcriptional repression [48]. Chromatin immunoprecipitation experiments show enrichment of DNMT1 and DNMT3a on the RARβ2 promoter. Interestingly, TSA and 5adC could partially restore transcriptional competence, and this was correlated with changes in the methylation status of the RARβ2 promoter (see Fig. 2). A surprising result is that RA treatment could reduce promoter methylation, suggesting that cooperation of the DNMT methylases

Table 2. Consequence of DNMT disruption

| Gene | Enzyme activity | Reduction in methylation content | Gene expression |
|---|---|---|---|
| DNMT1$^{-/-}$ | 96% | 20% | None |
| DNMT3b$^{-/-}$ | 87% | 3% | None |
| DNMT1/3b$^{-/-}$ | 99.9% | 95% | Expressed |

are central to carcinogenesis. Taken together, these results suggest a leukemia-promoting protein is directly associated with carcinogenesis by inducing gene hypermethylation and the recruitment of DNMTs. These observations clearly identify that DNA hypermethylation is associated with silencing of tumor susceptibility genes in several forms of cancer. However, direct proof that CpG hypermethylation and transcriptional silencing are the primary mechanisms of cellular transformation is currently lacking.

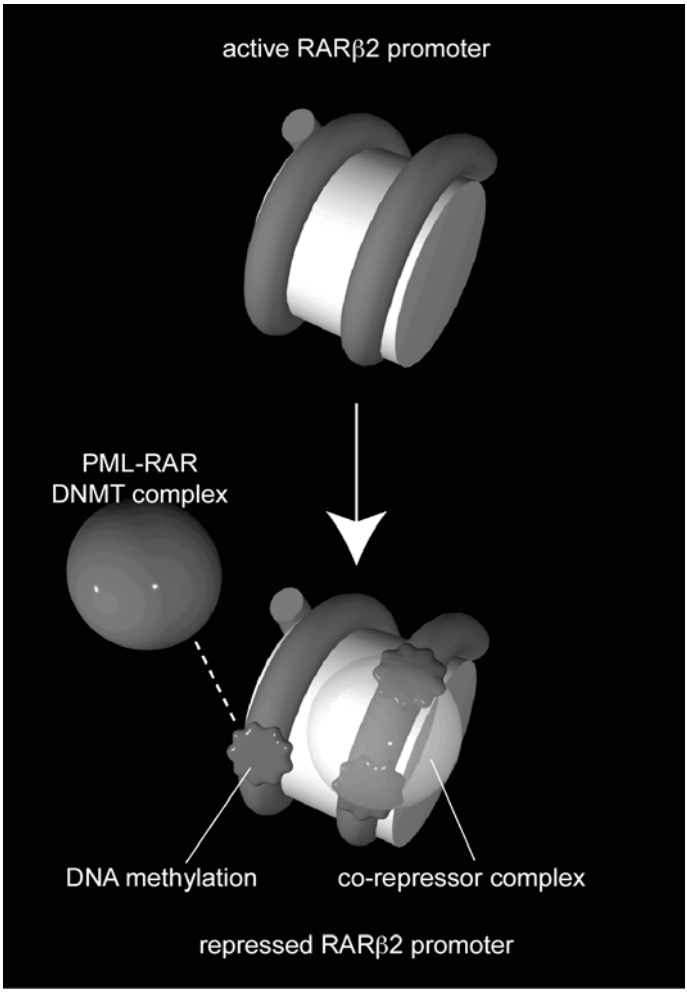


Figure 2. Recruitment model of PML-RAR/methyltransferase silencing on retinoic acid target genes. The active promoter of RARβ2 gene is targeted by the PML-RAR/DNMT methyltransferase associated complex and undergoes endogenous CpG methylation before recruitment of a methylation-dependent co-repressor complex and transcriptional silencing. Epigenetic modification induced by 5adC or RAR can reverse silencing by DNA demethylation.

If DNA methylation is inversely correlated to transcriptional repression, then recent findings that chromatin remodelling can change genomic methylation events pose some interesting questions on the antithetical nature of epigenetic modification [19, 34]. Lymphoid specific helicase (Lsh) belongs to the SNF2 subfamily of ATPase-dependent chromatin remodelling proteins [49, 50]. Results with $Lsh^{-/-}$ mice reveal substantial changes in genomic methylation levels, suggesting a role in regulating DNA methylation, histone tail modification and genetic instability during tumor progression [50–52]. In *Arabidopsis thaliana* the ddm1 (decrease in DNA methylation) gene is responsible for significant reductions in *de novo* methylation [53]. The models discussed in this review are by no means meant to represent the mechanistic riposte, nevertheless, the experimental findings expand our understanding of DNA methylation and highlights the diverse biological nature at a molecular level.

## *Conclusion*

It is clear that the study of epigenetics continues to attract widespread interest, both within basic and medical research. The future holds great promise and, given these recent research findings, may lead to the development of new therapeutic tools based on the pharmaceutical reversal of the methylation signal and/or regulation of the machinery responsible for methylation.

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