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Review

DNA methylation and demethylation probed by small molecules ☆

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ABSTRACT

DNA methylation is a covalent modification of DNA that plays an important role in setting gene expression programs during development. Recent evidence suggests that changes in DNA methylation patterns are involved in human disease through altering normal gene expression programming. In contrast to genetic changes aberrant DNA methylation patterns are potentially reversible raising the hope for DNA methylation based therapeutics. It was previously believed that the only relevant DNA methylation reaction in mature cells is DNA methyltransferase (DNMT), which accurately copies the DNA methylation pattern during cell division. The major effort in the field has therefore focused on developing DNMT inhibitors for cancer a disease of mitotic cells. However, recent evidence suggests that the DNA methylation state in both mitotic and postmitotic cells is a balance of DNA methylating and demethylating activities. This expands the scope of DNMT inhibitors to postmitotic tissues such as the brain. Since the identity of the DNA demethylating activity is still a mystery, the development of DNA demethylation inhibitors has been lagging. This review will discuss DNA methylation and demethylation machineries, and their therapeutic potentials as targets for small molecule inhibitors.

1. Introduction

Vertebrate DNA is covalently modified by addition of methyl residues at the 5' position of cytosines residing mostly in CG dinucleotides [1]. DNA methylation is unique amongst all the factors that are involved in programming gene expression since the methyl moiety is part and parcel of the chemical structure of the genome. Thus, our DNA contains two layers of information, the ancestral genetic information encoded by the 4 bases comprising the DNA sequence and the methyl moieties that bear epigenetic information. The genetic information is inherited and copied by the DNA replication enzymatic complex following Watson and Crick rules while the DNA methylation pattern is sculpted during embryonal development by an independent enzymatic machinery that includes a balance of methylating and demethylating enzymes and targeting proteins.

Not all CGs are methylated in vertebrate genome, the distribution of methylated and unmethylated CGs in the genome is tissue specific [2]. A sequence of global demethylation and methylation events accompany the process of development from the early demethylation of the male pronucleus few hours after fertilization to global *de novo* methylation during pregastrulation followed by gene and site specific demethylation during onset of activity of specific genes during cell differentiation [3]. It was originally believed that DNA methylation was involved in cell differentiation and organogenesis by setting up the organized tissue specific pattern of gene expression as well as X inactivation and parental imprinting. It was postulated therefore that the DNA methylation pattern remains stable post-differentiation because it was believed that methyl groups could not be removed from DNA by enzymatic demethylation and that *de novo* methylation does not occur in fully differentiated cells. This model did not exclude stochastic drifts in DNA methylation occurring during gestation, germ line development or in mitotic cells. However, it excluded any change in methylation in postmitotic cells or the possibility that DNA methylation inhibitors would have any impact on the brain. This dogma still dominates the way we think about the involvement of DNA methylation in cancer pathology as well as our approach to developing DNA methylation inhibitors. Since only maintenance DNA methylation during cell division was believed to occur in somatic cells, DNA methylation inhibitors were the main goal of drug development in this area and the therapeutic scope was limited to dividing cancer cells.

We suggested a decade ago that DNA methylation was a reversible signal like other biological signals [4]. If indeed DNA methylation patterns were reversible, then they could play a role in life-long genome adaptations. A reversible DNA methylation

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reaction would also imply that the demethylating reactions could serve as targets for pharmacological and therapeutic modulation. An increasing body of data supports this hypothesis. The intensity of maternal care in rodents [5] and early life adversity is associated with altered DNA methylation patterns in the adult brains [6]. Interestingly DNA methylation functions as a physiological signal during memory acquisition in fear conditioning in adult mice brains [7–10]. The emerging dynamic nature of DNA methylation in the brain extends the therapeutic scope of DNA methylation and demethylation inhibitors to organs and diseases that were previously thought to be out of range for pharmacological DNA methylation modifiers. In addition, these data point to the possibility that active DNA demethylation plays a role in cancer, and in the mechanism of action of DNA methylation modulators. This will be discussed below.

2. DNA methylation and its role in programming gene expression

DNA methylation patterns in vertebrates are distinguished by their correlation with chromatin structure. Active regions of the chromatin, which enable gene expression, are associated with hypomethylated DNA whereas hypermethylated DNA is packaged in inactive chromatin [11]. It has been known for more than two decades that DNA methylation in regulatory regions such as promoters and enhancers could silence gene expression and an inverse correlation between gene expression and DNA methylation in promoters was proposed [2]. Recent whole genome approaches revealed that promoters of vertebrate genes are generally devoid of DNA methylation and that there is an overall inverse correlation between promoter DNA methylation and gene expression [12].

Two important mechanisms for inhibition of gene expression by promoter DNA methylation are well established. Methyl cytosine in the recognition elements of transcription factors block binding resulting in reduced transcriptional activity [13,14]. A second mechanism involves recruitment of methylated DNA binding domain proteins (MBD) to methylated cytosines in promoters [15]. MBDs recruit histone-modifying complexes containing histone deacetylases (HDACs) such as the NurD complex and histone methyltransferases (HMTase) to promoters resulting in an inactive chromatin configuration around the genes [16].

The involvement of chromatin modification enzymes in the mechanism of gene silencing by DNA methylation points to possible synergistic interactions between DNA methylation inhibitors and chromatin modification inhibitors such as HDAC inhibitors. Indeed several studies have shown synergism between DNA methylation and HDAC inhibitors [16]. Combination of DNA methylation and HDAC inhibitors has been shown to be effective in preclinical studies [17,18].

Although most of the attention in the field has focused on promoter DNA methylation, recent data suggests that gene-body DNA methylation positively correlates with gene expression in plants [16] and vertebrates [12,19,20]. It is unclear whether gene body methylation is involved in regulating gene expression. It is possible that DNA methylation in gene bodies suppresses spurious firing of cryptic promoters including antisense promoters, thus facilitating firing from the correct start site. If indeed gene-body DNA methylation plays a positive role in regulating gene expression, this could confound the biological and therapeutic consequences of small molecule inhibition of DNA methylation. Indeed, a recent paper has shown that gene-body DNA demethylation causes reduced expression and processing of rRNA genes by allowing cryptic RNApolII firing [21]. It is possible therefore that DNA methylation inhibitors would result in a mixed effect on gene expression, activation of gene expression by demethylating promoters and silencing of gene expression by gene-body demethylation.

3. Enzymes involved in the DNA methylation reaction

3.1. DNA methyltransferases

The DNA methylation reaction is catalyzed by DNA methyltransferase(s) (DNMT) [11]. Methylation of DNA occurs immediately after replication by a transfer of a methyl moiety from the donor S-adenosyl-L-methionine (SAM, AdoMet) in a reaction catalyzed by DNA methyltransferases (DNMT) (Fig. 1). Three distinct phylogenic DNA methyltransferases were identified in mammals. DNMT1 shows preference for hemimethylated DNA *in vitro*, which is consistent with its role as a maintenance DNMT, whereas DNMT3a and DNMT3b methylate unmethylated and methylated DNA at an equal rate which is consistent with a *de novo* DNMT role [22]. Two additional DNMT homologs were

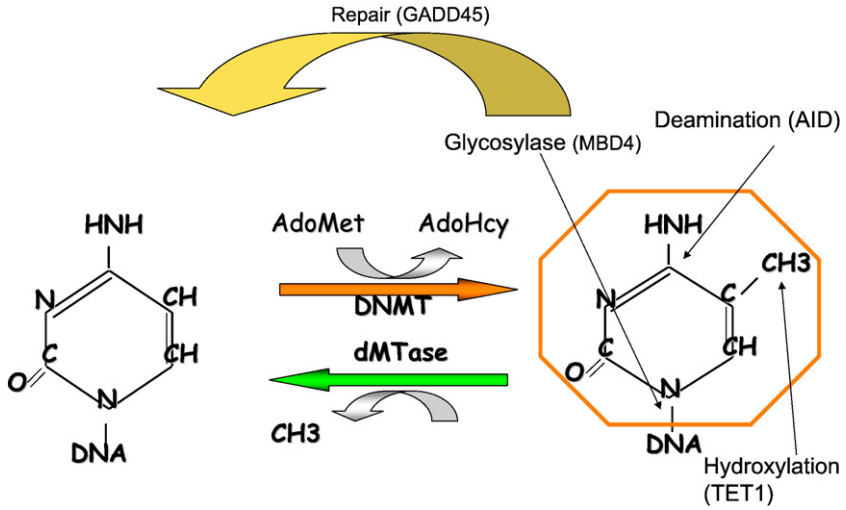

Fig. 1. DNA methylation and demethylation reactions. DNA is methylated by a transfer of a methyl moiety from the methyl donor S-adenosyl-L-methionine (AdoMet; SAM) to the 5' position on a cytosine ring by DNA methyltransferases (DNMT) releasing S-adenosyl-homocysteine. Several demethylation reactions were suggested. Direct demethylation by a demethylase enzyme (dMTase) could release a methyl moiety (CH3) in the form of either methanol or formaldehyde. Alternatively, the methyl cytosine ring could be modified by either deamination catalyzed for example by AID or hydroxylation of the methyl moiety catalyzed by TET1. The modified base is then excised and repaired. Alternatively, the bond between the sugar and the base is cleaved (by glycosylases such as MBD4) followed by repair. A repair protein shown to be associated with demethylation is GADD45(a and b).