Cell. Mol. Life Sci. (2009) 66:2249–2261
DOI 10.1007/s00018-009-0015-5

Cellular and Molecular Life Sciences

REVIEW

DNA hypomethylation in the origin and pathogenesis of human diseases

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Received: 26 January 2009 / Revised: 25 February 2009 / Accepted: 6 March 2009 / Published online: 27 March 2009
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Abstract The pathogenesis of any given human disease is a complex multifactorial process characterized by many biologically significant and interdependent alterations. One of these changes, specific to a wide range of human pathologies, is DNA hypomethylation. DNA hypomethylation signifies one of the major DNA methylation states that refers to a relative decrease from the "normal" methylation level. It is clear that disease by itself can induce hypomethylation of DNA; however, a decrease in DNA methylation can also have an impact on the predisposition to pathological states and disease development. This review presents evidence suggesting the involvement of DNA hypomethylation in the pathogenesis of several major human pathologies, including cancer, atherosclerosis, Alzheimer's disease, and psychiatric disorders.

Keywords DNA hypomethylation · G-specific hypomethylation · Cancer · Atherosclerosis · Alzheimer's disease · Psychiatric disorders

Introduction

"Epigenetics" is defined as heritable changes in gene expression associated with modifications of DNA or chromatin proteins that are not due to any alteration in the DNA sequence [1–3]. Such modifications include the best known and much studied methylation of DNA, a covalent addition of a methyl group (CH_3) to the cytosine residue at CpG sequences in mammals [4], and the modifications of the proteins that bind to DNA [5, 6]. These epigenetic modifications are essential for normal development and proper maintenance of cellular functions in adult organisms. Additionally, alterations in DNA methylation, both decreases and increases, are a frequent characteristic of a wide range of human pathologies. Although these alterations are well established and have been studied extensively [2, 3], until recently, most biomedical research has concentrated on the role and mechanisms of hypermethylation under normal physiological conditions, e.g., aging [7], or during pathological conditions [2, 8]. Much less attention has been devoted to the role and place of the disease-linked DNA hypomethylation [9]. This review presents evidence suggesting the involvement of DNA hypomethylation in the pathogenesis of several major human pathologies, including cancer, atherosclerosis, Alzheimer's disease, and psychiatric disorders.

DNA methylation

DNA methylation is the addition of a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the fifth carbon atom in the cytosine pyridine ring, resulting in the formation of 5-methylcytosine (5meC) [10] (Fig. 1a). This reaction is catalyzed by DNA methyltransferases (DNMTs) [11, 12]. In eukaryotes, this stable post-synthetic epigenetic mark is found exclusively at cytosine residues at CpG sequences [13]. DNA methylation is essential for normal development and the maintenance of cellular homeostasis and functions in adult organisms, particularly for X-chromosome inactivation in females

The views expressed in this paper do not necessarily represent those of the US Food and Drug Administration.

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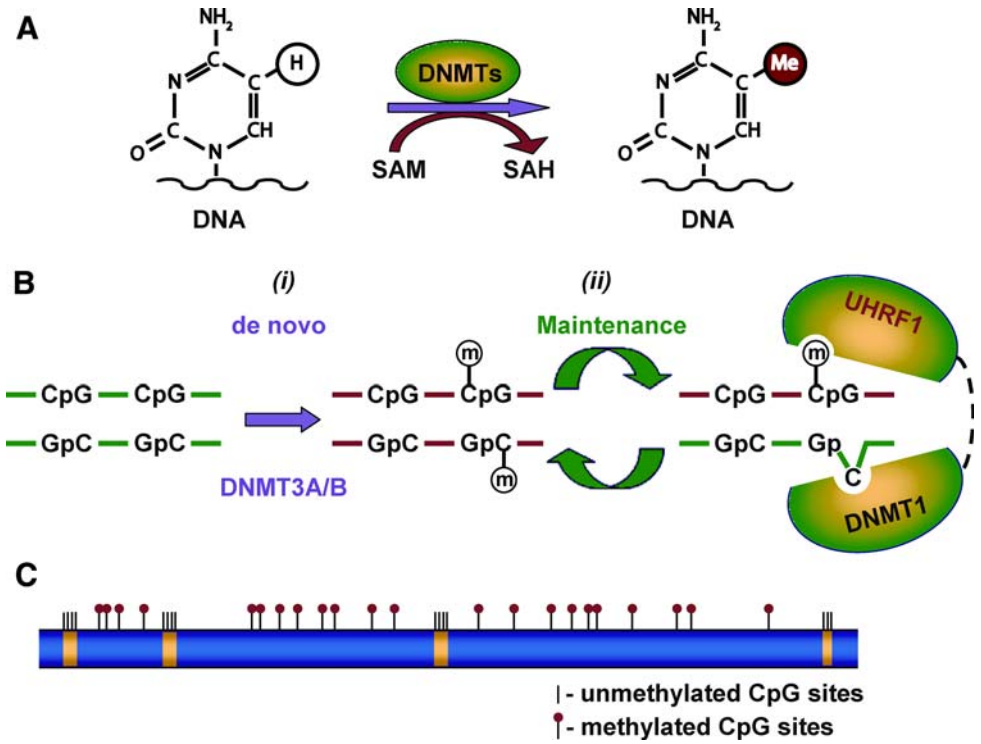

Fig. 1 Schematic model showing cytosine DNA methylation. **a** Cytosine residues in DNA at CpG sites are converted 5-methylcytosines by the addition of a methyl group from SAM to the fifth carbon atom in the cytosine pyridine ring. This reaction is catalyzed by the enzymatic activity of DNA methyltransferases (DNMTs). **b** Establishment and maintenance of the DNA methylation pattern during DNA replication. (*i*) DNA methylation is initiated and established during embryonic development by means of the de novo DNMT3A and DNMT3B DNA methyltransferases. (*ii*) Maintenance of DNA methylation. During DNA replication, DNA methylation is maintained by a complex coordinated action of the maintenance methyltransferase DNMT1 and UHRF1 [28–30]. The SRA domain of UHRF1 recognizes the hemimethylated CpG site and recruits DNMNT1, which transfers methyl group to the unmethylated cytosine residue on the newly synthesized DNA strand. **c** DNA methylation landscape in mammalian genomes. Methylation in normal mammalian cells occurs primarily at CpG sites located in repetitive sequences, exons other than first exons, and intergenic DNA (*blue*). The CpG islands that span the promoter and first exons of the majority of genes are usually unmethylated (*yellow*) in normal cells and embedded in a matrix of long methylated domains (*blue*) [33, 34]

[14], genomic imprinting [15], silencing of repetitive DNA elements [11, 16], regulation of chromatin structure [17], and proper expression of genetic information [18].

DNA methylation is initiated and established by means of the de novo DNA methyltransferase DNMT3 family (DNMT3A and DNMT3B) [11, 19] (Fig. 1b), whose expression is coordinated by DNMT3L [20], Lsh (lymphoid-specific helicase) [21], microRNAs [22], and piRNAs [23]. During DNA replication, DNA methylation is maintained by a complex cooperative interplay of maintenance methyltransferase DNMT1 with the de novo DNA methyltransferases DNMT3A and DNMT3B [24, 25], methyl-CpG-binding protein 2 (MeCP2) [26], histone-modifying enzymes [27], and the UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) protein [28–30] (Fig. 1b).

Total genomic DNA methylation refers to the overall content of 5meC in the genome. Approximately 70–90% of the CpG dinucleotides in the mammalian genome are methylated [31]; however, the CpG sites are not distributed uniformly across the genome [31, 32]. The methylation landscape of mammalian genomes consist of short (<4 kb) unmethylated domains embedded in a matrix of long methylated domains (Fig. 1c) [33, 34]. Promoters and first exons of the majority of genes in the genome are strongly enriched in unmethylated domains and depleted in methylated domains, which are found predominantly in interspersed and tandem repetitive sequences and exons other than first exons [33, 34]. The enrichment of CpG islands, genomic regions that contain the high G + C content and the high frequency of CpG dinucleotides [35], in unmethylated domains is the major difference between the unmethylated and methylated DNA regions [33, 34].

The accurate maintenance of DNA methylation patterns depends on the function and cooperation of several critical factors, including the activity and expression of DNMTs [11, 36], DNA demethylase [37], histone-modifying

enzymes [27, 38], the status of one-carbon metabolism [39, 40], DNA integrity [41, 42], and cell proliferation [43]. Disturbances in any or all of these factors may lead to an altered DNA methylation status, including DNA hypomethylation.

Mechanisms of DNA hypomethylation

DNA hypomethylation signifies one of the major DNA methylation states, the other being hypermethylation, and in most cases refers to a relative situation in which there is a decrease from the "normal" methylation level [31]. The mechanism of DNA hypomethylation is still unclear, and, very likely, there is not a single mechanism responsible for demethylation of DNA. However, it is well established that several factors may trigger and contribute to the loss of genomic methylation.

DNA methyltransferases and DNA hypomethylation

A large body of evidence clearly demonstrates that the proper function of DNMTs is crucial in the maintenance of faithful DNA methylation [36]. Reducing the expression of *Dnmt*s through gene-targeting of individual *Dnmts*, or a combination of *Dnmts*, is associated with markedly decreased global methylation levels [44–46]. For instance, the reduction of *Dnmt1* expression to 10% causes significant hypomethylation of centromeric and endogenous retroviral intracesternal A particle (IAP) repetitive sequences in mice [44]. Likewise, the loss of DNMT function secondary to the inhibition of its activity with demethylating agents, such as 5-aza-2′-deoxycytidine (5-aza-dC) [45], homocysteine, or its metabolite S-adenosyl-L-homocysteine (SAH) [46], results in rapid demethylation of DNA. Additionally, DNA demethylation caused by exposure to a number of environmental chemicals, e.g., arsenic [47], and nutritional and life-style factors, such as dietary bioflavonoids [48], alcohol [49], and cigarettes [50], is associated with an inhibitory effect on the expression and activity of DNA methyltransferases.

The normal status of DNA methylation also depends on cooperation between individual DNMTs [24, 25] and critical regulators of DNMTs function, including DNMT3L [20], Lsh [21, 51], microRNAs [22], and piRNAs [23]. Additionally, the results of a recent study have demonstrated the involvement of lysine-specific demethylase 1 (LSD1) for the maintenance of DNA methylation by regulation of the methylation status of DNMT1 and modulation of its stability [38]. Aberrations in any of these factors may compromise the DNMT function leading to DNA hypomethylation.

One-carbon metabolism and DNA hypomethylation

The methyl groups needed for all cellular biological methylation reactions, including DNA methylation, are acquired from SAM, the primary universal donor of methyl groups, which is derived from methionine through a one-carbon metabolic pathway [10]. This process indispensably connects faithful DNA methylation to the proper functioning of the one-carbon metabolic pathway, which has a great impact on DNA methylation [39, 40]. There are two groups of risk factors that may compromise the normal functioning of the one-carbon metabolic pathway and, subsequently, affect the DNA methylation profile. The first group consists of nonmodifiable genetic risk factors, such as genetic variations in genes encoding enzymes involved in the cellular one-carbon metabolism. Indeed, there are extensive amounts of data showing that single nucleotide polymorphisms in these genes are associated with aberrant DNA methylation [52, 53]. The second group consists of potentially modifiable factors, specifically essential nutrients involved in the metabolism of methyl groups, including methionine, choline, folic acid, and vitamin B_{12} [39, 40, 54]. Previously, we and others have demonstrated that long-term exposure to an inadequate supply of methionine, choline, folic acid, or vitamin B_{12} results in a profound loss of cytosine methylation in the livers of male rats and mice [55–57]. Additionally, it is believed that the loss of DNA methylation induced by exposure to arsenic [47, 58], diethanolamine [59], trichloroethylene [60], and alcohol [61], is associated with perturbations in cellular SAM homeostasis.

DNA integrity and DNA hypomethylation

The integrity of the genome is another critical factor that affects the normal status of DNA methylation. Every living organism is exposed to a variety of genomic insults on a daily basis from many endogenous and exogenous sources [62]. The results of several studies have demonstrated that the presence of unrepaired lesions in DNA induced by these factors substantially alters the methylation capacity of DNA methyltransferases, leading to DNA hypomethylation [41, 42, 57]. Specifically, the presence of 8-oxoguanine and 5-hydroxymethylcytosine in DNA, common DNA modifications resulting from oxidative damage to DNA, inhibits the binding of the MeCP2 protein and diminishes the ability of the DNMTs to methylate DNA [41, 63]. Likewise, the presence of pyrimidine photodimers, preferentially induced by sunlight at methylated CpG sites [64], reduces DNA methylation [65]. The significance of these processes in DNA hypomethylation increases progressively with age due to an age-dependent decrease in the proficiency of DNA repair [66].

DNA demethylases and DNA hypomethylation

It is believed that, in addition to a passive loss of DNA methylation through the blocking of methylation of cytosine residues, DNA methyl groups can be removed by active demethylation by means of DNA demethylases. DNA demethylase activity has been attributed to several proteins, including RNA-dependent 5meC glycosylase [67] with the involvement of DNA repair pathway [68], ribozyme-like demethylase [69], and methyl-binding domain 2 (MBD2) [70]. Furthermore, two recent reports claim that DNMT3A and DNMT3B may also act as DNA demethylases [71, 72]. Interestingly, all these proteins are characterized by quite different DNA demethylating mechanisms. However, despite efforts to identify unambiguously a DNA demethylase, the evidence for the existence of an active DNA demethylation in mammals remains inconclusive [37].

DNA hypomethylation and human diseases

DNA hypomethylation and cancer

Classically, the development of cancer in humans has been viewed as a progressive multistep process of transformation of normal cells into malignant cells driven by genetic alterations [73, 74]. However, a wealth of data in the past decade indicating the importance of epigenetic processes has largely changed the view on cancer as being a solely genetic disease [75]. Currently, cancer is recognized as a disease driven by both genetic and epigenetic alterations, and both of these components cooperate and complement each other at every stage of cancer development [75].

The loss of global DNA methylation, the first epigenetic abnormality identified in cancer cells more than a quarter century ago [76–78], continues to be a central feature and one of the most common molecular alterations in human cancers. This is evident by the fact that almost all of major human cancers, including colon [79, 80], gastric [80], lung [81], liver [82], breast [83], bladder [84], ovarian [85, 86], and endometrial [87], are characterized by a profound cancer-linked hypomethylation of the genome. More importantly, the association between the degree of DNA hypomethylation and the grade and stage of cancer gives a firm basis for its use as a biomarker for the diagnosis and prognosis of disease [84–86]. Indeed, the results of several studies have demonstrated that DNA hypomethylation is a more informative prognostic marker than tumor stage or grade [85]. However, a decrease in DNA methylation, by itself, is not sufficient to address precisely the role of DNA hypomethylation in tumorigenesis [88] because it could simply be a secondary consequence of malignant cell transformation reflecting the undifferentiated state of tumors. To provide evidence that hypomethylation has a significant role in cancer development, it is necessary to demonstrate the following: (1) the loss of DNA methylation occurs at a considerable frequency at early stages of carcinogenesis, (2) changes that occur at preneoplastic stages are also present during later stages of cancer, (3) additional changes in methylation are acquired during tumor progression, and (4) a mechanistic link exists between the hypomethylation of DNA and cancer development. The results of numerous studies demonstrating (1) the frequent loss of DNA methylation during premalignant pathological states or during early preneoplastic stages of tumorigenesis [81–84], (2) a greater degree of DNA hypomethylation in tumors compared to preneoplastic tissues [83–87], and (3) cumulative methylation changes during cancer progression from normal to stage IV disease in various cancers [86] provide convincing evidence that loss of DNA methylation in cancer is not a secondary event. Furthermore, a recent large case-control study has furnished solid evidence for an association between DNA hypomethylation and an increased risk of bladder-cancer development [89]. In addition, a decrease in DNA methylation by gene-targeting of *Dnmt1* [44, 90, 91] and *Lsh* [92] results in tumor induction, providing strong evidence for a causative role of DNA hypomethylation in the origin of cancer.

The mechanistic link between the loss of DNA methylation and cancer development, including induction of chromosomal instability, reactivation and transposition of retrotransposable elements, loss of imprinting, and activation of normally silenced genes, is directly related to the DNA methylation landscape of the mammalian genome and the function of DNA methylation in normal cells. As mentioned previously, the mammalian genome consists of relatively short unmethylated domains embedded in a matrix of long stably methylated domains, in which methylation occurs at repetitive elements and within the body of genes [33, 34]. Because of this, loss of DNA methylation largely affects only these areas of the genome. Evidence for this is provided by the strong correlation between the loss of global DNA methylation and the demethylation of repetitive sequences, such as long interspersed nucleotide elements (LINE), short interspersed nucleotide elements (SINE), IAP, and Alu elements in tumors. Furthermore, loss of LINE-1 methylation has been proposed as a surrogate marker for cancer-linked genome demethylation [93].

There are two well-established consequences associated with the loss of DNA methylation at repetitive sequences that may contribute to tumorigenesis. First, demethylation of repetitive sequences located at centromeric, pericentromeric, and subtelomeric chromosomal regions may cause

the induction of chromosomal abnormalities. For example, recent findings have demonstrated that DNA hypomethylation at the centromeric region causes permissive transcriptional activity at the centromere [94] and the subsequent accumulation of small minor satellite transcripts that impair centromeric architecture and function [95]. Likewise, hypomethylation of the subtelomeric regions is associated with enhanced transcription of the telomeric region [96, 97]. Second, hypomethylation of LINE-1, SINE, Alu, and IAP retroviral elements causes their activation and transposition [98] that may lead to genomic instability. An integral role of the loss of DNA methylation and the presence of these alterations in the neoplastic process is now commonly accepted.

One of the main functions of DNA methylation in normal cells is genomic imprinting [15], a parent-of-origin-dependent allele-specific expression of a small number of genes (approximately 90). Loss of imprinting (LOI), a loss of monoallelic regulation of imprinted gene expression, is frequently detected in human tumors and currently is considered as one of the most frequent alterations in cancer [99]. The first imprinted gene that exhibited LOI in human cancer was the insulin-like growth factor-II (*IGF2*) gene [100]. Initially, LOI of *IGF2* was linked to increased methylation; however, a number of studies have established that hypomethylation is the reason for LOI of the *IGF2* gene in colorectal [101, 102], breast [102], liver [103], and bladder [104] cancers; the *H19* gene in colon [102] and lung [105] cancers; and the *KCNQ1* gene in breast, liver, and colon cancers [106, 107].

It is well established that more than 70% of the genes in the human genome normally contain unmethylated CpG islands in their promoters [108]. However, a recent analysis of 5,549 autosomal genes with dense CpG island promoters indicates that about 4% of these genes are methylated and silenced under normal conditions [109]. Until recently, the majority of the studies in the field of cancer research have focused on the role of promoter hypermethylation and gene silencing in cancer, which overshadowed the significance of the hypomethylation of normally methylated genes in cancer development. However, mounting evidence indicates that gene-specific hypomethylation also plays an important role in cancer. Table 1 lists selected hypomethylated and overexpressed genes in various human cancers.

This list is noticeably shorter than the number of hypermethylated genes in human cancers [153] and even in any specific type of cancer, e.g., breast cancer [154]. This is because the number of genes that can potentially be demethylated (normally methylated) is substantially smaller than the number of genes that can potentially be methylated (normally unmethylated), which is directly predetermined by the methylation landscape of the genome. Despite the different number of cancer-linked hypomethylated and hypermethylated genes, the dynamic of gene-specific methylation changes during tumorigenesis is identical: the progressive accumulation of hypomethylated or/and hypermethylated alterations during tumor development.

DNA hypomethylation, carcinogen exposure, and cancer risk assessment

Environmental exposure to natural and man-made chemical and physical agents is one of the major causes of human cancer [155]. The need for the rapid identification and appropriate regulation of human carcinogens before their dissemination into society is of prime importance for the primary prevention of neoplasia in humans. Until now, research emphasis in cancer risk assessment and cancer epidemiology has focused on the measurement of DNA damage, DNA adduct formation, and mutations induced by specific agents or exposures [156]. The recognition of the role of epigenetic mechanisms in carcinogenesis and results of studies documenting that environmental exposures can alter expression of genetic information not only by genetic but also by epigenetic mechanisms [157] have challenged our current approach to carcinogenicity testing and indicated the need for a new generation of exposure biomarkers [158]. The results obtained in numerous animal studies have demonstrated that early indicators of carcinogenic exposure are epigenetic alterations and the emergence of epigenetically reprogrammed cells with epigenetic alterations similar to those found in malignant cells [155, 159]. Furthermore, it has been proposed that epigenetic alterations, including genomic and repeat-associated hypomethylation, may precede genetic alterations [159, 160]. Additionally, considering the stability and inheritance of epigenetic alterations through transmission of carcinogen-induced aberrant epigenetic patterns from one cell generation to the next, epigenetic alterations may be better biomarkers of carcinogenic exposure. The results of recent human studies have provided strong support for this suggestion [161–164]. For instance, low-level occupational exposure of gas-station attendants and traffic police to benzene has resulted in significant epigenetic alterations, as characterized by a significant reduction of LINE-1 and *MAGE1* gene methylation in blood DNA samples, compared to unexposed subjects [161]. Importantly, the aberrant DNA methylation patterns in exposed individuals highly reproduce the aberrant epigenetic patterns found in acute myelogenous leukemia patients. Similar DNA methylation changes in the blood have been found in humans exposed chronically to organic pollutants [162], arsenic [163], and traffic-derived particles [164].

Table 1 Selected list of the hypomethylated genes in human cancers

Gene	Official gene name	Tumor	References
S100A4	S100 calcium binding protein A4	Colon, endometrial, pancreatic	[110–112]
CYP2W1	Cytochrome P450, family 2, subfamily W, polypeptide 1	Colon	[113]
CDH3	Cadherin 3 (P-cadherin)	Colon, breast	[114, 115]
BAGE	B melanoma antigens	Colon	[116]
DCN	Decorin	Colon	[117]
MAGE-A1	Melanoma antigen, family A, 1	Colon, gastric	[118, 119]
MAGE-A3	Melanoma antigen, family A, 3	Colon, gastric	[118, 119]
XAGE-1	X antigen family	Gastric	[120]
CCND2	Cyclin D2	Gastric	[121]
SERPINB5	Serpin peptidase inhibitor, clade B, member 5 (Maspin)	Gastric, pancreatic, thyroid	[112, 122–124]
MUC2	Mucin 2	Gastric	[125]
NGALR	Neutrophil gelatinase-associated lipocalin receptor	Esophagus	[126]
CD133	Cell surface protein CD133	Brain	[127]
NAT1	*N*-Acetyltransferase	Breast	[128]
FEN1	Flap endonuclease 1	Breast	[129]
SNCG	Synuclein gamma	Breast, ovarian	[130, 131]
UPA	Plasminogen activator, urokinase	Breast, prostate	[132]
CAV1	Caveolin 1	Breast	[133]
ZEB2	Zinc finger E-box binding homeobox 2	Breast	[134]
TFF3	Trefoil factor 3	Pancreatic, liver	[112, 135]
CLDN4	Claudin 4	Pancreatic, ovarian	[112, 136]
LCN2	Lipocalin 2	Pancreatic	[112]
PAX2	Paired box 2	Endometrial	[137]
DNMT3L	DNA (cytosine-5-)-methyltransferase 3-like	Endometrial	[138]
CAGE	Cancer/testis antigen	Endometrial	[139]
ER-α	Estrogen receptor-alpha	Endometrial	[140]
HNF-1β	Hepatocyte nuclear factor-1 beta	Ovarian	[141]
BORIS	Brother of the regulator of imprinted sites	Ovarian	[142]
CA9	Carbonic anhydrase IX	Renal	[143]
GLIPR1/RTVP-1	Glioma pathogenesis-related 1/related to testis-specific, vespid, and pathogenesis proteins 1	Wilms tumors	[144]
HPSE2	Heparanase 2	Prostate	[145]
PRAME	Preferentialy expressed antigen of melanoma	Myeloid leukemia	[146]
DDX43	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43 (HAGE)	Myeloid leukemia	[147]
PRDM16	PR domain containing 16 (MEL1)	T-cell leukemia	[148]
BCL2	B-cell CLL/lymphoma 2	B-cell lymphocytic leukemia	[149]
TCL1	T-cell leukemia/lymphoma 1A	T-cell lymphocytic leukemia	[150]
FGFR1	Fibroblast growth factor receptor 1	Rhabdomyosarcoma	[151]
TNFRSF8	Tumor necrosis factor receptor superfamily, member 8 (CD30)	Hodgkin lymphoma	[152]

DNA hypomethylation and cardiovascular diseases

Atherosclerosis and its complications are a major cause of mortality, morbidity, and disability in developed Western countries [165]. Atherosclerosis is characterized by the infiltration of lipid particles into the arterial wall, accompanied by the recruitment of inflammatory and immune cells, migration and proliferation of smooth muscle cells, synthesis of extracellular matrix, and development of fibrocellular lesions [166]. In contrast to cancer research, where the role of DNA hypomethylation has been studied for decades, the involvement of DNA hypomethylation in the context of atherosclerosis was first formulated by Newman in 1999 [167]. The hypothesis was based on evidence suggesting that elevated plasma homocysteine is a risk factor for atherosclerosis [168] and that homocysteine and SAH efficiently inhibit DNA methyltransferases, causing hypomethylation of DNA. The significance of the

loss of DNA methylation in atherosclerosis is widely documented [169–171]. Substantial global DNA hypomethylation has been found in peripheral white blood cells [172], smooth muscle cells [170, 173], and atherosclerotic lesions [174] in patients with atherosclerosis. These correlative studies, without disputing the underlying role of homocysteine as a risk factor for atherosclerosis, suggest that hypomethylation during atherosclerosis may be a secondary event induced by elevated homocysteine. However, it should be noted that the result of a recent study, in which the occurrence of global DNA hypomethylation prior to the formation of atherosclerotic lesions in genetically atherosclerosis-prone *Apoe*$^{-/-}$ mice, clearly demonstrated the significance of DNA hypomethylation in the pathogenesis of atherosclerosis and in susceptibility to the disease [175]. Furthermore, transcriptional up-regulation of the *5-lipoxygenase* and *15-lipoxygenase* genes, key enzymes implicated in the pathogenesis of atherosclerosis [176], is mediated by promoter hypomethylation [177, 178].

DNA hypomethylation and neurodegenerative diseases and psychiatric disorders

Alzheimer's disease is an age-related progressive neurodegenerative disorder characterized by the presence of amyloid plaques and intracellular tangles in the brain [179]. The biogenesis and accumulation of amyloid plaques, which consist primarily of 40- to 42-residue β-amyloid peptides (Aβ40 and Aβ42) derived from amyloid precursor protein (APP) as a result of sequential proteolic processing by β-secretase (BACE1) and γ-secretase complex [180], is a key event in Alzheimer's disease. An association between DNA hypomethylation and Alzheimer's disease has been noted in several studies. For example, hypomethylation-associated overexpression of the *APP* gene has been demonstrated in the brain of an Alzheimer's patient [181] and, in another study, substantial age-dependent *APP* promoter demethylation has been demonstrated in the cortex from Alzheimer's patients [182]. Specifically, the frequency of methylation of cytosine residues at -207, -204, -200, and -182 in the *APP* promoter region in subjects younger than 70 years was substantially greater (55%) compared to subjects older than 70 years (5%) [182]. Additionally, expression of the *presenilin 1* (*PS1*) gene, which encodes a key component of the γ-secretase complex, is regulated by methylation [183]. In light of these considerations, the following hypothetical model is proposed for the pathogenesis of Alzheimer's disease driven by the DNA hypomethylation events (Fig. 2). First, the age-related hypomethylation of the *APP* promoter provokes an over-expression of the *APP* gene, leading to greater levels of APP in brain. Second,

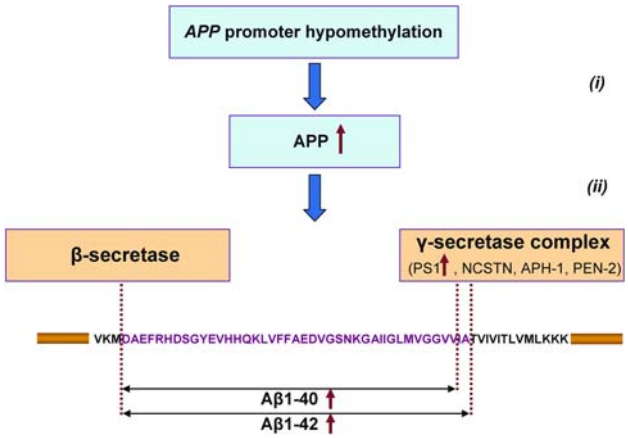

Fig. 2 Hypothetical model of the pathogenesis of Alzheimer's disease driven by DNA hypomethylation events. Involvement of DNA hypomethylation in the biogenesis and processing of APP in the human brain. First, hypomethylation of the *APP* promoter provokes an overexpression of the *APP* gene, leading to greater levels of APP protein in the brain (*i*). Second, the hypomethylation and up-regulation of the *PS1* gene induces the activity of the γ-secretase complex and stimulates the proteolytic cleavage of APP (*ii*), leading to the accumulation of Aβ40 and Aβ42. Note that genes encoding nicastrin (*NCSTN*), anterior pharynx defective 1 (*APH-1*), and presenilin enhancer 2 (*PEN-2*), three other components of the γ-secretase complex, also contain CpG islands according to http://cpgislands.usc.edu [35] and may be regulated by DNA methylation

hypomethylation and up-regulation of the *PS1* gene activates the γ-secretase complex and stimulates the proteolytic cleavage of APP, leading to the accumulation of Aβ40 and Aβ42. Importantly, this model brings together the two most widely accepted hypotheses of Alzheimer's disease, the amyloid and presenilin hypotheses, into a single mechanism.

Despite the strong evidence that supports a genetic origin of major human psychiatric disorders, no specific gene associated with the development of these disorders has been identified [184]. In contrast, a growing body of evidence suggests the involvement of aberrant epigenetic mechanisms in the pathogenesis of major psychiatric disorders, including schizophrenia and bipolar disorder. For instance, the involvement of promoter hypermethylation of the reelin (*RELN*) gene in the pathogenesis of schizophrenia is well-established [185]. Another critical gene that has been implicated in the etiology of psychiatric disorders is a catechol-*O*-methyltransferase (*COMT*) [184]. The results of recent studies have demonstrated a crucial role of promoter hypomethylation of membrane-bound COMT, a predominant form of COMT that is involved in the degradation of synaptic dopamine in the human brain, in the pathogenesis of schizophrenia and bipolar disorder [186]. Additionally, analysis of leukocyte DNA methylation in 124 male patients with schizophrenia has demonstrated a

significant hypomethylation of DNA compared to healthy subjects [187].

DNA hypomethylation and other human pathologies: autoimmune and chronic kidney diseases, and age-related macular degeneration

Similar global and gene-specific hypomethylation changes have been found in patients with uremia [188], systemic lupus erythematosus [189, 190], rheumatoid arthritis [191, 192], and age-related macular degeneration [193]. For example, age-related macular degeneration, the leading cause of irreversible blindness in people 50 years and older [194], is associated with hypomethylation-induced overexpression of the clusterin (*CLU*) gene [193] that encodes one of the major proteins of drusen, the deposition of which between pigment epithelium and Bruch's membrane causes blindness.

Concluding remarks

The pathogenesis of any given human disease is a complex multifactorial process characterized by many biologically significant and interdependent alterations. One of these changes, which is specific to many human diseases, is the alteration of DNA methylation, including hypomethylation. It is clear that disease by itself can induce hypomethylation of DNA; however, the loss of DNA methylation can also have an impact on the predisposition to pathological states and disease development. Interestingly, one of the common features of the previously described human chronic pathological states is their association with aging. It is well-established that levels of DNA methylation are markedly decreased upon aging [7]. DNA methylation is a crucial biological process that programs a proper expression of genetic information in mammals. The accurate status of DNA methylation is balanced in mature cells, but with age this balance is strongly shifted in favor of DNA demethylation. Therefore, DNA hypomethylation that occurs during normal aging appears to be a critical risk factor contributing to the development of chronic age-related human pathological states. In addition to age-related hypomethylation, DNA hypomethylation can be caused by various endogenous and exogenous factors, including environmental chemicals and physical agents, lifestyle factors, and infections. This induced DNA hypomethylation may predispose individuals to disease development. However, considering the fact that a remarkable feature of epigenetic abnormalities, including DNA hypomethylation, is their potential reversibility, timely correction and proper maintenance of DNA methylation levels are promising avenues to prevent the development of chronic human diseases.

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