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Review

DNA methylation in development and human disease

Suhasni Gopalakrishnan, Beth O. Van Emburgh, Keith D. Robertson*

Department of Biochemistry & Molecular Biology, UF-Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida, College of Medicine, Box 100245, 1600 S.W. Archer Road, Gainesville, FL 32610, United States

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ABSTRACT

DNA methylation is a heritable and stable epigenetic mark associated with transcriptional repression. Changes in the patterns and levels of global and regional DNA methylation regulate development and contribute directly to disease states such as cancer. Recent findings provide intriguing insights into the epigenetic crosstalk between DNA methylation, histone modifications, and small interfering RNAs in the control of cell development and carcinogenesis. In this review, we summarize the recent studies in DNA methylation primarily focusing on the interplay between different epigenetic modifications and their potential role in gene silencing in development and disease. Although the molecular mechanisms involved in the epigenetic crosstalk are not fully understood, unraveling their precise regulation is important not only for understanding the underpinnings of cellular development and cancer, but also for the design of clinically relevant and efficient therapeutics using stem cells and anticancer drugs that target tumor initiating cells.

Contents

1. Introduction

DNA methylation is a covalent chemical modification of DNA occurring at cytosine residues in CpG dinucleotides. DNA methyltransferases (DNMTs) catalyze genome-wide DNA methylation and are associated with histone modifying enzymes (e.g. histone deacetylases (HDACs)), histone methyltransferases (SUV(39)H1/2 and EZH2), and ATP dependent chromatin remodeling enzymes (e.g. hSNF2H, LSH) (Fig. 1) [1,2]. DNA methylation is a stable epigenetic mark that regulates chromatin structure and gene expression involved in processes such as X chromosome inactivation, imprinting, embryogenesis, gametogenesis, and silencing of repetitive DNA elements [3]. In this review, we emphasize the dynamic nature of epigenetic gene regulation with a focus on the role of DNA methylation during mammalian development and human disease, and provide examples on how DNA methylation, together with other epigenetic silencing mechanisms such as histone methylation and chromatin remodeling

* Corresponding author. Tel.: +1 352 392 1810; fax: +1 352 392 2953.
E-mail address: keithr@ufl.edu (K.D. Robertson).

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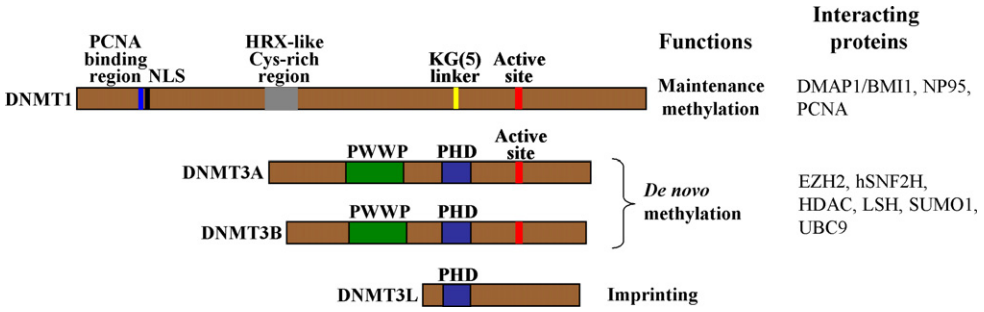

Fig. 1. The DNA methylation machinery. The mammalian DNA methyltransferase family consists of DNMT1, DNMT3A, DNMT3B, and DNMT3L. DNMT1 is considered the maintenance methyltransferase due to its high activity and preference for hemimethylated DNA during DNA replication. DNMT3A and DNMT3B are *de novo* methyltransferases. Shown in this figure is a schematic of the DNA methyltransferases with their key functional domains and protein–protein interaction domains indicated. All of the active DNA methyltransferases contain the active site motif IV in the C-terminal region (red box). DNMT1 contains a region required for its interaction with PCNA, which is adjacent to the nuclear localization signal (NLS). The N-terminal region of DNMT1 also contains a cysteine-rich HRX-like region and a lysine-glycine repeat (KG(5)) region. DNMT3A and DNMT3B contain plant homeodomain (PHD) and PWWP domains. These two domains are required for targeting DNMT3A and DNMT3B to pericentromeric heterochromatin and contribute to protein–protein interactions by recognition of histone modifications. Interacting proteins relevant to this review are also listed.

factors, enables precise control over transcriptional gene silencing.

2. The mammalian DNA methylation machinery

In mammals, DNMT3A and DNMT3B (Fig. 1) are responsible for establishing new DNA methylation patterns largely associated with retrotransposon sequences and satellite repeats in pericentromeric regions and differentially methylated regions (DMRs) of imprinted loci [1]. DNMT1, in contrast, copies existing methylation patterns following DNA replication and hence is predominantly considered the maintenance methyltransferase (Fig. 1) [1]. Mice lacking *Dnmt3a* developed to term, but died at about four weeks of age. In contrast, *Dnmt3b* null mice were not viable (embryonic lethality at E14.5–18.5) and showed multiple developmental defects, demonstrating that *de novo* methylation is an essential process for mammalian development [3]. DNMT3L is homologous to DNMT3A and DNMT3B within the N-terminal regulatory region and is highly expressed in germ cells. Although catalytically inactive, DNMT3L regulates DNMT3A and DNMT3B by stimulating their catalytic activity *in vivo* (Fig. 1) [4] and DNMT3L, like DNMT3A, is required for establishing genomic imprints. Mice deficient for *Dnmt3L* display genome-wide demethylation and developmental arrest at E8.5 and lack of *Dnmt3L* leads to a failure to establish maternal DNA methylation imprints in oocytes and male sterility due to defects in spermatogenesis [1].

3. DNA methylation in pluripotency and differentiation

Differentiation is an epigenetic process associated with the selective temporal activation of lineage specific genes and the regulated silencing of pluripotency genes. Dynamic regulation of *de novo* DNA methyltransferase expression occurs during development with higher levels in undifferentiated cells and reduced expression upon differentiation [5]. DNMTs are required for cellular differentiation during early embryonic development to regulate the systematic transcriptional inactivation of particular genes by promoter methylation. Key transcription factors, such as Oct4 and Nanog, form a transcriptional regulatory network that selectively activates genes essential for murine ES cell survival and proliferation while selectively repressing genes required for cell differentiation [6]. The *Oct4* enhancer/promoter region is hypomethylated in mouse ES cells while it is hypermethylated in trophoblast stem cells demonstrating epigenetic control of Oct4 expression during early embryogenesis by DNA methylation and chromatin modification in a stage- and cell type-specific manner [7]. Subsequent studies showed that *Oct4* and *Nanog* expression was progressively silenced by acquisition of histone H3 lysine 9 (H3K9) methylation, mediated by the SET domain histone methyltransferase protein G9a, leading to alterations in chromatin structure. Local heterochromatinization, due to the recruitment of heterochromatin protein 1 (HP1) at H3K9 methylated chromatin, is required for subsequent *de novo* methylation at the *Oct4* and *Nanog* promoters. Moreover, *in vitro* differentiation of pluripotent cells lacking G9a or the orphan nuclear receptor family member GCNF (also a transcriptional repressor of *Oct4* [8]) leads to activation of *Oct4* expression due to promoter hypomethylation [9,10]. Interestingly, both Dnmt3a and Dnmt3b function synergistically within a protein complex and stimulate each other's activity to methylate the *Oct4* and *Nanog* promoters in differentiating mouse embryonic carcinoma (EC) and ES cells [11]. A study of *Oct4* transcription by injecting mammalian somatic cell nuclei into *Xenopus* oocytes demonstrated that DNA demethylation is necessary for *Oct4* transcription and subsequent epigenetic reprogramming, suggesting selective promoter demethylation precedes gene reprogramming [12,13]. This finding, in turn, suggests that reprogramming deficiencies in cloned embryos arise from abnormal removal of repressive marks and impaired DNA methylation patterns [14]. Therefore, DNA methylation may augment or lock in stable repression of pluripotency-associated genes [15,16].

A comprehensive DNA methylation analysis of 16,000 human gene promoters using methylated DNA immunoprecipitation (MeDIP) combined with high-density DNA microarrays revealed large numbers of CpG island promoters to be hypomethylated even when transcriptionally inactive in somatic cells. These regions also contained elevated levels of H3K4 dimethylation that may serve as a defensive marker against DNA methylation in high CpG-density promoters [17]. The recent finding that Dnmt3L recognizes unmethylated lysine 4 on histone H3 and induces *de novo* DNA methylation by recruitment or activation of Dnmt3a2 [18,19], provides a novel mechanistic link between these two epigenetic pathways and provides insights into how DNA methylation may complement other epigenetic regulatory mechanisms. Dnmt3L may help interpret preexisting histone marks established at different genomic regions thus enabling acquisition of DNA methylation for long-term silencing. Therefore, DNA methylation represents a unique transcription program that forms the basis of cell differentiation.

More insights into the regulation of pluripotency by DNA methylation come from recent studies involving the mammalian RNase III family nuclease Dicer. Dicer initiates RNA interference (RNAi) by processing the small RNAs that determine the specificity of