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Review article

DNA methylation in human diseases

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ABSTRACT

Aberrant epigenetic modifications, particularly DNA methylation, play a critical role in the pathogenesis and progression of human diseases. The current review aims to reveal the role of aberrant DNA methylation in the pathogenesis and progression of diseases and to discuss the original data obtained from international research laboratories on this topic. In the review, we mainly summarize the studies exploring the role of aberrant DNA methylation as diagnostic and prognostic biomarkers in a broad range of human diseases, including monogenic epigenetics, autoimmunity, metabolic disorders, hematologic neoplasms, and solid tumors. The last section provides a general overview of the possibility of the DNA methylation machinery from the perspective of pharmaceutic approaches. In conclusion, the study of DNA methylation machinery is a phenomenal intersection that each of its ways can reveal the mysteries of various diseases, introduce new diagnostic and prognostic biomarkers, and propose a new patient-tailored therapeutic approach for diseases.

1. Introduction

The last two decades were the milestone in the era of the molecular biology of diseases as the footprints of epigenetic modifications became evident in the progression of a wide range of human diseases. Despite the common belief, the advent of whole-genome sequencing methods has demonstrated that the frequency of genetic alterations is notably low in many diseases. All genetic predispositions do not necessarily lead to disease development because the incidence of some diseases is entirely different in genetically identical monozygotic twins [1,2]. These new observations have raised a question: What can explain this genotype-phenotype discrepancy? Soon after, the answer showed itself in the cover of epigenetic alterations –a group of heritable changes in the genome that adapt themselves to the needs of cells according to environmental variations. In plain words, epigenetics transmits environmental signals to the genome without any alterations in the primary nucleotide sequence of DNA [3,4]. Epigenetic alterations include DNA methylation, histone modifications, and non-coding RNAs that are fundamental in the early stages of development as they can readily regulate the expression of genes, parental imprinting, and X chromosome inactivation. Moreover, these alterations have a

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solid foot in maintaining the stability and integrity of the genome [5].

Among different modifications, DNA methylation is one of the most popular epigenetic modifications that may recruit to change the genome signature and thus alter gene expression. DNA methylation, or what is now known as the DNA methylation machinery, was first explained by Halliday and Pugh in 1975 [6] as a process in which a group of enzymes adds a methyl group (-CH3) from S-adenosyl methionine (SAM) to the fifth carbon position of cytosine in a CpG dinucleotide [7]. They described DNA methylation as a gene expression regulatory mechanism. However, it was not long before the importance of this modification in vital biological processes such as embryogenesis [8] and aging [9] became clear. What we know now about DNA methylation is more complicated than what has been described previously; recently, it seems that advances in studying DNA methylation alteration not only might help uncover the understanding of many biological events but also may revolutionize the conventional concept of disease pathogenesis. In the present review, we aim to discuss the fundamental functions of DNA methylation in the epigenetic regulation of genes and also update the recent findings of the DNA methylation alterations in the pathogenesis/progression of different human diseases, ranging from autoimmune to metabolic disorders as well as various human malignancies.

2. Methods

To perform the literature review, our initial search consisted of an AND/OR combination of DNA methylation, DNA methyltransferases, Methyl-CpG-binding domain proteins, DNA-demethylating enzymes, diagnosis, prognosis, minimal residual disease, response, pathogenesis, survival, Imprinting disorders (Silver–Russell syndrome; Beckwith–Wiedemann syndrome; Prader–Willi syndrome; Angelman syndrome), Single-gene disorders (Immunodeficiency, centromeric instability, and facial anomalies syndrome; Hereditary sensory autonomic neuropathy 1E with dementia and hearing loss; Autosomal dominant cerebellar ataxia with deafness and narcolepsy, …), Autoimmune diseases (Systemic lupus erythematosus; Rheumatoid arthritis; Systemic sclerosis; Multiple sclerosis), Metabolic disorders (Type 2 diabetic Mellitus; Obesity), Hematological malignancies (Myeloproliferative neoplasms; myelodysplastic syndrome; Acute myeloid leukemia; T cell lymphoma; Adult T acute lymphoblastic leukemia), Solid tumors (Bladder cancer; Breast cancer; Colorectal cancer; Cervix cancer; Cancer of unknown primary; Glioblastomas; Gastric cancer; Hepatocellular carcinoma; Head and neck squamous cell carcinoma; Lung cancer; Non-small-cell lung cancer; Ovarian cancer; Prostate cancer; Pancreatic cancer; Small cell lung cancer), DNA methylation-based drugs (DNMT inhibitors; IDH1/2 inhibitors, …). We further extended our search domain by investigating scientific articles of electronic resources (Google Scholar, PubMed, Science Direct, Wiley, Scopus, and

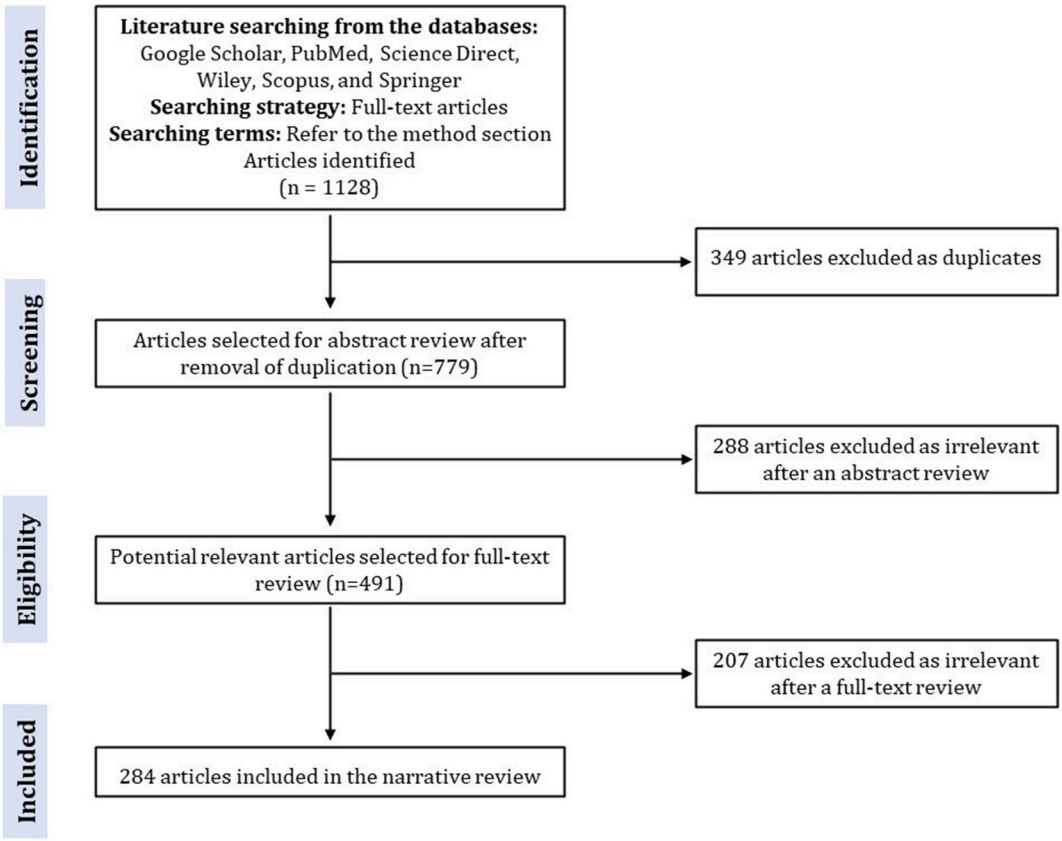

Fig. 1. The flowchart of the literature search process.

Springer) by the abbreviated form of the names. Ultimately, we investigated the results and selected the most relevant publications for review (Fig. 1).

3. DNA methylation machinery

DNA methylation is a triphasic process in which DNA methylation groups are established (*de novo* DNA methylation), preserved, and removed. The equilibrium between methyltransferases, which are known as "writers", and DNA demethylases (erasers) keeps the integrity of the process. DNA methylation machinery also needs a group of proteins known as "readers" to translate annotation into functional information [10].

3.1. DNA methyltransferases; writers

The family of DNA methyltransferases (DNMTs) is responsible for the methylation of the fifth carbon of cytosines (5 mC) in CpG dinucleotides [7]. Apart from their role in the establishment phase, DNMTs also have a hand in the maintenance of methyl groups in the DNA methylation process. There are five DNMTs in mammals, DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT3A and DNMT3B, which mostly take part in *de novo* methylation, contain two chromatin reading domains, a Pro-Trip-Trip-Pro (PWWP) domain, and ATRX- DNMT3- DNMT3L (ADD) and a highly conserved DNMT (MTase) domain in the carboxy terminus [11]. Whereas, DNMT1 and UHRF1 hold a respectable share in copying the methylation group of template strands in daughter strands after replication, a process that is observed in the maintenance phase. DNMT1, a maintenance DNMT, contains an MTase domain in the carboxy terminus and a large N-terminal regulatory region consisting of a replication foci targeting sequence (RFTS) domain, a CXXC zinc finger domain, and a pair of bromo adjacent homology (BAH) domains [12]. Either participating in the establishment or maintenance phase, DNMTs repress gene expression through direct or indirect mechanisms; prevent the binding of transcription factors (TFs) to the promotor of genes, or recruit Methyl-CpG-binding domain (MBD) proteins, which can either inhibit the binding of TFs or recruit transcriptional repression complex [10]. While the function of DNMT1, DNMT3A, and DNMT3B is canonically related to transferring methyl groups to CpG sites, DNMT2 and DNMT3L seem to have non-canonical functions. DNMT3L forms a heterotetramer with DNMT3A to increase its catalytic activity [13]. DNMT2, on the other hand, participates in methylating the cytosine of RNAs, especially transfer RNAs (tRNAs) [14].

3.2. Methyl-CpG-binding domain proteins (MBDs); readers

To repress the expression of genes, methylated CpG sites, eventually in the form of methylated CpG islands, should be translated into the function [15]. For this purpose, the methylation status of the CpGs should first be read by a group of proteins known as readers which are the MBD family (including MECP2 and MBD1-4) [15], the Kaiso and Kaiso-like proteins [16], as well as the SET and ring finger-associated (SRA) domain family [17]. Among them, MECP2, MBD1, and MBD2 seem to have a fundamental role in gene silencing. These proteins bind to methyl groups in the CpG island via their MBD domains and recruit co-repressor complexes such as histone-modifying enzymes or chromatin remodeling complexes via their transcriptional repression domains (TRD) to prevent transcription of a specific gene [15]. Other MBD proteins such as MBD3, MBD4, MBD5/6, SETDB1/2, and BAZ2A/B have also been identified as important elements in the methylation machinery, although they might have different structures [18].

In addition to MBDs, other reader proteins such as ZBTB4, ZBTB38, and ZBTB33/Kaiso recognize methylated groups in the CpG dinucleotides and interact through their zinc-finger (ZnF) domains. Unlike ZBTB4 and ZBTB38 which only need one methylated CpG as a landing site, the binding of Kaiso would not occur unless at least two methyl groups are presented on the CpG dinucleotides [16]. ZFP57 is another ZnF protein that protects the DNA methylation imprints during early embryogenesis against DNA demethylation enzymes via binding to methylated hexanucleotide (TGCCGC) in imprinting control regions (ICRs) [19]. Another reader in the DNA methylation machinery, UHRF1, is a multi-domain protein consisting of an N-terminal ubiquitin-like (UBL) domain, a tandem Tudor domain (TTD), a plant homeodomain (PHD), a SET- and RING-associated (SRA) domain, and a C-terminal RING finger domain. This protein is more involved in the maintenance of DNA methylation than silencing gene expression and is an essential agent for recruiting DNMT1 to replication foci [20].

3.3. DNA-demethylating enzymes; erasers

It was in acute myeloid leukemia (AML) molecular investigations that a group of novel proteins named ten-eleven translocation (TET) was identified as a result of chromosomal rearrangement t(10; 11)(q22; q23). It firstly claimed that this newly identified gene was responsible for encoding the histone H3 lysine 4 (H3K4) methyltransferases [21]; however, very soon, it became evident that the TET family could also carry out active DNA demethylation [22]. TET proteins showed the ability to bind to unmethylated CpGs and maintain them in hypomethylated status [23]. Moreover, they can use alpha-ketoglutarate (α-KG) and oxygen as substrate and Fe (II) as a cofactor to oxidize 5mCs to create 5-hydroxymethylcytosine (5hmC), which consequently converts into formyl-(5-fc) and carboxyl-(5caC) derivatives. After the formation of such derivatives within the DNA, DNA repair proteins such as thymine glycosylase (TDG) come into action to exit them by engaging the base excision repair (BER) pathway [24]. Thus far, three members of the TET family have been identified. TET1 erases imprinting marks in primordial germ cells [25], TET2 regulates hematopoiesis [26], and TET3 is a paramount mediator of preimplantation development via removing methyl groups in the zygote [27]. To provide a well-conceptualized perspective of the DNA methylation machinery, we summarized the mechanisms of DNA methylation and

demethylation in Fig. 2.

4. Dysregulation in the DNA methylation machinery; a missing piece in the puzzle of human diseases

The footprint of DNA methylation dysregulation in the pathogenesis of a wide range of human diseases emphasizes that methylation machinery should be positioned at the center of attention in many diseases. The results of new sequencing methods have revealed the high incidence of mutation in genes encoding DNMTs and TET in several human diseases. For example, mutations in genes encoding *DNMT1*, *DNMT3A*, and *DNMT3B,* have been identified in different diseases, such as ICF syndrome. In addition, *MECP2* mutations have been well-detected in many neurological disorders, such as Rett syndrome. The following part of this review aims to update recent advances about the role of DNA methylation abnormalities in different human diseases.

4.1. DNA methylation in monogenic epigenetic diseases

4.1.1. DNA methylation in imprinting disorders

Imprinting disorders were the first human diseases in which the footprint of DNA methylation was identified. In the human genome, more than a hundred imprinted genes have been recognized, and their expressions can be mediated in mono-allelic manners. This happens due to the induction of *de novo* methylation in one germ cell that inhibits the expression of one copy of the gene in the offspring [28]. Due to the importance of imprinting genes in human development, it is not surprising that any dysregulation in the expression of these genes leads to life-threatening congenital disorders [29]. Imprinting disorders can result from four specific causes: epimutations, uniparental disomy (UPD), deletions, and mutations in the imprinting control regions (ICRs). These molecular alterations result in the expression of the inactive allele or aberrant silencing of the active allele [30].

Epimutation can be caused by alternations of DNA methylation in the ICRs or disruptions of histone modifications among the imprinted loci. The best evidence of the mechanism through which imprinting abnormalities cause a disorder can be detected on chromosome 11p15.5, where the insulin-like growth factor 2 *(IGF2)/H19* is placed. Of note, the adjusted intracellular levels of *IGF2* and *H19* are of such importance that the excessive production of each protein is ceased through the imprinting process. Silver–Russell

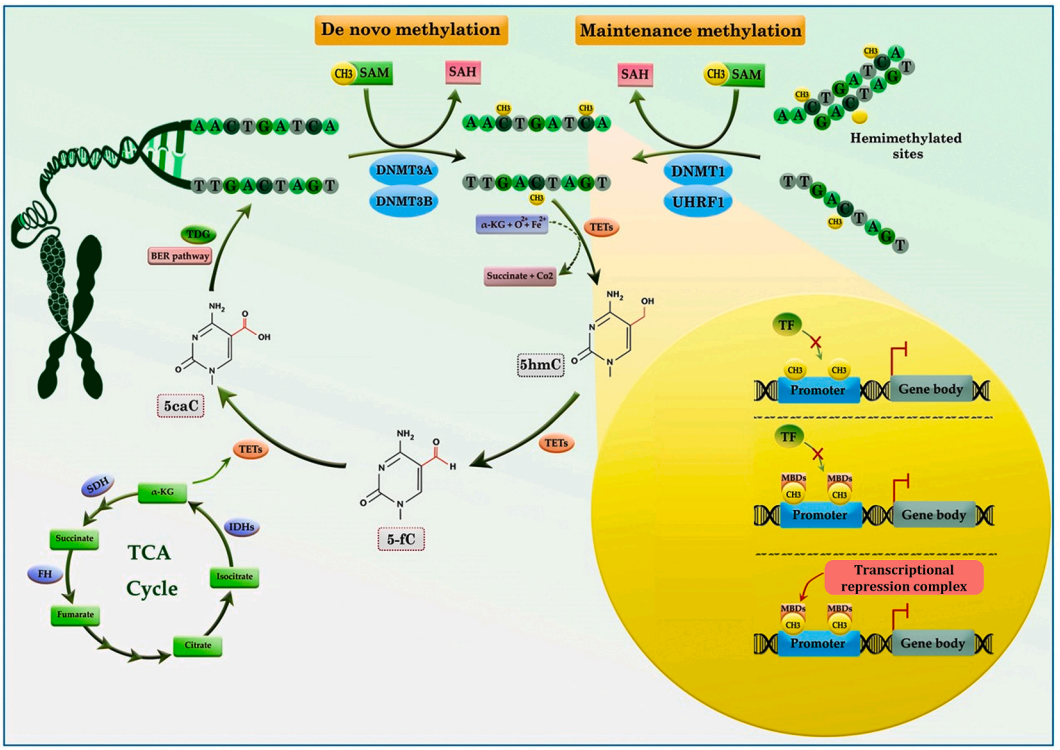

Fig. 2. A schematic representation of mechanisms of DNA methylation and demethylation. DNMT3A and DNMT3B establish *de novo* DNA methylation, whereas DNMT1 and UHRF1 add a methylation group on daughter strands after replication during the maintenance phase. DNMTs repress gene expression by inhibiting transcription factors (TFs) binding to the gene promoters or recruiting methyl-CpG-binding domain (MBD) proteins. In active demethylation, TETs oxidize 5mCs to 5-hydroxymethylcytosine (5hmC), which consequently converts into formyl-(5-fc) and carboxyl-(5caC) derivatives. Then thymine glycosylase (TDG) removes 5-fc (not shown) and 5caC during the base excision repair (BER) pathway. In the tricarboxylic acid (TCA) cycle, isocitrate dehydrogenases (IDHs) convert isocitrate to α-KG, an essential cofactor for dioxygenases, such as TET proteins. FH: fumarate hydrogenase; SAM: s-adenosyl methionine; SDH: succinate dehydrogenase.

Table 1
DNA methylation in monogenic epigenetic diseases.

Diseases [OMIM]	Locus	Molecular defect (frequency)	Major features	Ref
Imprinting disorders[a]				
SRS [180860]	11p15 7 12q14 8q12	*H19/IGF2*: paternal ICR1 LoM (50 %) Maternal UPD7 (10 %) Maternal UPD (11p15) (rare) 11p15 CNVs (<1 %) *CDKN1C, IGF2*, *HMGA2*, *PLAG1* point mutations (rare)	Intrauterine and postnatal growth restriction, dwarfism, triangular face, congenital hemihypertrophy (asymmetric body), low birth weight, and feeding difficulties	[31,64]
BWS [130650]	11p15	*CDKN1C/KCNQ1*: maternal ICR2 LoM (50–60 %) Paternal UPD (11p15) (20–25 %) *H19/IGF2*: maternal ICR1 GoM (5–10 %) 11p15 CNVs (2–4 %) *CDKN1C* point mutations on maternal allele (5 % sporadic; 50 % in families)	Lateralized overgrowth, macroglossia, placental overgrowth, exomphalos, neonatal hypoglycemia, hyperinsulinism, adrenal cortex cytomegaly, pancreatic adenomatosis, and a predisposition to embryonal tumors (primarily Wilms tumor, nephroblastomatosis)	[32,33, 65]
PWS [176270]	15q11.2–q13	15q11–q13 Paternal deletion (70–75 %) Maternal UPD15 (25–30 %) *SNURF/SNRPN*: paternal PWS/ICR GoM (~1 %)	Developmental delay, Hypotonia, short stature, delayed cognitive development, behavioral complications, pediatric obesity, and hyperphagia	[34,38]
AS [105830]	15q1.21–q13	15q11–q13 maternal deletion (75 %) *UBE3A* point mutations on maternal allele (5–10 %) Paternal UPD15 (1–2 %) *SNURF/SNRPN*: maternal PWS/ICR LoM (3 %)	Developmental delay, microcephaly, severe intellectual disability, absent or limited speech, ataxia, seizures, scoliosis, unmotivated laughing, hyperactivity, happy facial appearance, and autistic traits	[35,37]
Single-gene disorders				
ICF [602900]	20q11.21	AR mutations of the *DNMT3B* in ICF1 (50 %); *ZBTB24* in ICF2; *CDCA7* in ICF3; *HELLS* in ICF4 (50 %)	Immunodeficiency, centromeric instability, failure to thrive, mental retardation, and facial anomalies (hypertelorism, epicanthic folds, a flat nasal bridge, and low-set ears)	[41–43]
HSAN1E [614116]	19p13.2	*DNMT1*: AD mutations in the amino-terminal and middle part of the RFTS domain	Hearing and sensory loss, reduction in cognitive function, ataxia, brain atrophy, and dementia	[59]
ADAC-DN [604121]	19p13.2	*DNMT1*: AD mutations in the carboxy terminus of the RFTS domain	Cerebellar ataxia, deafness, narcolepsy, progressive cognitive and behavioral deterioration	[60]
TBRS [602729]	2p23.3	*DNMT3A*: Heterozygous *DNMT3A* haploinsufficiency mutations	Macrocephalic overgrowth, distinctive facial appearance, and moderate intellectual disability, and leading to AML in approximately 25 % of patients	[62]
MD	2p23.3	*DNMT3A*: GoF mutations in the PWWP domain	Intrauterine and postnatal growth restriction, a profound yet proportionate reduction in body size and head size	[63]
c-RTT [312750]	Xq28	*MECP2*: LoF mutation (over 90 % of the case)	A severe neurological disorder with intellectual, language, motor impairments, and autistic traits	[66]
MDS	Xq28	*MECP2*: GoF mutation (*MECP2* duplication)	Infantile hypotonia, poor speech development, severe to profound mental retardation, progressive spasticity, recurrent infections, epilepsy, autistic features	[67]
TET3 deficiency	2p13.1	*TET3*: Reduction in *TET3* catalytic activity	Intellectual disability and/or global developmental delay, hypotonia, autistic traits, movement disorders, growth abnormalities, and facial dysmorphism	[68]
Other monogenic epigenetic diseases				
FXS [300624]	Xq27.3	The aberrant repeat of CGG trinucleotide in the 5′ UTR of the *FMR1* gene and silencing of the *FMR1* gene (<1 % point mutation or deletion of *FMR1* gene)	Intellectual disability, autistic phenotypes, elongated face, protruding ears, larger testes, stereotypic movements	[66]

[a] Imprinting disorders can result from specific four causes; epimutations, uniparental disomy (UPD), deletions, and mutations in the imprinting control regions (ICRs). AS: angelman syndrome; ADAC-DN: autosomal-dominant cerebellar ataxia, deafness and narcolepsy; AR: autosomal recessive; AD: autosomal-dominant; AML: acute myeloid leukemia; BWS: beckwith–wiedemann syndrome; CNVs: copy number variation; *CDKN1C*: cyclin dependent kinase inhibitor 1C; CDCA7: cell division cycle associated 7; MD: microcephalic dwarfism; *DNMT*: DNA (cytosine-5)-methyltransferase; FXS: fragile X syndrome; *FMR1*: fragile X mental retardation 1; GoM: gain of methylation; GoF: gain of-function; HSAN1E: hereditary sensory autonomic neuropathy 1E; *HMGA2*: high mobility group A2; *HELLS*: helicase lymphoid specific; ICF: immunodeficiency, centromeric instability and facial anomalies syndrome; *IGF2*: insulin-like growth factor 2; ICR: imprinting control region; *KCNQ1*: potassium voltage-gated channel subfamily Q member 1; LoM: loss of methylation; LoF: loss of function; *MECP2*: methyl CpG binding protein 2; MDS: *MECP2* duplication syndrome; PWS: prader–willi syndrome; *PLAG1*: pleomorphic adenoma gene 1; PWWP: ProTrpTrpPro; c-RTT: classical rett Syndrome; RFTS: replication foci targeting sequence; SRS: silver–russell syndrome; *SNURF*: *SNRPN* upstream reading frame; *SNRPN*: small nuclear ribonucleoprotein polypeptide N; TBRS: tatton-brown–rahman syndrome; TET3: tet methylcytosine dioxygenase 3; UPD: uniparental disomy; *UBE3A*: ubiquitin-protein ligase E3A; UTR: untranslated region; *ZBTB24*: zinc finger and BTB domain containing 24.

syndrome (SRS) and Beckwith–Wiedemann syndrome (BWS) are two imprinting disorders that arise from defective methylation of ICR1 in the *H19/IGF2* cluster. In SRS, the mistaken hypomethylation of parental ICR1 leads to the downregulation of *IGF2* [31]. On the other hand, both paternal and maternal ICR1 display hypermethylation form in BWS, an event that leads to unnecessary up-regulation of *IGF2* in neonates [32]. Within *H19/IGF2* cluster, there is another imprinted gene named *CDKN1C/KCNQ1* cluster. The regulation of this cluster is in the hands of ICR2 (KvDMR1). In half of BWS cases, the maternal ICR2 appears in the hypomethylated form, which in synergism with hypomethylated paternal ICR2 profoundly reduces the intracellular amount of *CDKN1C* in patients [33].

Induction of paternal and maternal deletion in 15q11.2-q13 causes two other imprinting disorders named Prader–Willi syndrome (PWS) and Angelman syndrome (AS), respectively [34,35]. The methylation of PWS and AS ICRs controls the expression of small nuclear ribonucleoprotein polypeptide N (*SNRPN*) and *SNURF* in the *SNURF/SNRPN* cluster within chromosome 15q11.2–q13, which in turn, determines the expression of ubiquitin-protein ligase E3A (*UBE3A*) [36] –a protein that is responsible for the function of neurons and the typical development of synapses. In normal conditions, only AS/PWS ICRs of the maternal allele are methylated, which allows the expression of *UBE3A* in the brain. AS is caused by the loss of maternal *UBE3A* expression in neuronal cells [37], whereas PWS is caused by the loss of paternal expression of *SNRPN*, *snoRNAs*, and other genes [38].

Apart from inherited abnormalities in the imprinting process which eventually lead to the development of several imprinting disorders, evidence suggested that some assisted reproductive technologies (ARTs) that are widely used for the solving of infertility problems in recent decades can cause dysregulation in the imprinting process. The incidence of BWS, AS, and SRS in ART-born children has shed light on the detrimental impact of these technologies on CpG methylation in the imprinting genes [39]. Nevertheless, it should not be forgotten that these techniques are entirely new, and still, many questions remain unanswered in this field due to the differences in study design and the small size of cohorts.

4.1.2. Single-gene disorders of the DNA methylation machinery

Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is an atypical immunoglobulin deficiency disorder with life-threatening infections in which DNA methylation plays a crucial role in its pathogenesis [40]. According to genetic mutations, ICF is discussed in four groups ICF1, ICF2, ICF3, and ICF4 [41–43]. The molecular hallmarks of ICF typically include instability of the juxtacentromeric heterochromatin regions of chromosomes 1, 9, and 16 enriched with specific hypomethylation of pericentromeric satellite repeats II and III [44]. A wealth of evidence declared that *DNMT3B* mutations, heavily methylated α-satellites [45], and the hypomethylation of subtelomeric repeats [46] that is detectable in 50 % of patients with ICF can be used to distinguish ICF1 from other

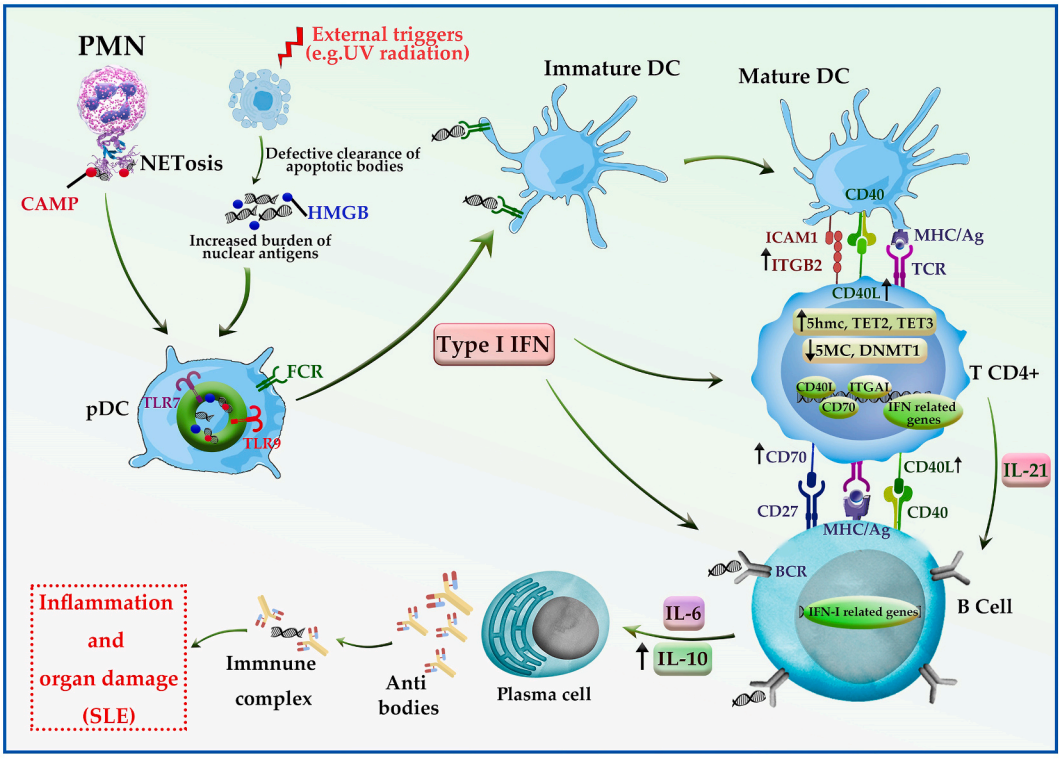

Fig. 3. A schematic representation of DNA methylation alterations involved in the pathogenesis of SLE. DNA methylation alterations in SLE immune cells (especially T helper cells) may contribute to more inflammation and autoimmunity by increased expression of genes involved in the interaction of T helper cells with B cells and DCs. BCR: B-cell receptor; CAMP: cathelicidin antimicrobial peptide; DCs: dendritic cells; FCR: Fc receptor; HMGB1: high mobility group box 1 protein; ICAM-1: intercellular adhesion molecule 1; IFNs: type I interferons; ITGB2: integrin subunit beta 2; MHC: major histocompatibility complex; pDCs: plasmacytoid dendritic cells; TCR: T-cell receptor; TLR: toll-like receptor.

types of ICF syndrome. Notably, patients with ICF1 uniquely demonstrate promoter hypomethylation of germline genes [47] and loss of methylation at X-linked genes [48], in agreement with a function for DNMT3B in establishing DNA methylation in early development. The aberrancies in other components of the DNA methylation machinery include genes encoding *ZBTB24*, *CDCA7*, and *HELLS* detected in ICF2, ICF3, and ICF4, respectively. Moreover, it has been indicated that these types of ICFs −in contrast to ICF1− display hypomethylated α-satellite [49] without any aberrations of DNA methylation in subtelomeric repeats [46]. This data suggests that ZBTB14, CDCA7, and HELLS may have a role in recruiting a DNMT other than DNMT3B to these sequences, although their associations with DNA methylation are not fully understood.

Recent studies have reported that the absence of ZBTB24, CDCA7, or HELLS leads to a decrease in DNA methylation at satellite repeats in the human embryonic kidney (HEK) 293T cells [50] and mouse embryonic fibroblasts (MEFs) [43]. In the mouse embryonic stem cells (mESCs)-based *ZBTB24* or *CDCA7* knockout model, fibroblasts and T cells derived from ICF2 patients, it has been shown that ZBTB24, a C2H2 zinc finger (ZF) transcription factor, directly binds to a sequence in the *CDCA7* promotor and regulates its expression [51,52]. Moreover, the studies indicated that CDCA7 is an essential agent for the recruitment of HELLS to chromatin and can stimulate HELLS nucleosome remodeling activity, facilitating the access of the DNA methylation machinery to genomic regions [53]. Aberrant DNA methylation patterns in ICF3 patients could be due to a defect in recruiting HELLS to chromatin [53]. Therefore, it seems that ZBTB24, CDCA7, and HELLS can serve as guides for the DNA methylation machinery.

However, it remains elusive how reduced DNA methylation can cause the phenotypes of ICF patients. Perhaps the simplest explanation is that DNA methylation alterations associated with gene expression changes play a role in the pathogenesis of ICF syndrome. For example, aberrant DNA methylation associated with dysregulation of the clustered protocadherins (*PCDH*) may be related to the neural defects observed in all patients with ICF [49]. A recent study has reported that CDCA7 and HELLS have a role in the classical non-homologous end joining (c-NHEJ) pathway and the deficiency of CDCA7 or HELLS in HEK293T cells triggers the C-NHEJ defect and delay in Ku80 accumulation at DNA damage sites. Defects in the C-NHEJ pathway are associated with the phenotypes of ICF patients, including abnormal chromosome configuration, instability of satellite repeats, reduced replication rates, and apoptosis [50]. Since the C-NHEJ pathway is required for both V(D)J- and class switch-recombination (CSR) [54,55], perhaps impaired IgE class switch induction in a non-ICF1 patient could be explained by a defect in the C-NHEJ pathway [56]. Finally, how aberrant DNA methylation can lead to immunodeficiency in ICF patients is unclear. Due to embryonic lethality and/or lack of immunoglobulin

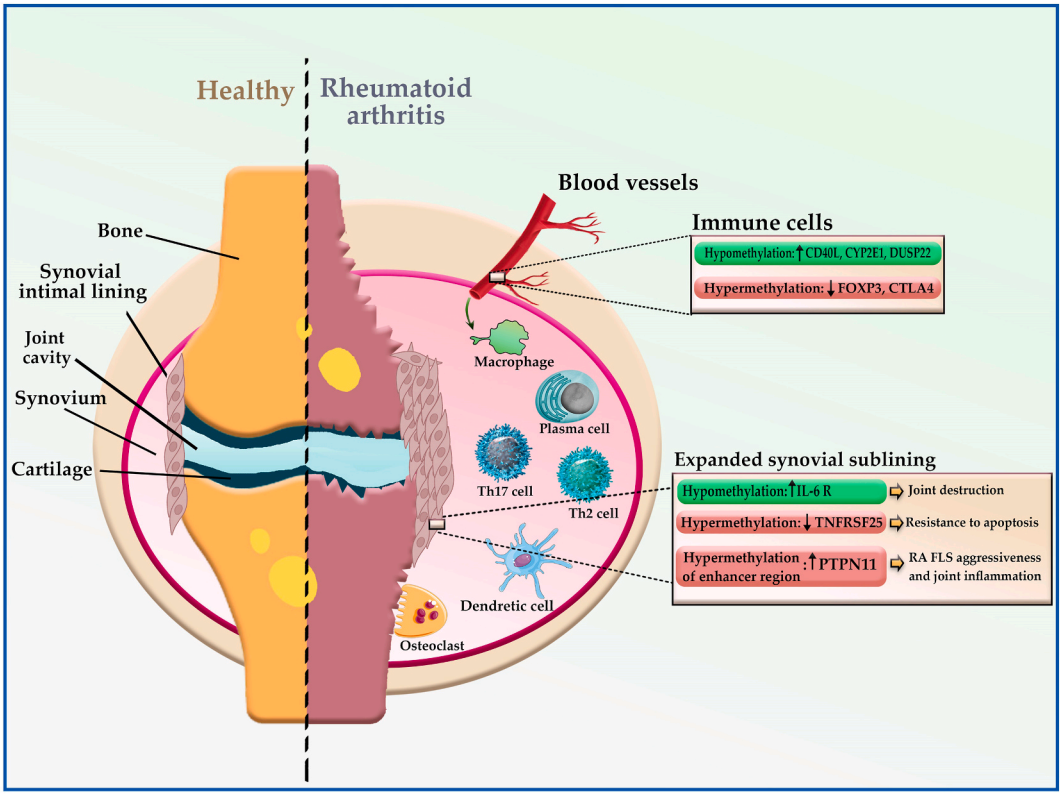

Fig. 4. A schematic representation of DNA methylation alterations involved in the pathogenesis of RA. DNA methylation alterations contribute to RA pathogenesis by affecting the FLSs and immune cells. DNA methylation in RA FLSs transforms a normal FLS into an apoptotic-resistant and aggressive phenotype that promotes inflammation and destruction of joints. On the other hand, DNA methylation in immune cells (especially regulatory T cells) may remove the immune brake and promote inflammation. . CTLA4: cytotoxic T-Lymphocyte Associated Protein 4; FLSs: fibroblast-like synoviocytes.

deficiency, mouse modeling for ICF patients is poorly informed [51,57]. There is only a limited study of data on the pericentromeric SAT1 hypomethylation that activates the innate immune system by derepressing SAT1-associated transcripts during embryonic development in the *ZBTB24*-mutant zebrafish. Such animal models may help identify the mechanisms involved in the pathogenesis of ICF syndrome in humans [58].

Apart from ICF, defects in the DNA methylation machinery have been detected in other autosomal-dominant progressive cognitive and behavioral deterioration. Hereditary sensory autonomic neuropathy 1E with dementia and hearing loss (HSNA1E; OMIM 614116) [59], and autosomal dominant cerebellar ataxia with deafness and narcolepsy (ADCA-DN; OMIM 604121) [60] all share a similarity in the presence of heterozygous mutations in *DNMT1*. Interestingly, the mutations of HSNA1E mostly affected the RFTS domain of *DNMT1* leading to cytoplasmic aggregation of this enzyme. In response to cellular stress and aging, it is believed that these aggregates induce toxicity, an event that leads to the degeneration of the central and peripheral nervous systems [61]. *DNMT1* mutation in HSNA1E and ADAC-DN, however, occur in the N-terminal and C-terminal of the RFTS domain, respectively [61]. Tatton--Brown–Rahman syndrome is another genetic disorder in which heterozygous haploinsufficiency mutations in *DNMT3A* have been reported [62]. Individuals with microcephalic dwarfism also showed to have either missense or gain-of-function mutations in the PWWP domain of *DNMT3A* [63]. The investigation of DNA methylation alterations in single-gene disorders has a long way to go; however, the identification of such mutations suggests the auxiliary impact of the DNA methylation machinery in the pathogenesis of these diseases. Table 1 summarizes DNA methylation aberrancy in monogenic epigenetic diseases.

4.2. DNA methylation in autoimmune diseases

4.2.1. Systemic lupus erythematosus (SLE)

SLE is a chronic autoimmune disease characterized by autoantibody production and immune complex deposition. External triggers (e.g., UV radiation) cause the apoptosis of cells. Inadequate clearance of the nuclei of these cells increases the burden of nuclear antigens and may lead to impaired ability to tolerate autoreactive B and T lymphocytes in susceptible individuals. In response to the self-antigens, activated plasmacytoid dendritic cells (pDCs) release type I interferons (IFNs), which increase antigen presentation by dendritic cells (DCs), thereby activating T cells. B cells are activated by exposure to self-antigens and interaction with T cells, producing autoantibodies. Eventually, the generation of immune complexes triggers increased inflammation and organ damage (Fig. 3).

At the primary stages of the disease and along with the formation of SLE, decreased expression of *DNMT1* together with induction of DNA hypomethylation in $CD4^+$ T cells increases the expression of inflammatory cytokines; an event which in turn leads to unnecessary

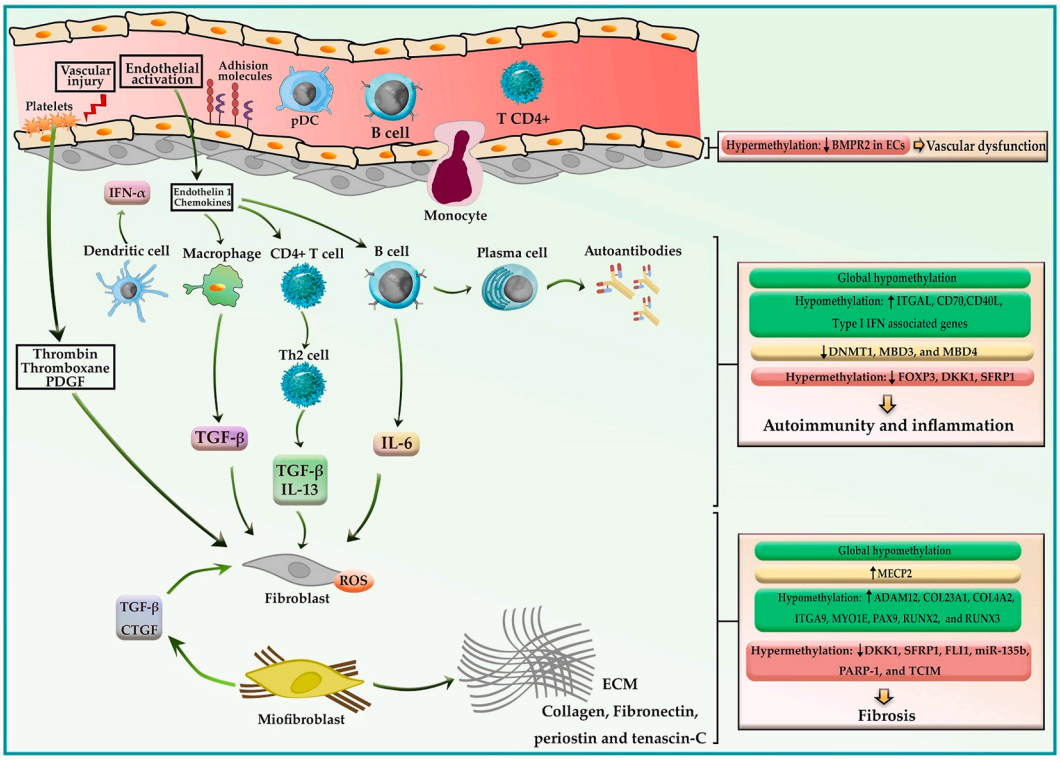

Fig. 5. A schematic representation of DNA methylation alterations involved in the pathogenesis of SSc. DNA methylation alterations contribute to SSc pathogenesis by affecting the endothelial cells, immune cells, and fibroblasts, resulting in vascular dysfunction, autoimmunity, inflammation, and fibrosis. ROS: reactive oxygen species.

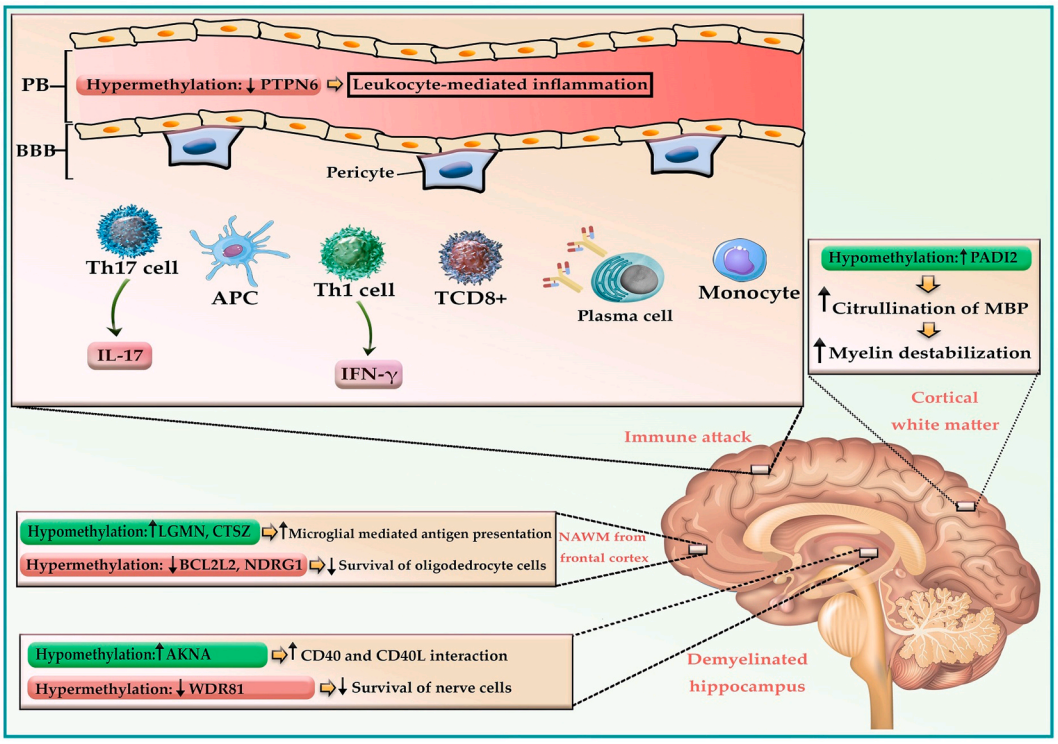

Fig. 6. A schematic representation of DNA methylation alterations involved in the pathogenesis of MS. DNA methylation alterations contribute to MS pathogenesis by affecting the immune and neurological compartments. In the immune compartment, DNA methylation may increase leukocyte-mediated inflammation through hypermethylation of the *PTPN6* gene (also known as *SHP-1*), which encodes an anti-inflammatory tyrosine phosphatase. In the various regions of the brain, specific hypomethylated and hypermethylated genes increase antigen presentation and myelin destabilization, while reducing the survival of nerve and oligodendrocyte cells.

stimulation of B cells [69]. As the disease progressed, DNA hypomethylation induced polarization of T helper 2 (Th2) cells by elevating the expression of IL-4, IL-5, and IL-13. Noteworthy, the shift of Th1/Th2 equilibrium in favor of Th2 reinforces the vicious cycle of auto-reactive antibody production [70]. These findings have been confirmed from another perspective, as well. The exposure of healthy mice to several DNA methylation inhibitors such as procainamide and hydralazine or injection of azacitidine (5-AC)-treated healthy $CD4^+$ T cells to the mice were all coupled with the development of SLE [71]. These make a consensus on the importance of $CD4^+$ T cells' methylation status in SLE pathogenesis. It has been also declared that the hypomethylation at two CpG sites within *IFI44L* can distinguish SLE patients from healthy individuals [72]. Besides, increased levels of 5hmC were associated with elevated expression of *TET2* and *TET3* in $CD4^+$ T cells from patients with SLE [73]. The evidence supporting the higher frequency of demethylation of the *CD40L* gene, which is located on chromosome X in females, also answers the question of why the incidence of SLE is higher in females [74]. As a straightforward interpretation of these events, DNA methylation modifications not only seem to contribute to SLE formation but also can guarantee disease progression.

4.2.2. Rheumatoid arthritis (RA)

RA is a chronic autoimmune disease characterized by inflammation of the synovium associated with the destruction of joint cartilage and bone erosion. Stressors cause post-translational modifications (especially citrullination) in mucosal sites. The citrullinated proteins trigger the immune response and autoantibody formation in susceptible individuals. Activated immune cells enter joints, respond to the self-proteins, and mainly trigger tissue injury by cytokine secretion and perhaps by antibody-dependent effector mechanisms. The pro-inflammatory environment in RA joints strongly activates fibroblast-like synoviocytes (FLSs) and leads to expanded synovial sublining. FLSs produce inflammatory cytokines, chemokines, and pro-angiogenic factors (such as IL-6, CXCL10, and VEGF) that promote and maintain joint inflammation. These cells also produce matrix metalloproteinases (MMPs) which lead to cartilage destruction. FLSs help bone erosion by facilitating osteoclastogenesis and interfering with bone repair (Fig. 4).

RA is one of the most important autoimmune diseases with genetic predispositions in the *HLA* gene [75]. However, it was in 2013 that the results of a study comparing the blood samples of RA patients and healthy counterparts revealed the presence of two methylation sites within the *MHC* region; opening a new chapter in the pathogenesis of the disease: this time from the perspective of DNA methylation regulation [76]. It should be noted that both RA and SLE may share similar methylation patterns in genes encoding *CD40L* [77] and *FOXP3* [78,79]. The hypermethylation of *CTLA4* in RA patients may also play an essential role in the auto-reactivity of immune responses against host antigens [80]. Noteworthy, the correlation between the amount of DNA methylation and the response

Table 2
Potential DNA methylation markers in autoimmune diseases.

Diseases	Cell/tissue type	Main findings	Ref
SLE	$CD4^+$ T cells from SLE patients and HCs	Disease activity was correlated with hypomethylation of genes involved in T cell activation and differentiation, and hypermethylation in genes involved in the inhibitory TGF-β signaling pathway.	[70]
	$CD4^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes from SLE patients and HCs	DMCpGs: 166 in B-cells, 97 in monocytes, and 1033 in T-cells; significant hypomethylation of IFN-regulated genes was detected in monocytes, T cells, and B cells of SLE patients relative to HCs.	[98]
	$CD4^+$ T cells, monocytes, granulocytes, and B-cells from discordant MZ and DZ twins for SLE	Significant hypomethylation of IFN-regulated genes was detected in all cell types, which was more pronounced in twins with disease flare within the past two years.	[99]
	PBMNs from AA SLE patients and EA SLE patients and HCs	85 % of 41 DMCpGs were hypomethylated in SLE patients relative to HCs. The number of hypomethylated CpG sites relevant to IFN-related genes was higher in AA SLE patients and SLE patients with SLEDAI>6 compared with EA SLE patients and SLE patients with SLEDAI≤6.	[100]
	Naïve $CD4^+$ T cells from HCs and SLE patients with and without a history of renal involvement	191 DMCpGs (121 DAGs) were only identified in SLE patients with a history of renal involvement. A hypomethylated CpG site in the gene *CHST12* had a sensitivity of 85.7 % and a specificity of 71.4 % to detect renal involvement in SLE patients.	[101]
	PMNCs from SLE patients and HCs	The methylation levels of *IL-10* and *IL-1R2* genes significantly reduce in SLE relative to HCs and correlate with higher disease activity.	[102]
	WB from SLE, RA, pSS patients, and HCs	Significant hypomethylation of two CpG sites within the *IFI44L* promoter can distinguish SLE patients from RA patients, pSS patients, and HCs, and was correlated with high disease activity and renal damage in SLE.	[72]
	PB from HCs, SLE, and DLE patients	The promoter hypomethylation of *IFI44L* and higher serum levels of IFN-a1 were detected in SLE patients relative to DLE patients and HCs, which may potentially serve as biomarkers to distinguish SLE from DLE.	[103]
	T cells from SLE patients and HCs	Promoter hypomethylation and increased expression of *CD70* were detected in T cells of patients with SLE compared to HCs and may lead to overstimulation of B cells.	[104]
	T cells from active SLE patients, inactive SLE patients, and HCs	Hypomethylation of specific sequences flanking the *ITGAL* promoter increases the expression of *ITGAL* (also known as *CD11a*) in T cells from patients with active lupus compared to patients with inactive lupus and HCs.	[105]
RA	$CD14^+$ monocytes, $CD19^+$ B cells, $CD4^+$ naive T cells, and $CD4^+$ memory T cells from RA patients and HCs	Promoter hypomethylation of *CYP2E1* and *DUSP22* genes were correlated with active and erosive disease, respectively.	[106]
	WB from pre-treatment RA patients	Five differentially methylated CpG sites at diagnosis time can distinguish RA patients with a good response from RA patients without response to 3 months of TNFi therapy.	[107]
	T lymphocytes from pre-treatment RA patients	The methylation levels of two CpG sites at diagnosis time can distinguish RA patients with a good response from RA patients with a poor response to 6 months of DMARDs therapy.	[108]
	WB from early RA patients	A higher baseline global leukocyte DNA methylation was associated with a decreased clinical response to MTX therapy.	[81]
	WB DNA at baseline and following 4 weeks of MTX therapy from RA patients	The methylation status of four CpGs after 4 weeks of MTX initiation was associated with disease improvement after 6 months of therapy.	[109]
	Synovial tissue from RA patients and OA patients	Promoter hypermethylation and the decreased protein expression of the *TNFRSF25* gene (also known as *DR3*), a member of the apoptosis-inducing Fas gene family, may be associated with resistance to apoptosis in RA synovial cells.	[110]
	FLSs from synovial tissues of patients with RA and OA	Hypomethylation downstream of the TSS of the *IL-6R* gene increases the expression of IL-6R in RASFs compared with OASFs and may be associated with an increase in the risk of joint destruction in RA.	[111]
	Synovial tissue from patients with OA and RA	Hypermethylation of two CpGs in an intronic enhancer region in the *PTPN11* gene (also known as *SHP2)* increases the expression of *PTPN11* in RA FLSs compared with OA FLSs and promotes RA FLSs aggressiveness and joint inflammation.	[112]
	FLSs from HCs and patients with RA in very early, resolving, established stages	Differentially methylation in the promoter of genes involved in pathways related to integrin, cadherin, Wnt signaling, the actin cytoskeleton, and components of the antigen presentation was found in FLSs from patients with very early, resolving, or established RA compared with HCs.	[113]
SSc	Fibroblasts from biopsies of lesional skin and PB from patients with SSc and HCs	Promoter hypermethylation and the decreased expression of *DKK1* and *SFRP1* genes in fibroblasts and PMNCs of patients with SSc may lead to aberrant activation of the Wnt signaling pathway.	[82]
	Dermal fibroblasts of SSc patients and HCs	Promoter hypermethylation and the decreased expression of the *FLI1* gene, a potent repressor of the type I collagen gene, were detected in dermal fibroblasts of SSc patients compared with HCs.	[83]
	Dermal fibroblasts from SSc patients and HCs	miR-135b, which reduces collagen induction in fibroblasts, was downregulated in dermal fibroblasts from SSc patients compared with	[84]

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Table 2 (*continued*)

Diseases	Cell/tissue type	Main findings	Ref
		HCs. Its downregulation may result from methylation and elevated levels of MECP2.	
	Dermal fibroblasts from SSc patients and HCs	Promoter hypermethylation and the decreased expression of the *TIPARP* gene (also known as *PARP-1*) were detected in the dermal fibroblasts from SSc patients compared with HCs. TIPARP downregulation may contribute to hyperactive TGF-β signaling and continuous fibroblast activation in SSc.	[85]
	Dermal fibroblasts from SSc patients and HCs	The level of MECP2 protein was elevated in fibroblasts from SSc patients compared with HCs. Increased binding of MECP2 to *SFRP-1* promoter leads to transcriptional silencing of *SFRP-1* and increased pro-fibrotic Wnt signaling.	[86]
	$CD4^+$ T cells from SSc patients and HCs	The global DNA methylation levels and *DNMT1*, *MBD3*, and *MBD4* expression were reduced in $CD4^+$ T cells from SSc patients compared with HCs.	[87]
	$CD4^+$ T cells from SSc patients and HCs	Decreased methylation in regulatory elements and increased expression of the *ITGAL* gene, encoding CD11a, was detected in $CD4^+$ T cells from SSc patients compared with HCs and may be associated with disease activity.	[88]
	$CD4^+$ and $CD8^+$ T cells from SSc patients and HCs	Hypomethylation and increased expression of type I IFN-associated genes were observed in $CD4^+$ and $CD8^+$ T cells from SSc patients compared with HCs.	[89]
	$CD4^+$ T cells from SSc patients and HCs	Decreased methylation of the promoter region and increased expression of the *TNFSF7* gene, encoding CD70, was detected in $CD4^+$ T cells from SSc patients compared with HCs.	[90]
	$CD4^+$ T cells from SSc patients and HCs	Decreased methylation of the *CD40L* regulatory sequence on the inactive X chromosome leads to increased expression of the *CD40L* in $CD4^+$ T cells from female patients with SSc compared with female HCs.	[91]
	$CD4^+$ T cells from SSc patients and HCs	Increased methylation of regulatory elements and the decreased expression of the *FOXP3* were detected in $CD4^+$ T cells from SSc patients compared with HCs.	[114]
	MVECs of skin biopsies from SSc patients and HCs	Promoter hypermethylation and the decreased expression of the BMPR2 gene, a member of the TGF-β superfamily of proteins that coordinate cell proliferation, differentiation, and survival, may contribute to inducing apoptosis in MVECs of SSc patients.	[115]
	Fibrotic lung tissue from SSc patients and control tissue from histologically normal areas of a peripheral lung removed at lung cancer resection as HCs	Hypermethylation and the decreased expression of the *TCIM* (also known as *c8orf4*) gene were detected in fibrotic lung fibroblasts from SSc patients compared with HCs fibroblasts. The aberrant expression of *c8or4* decreases expression of anti-fibrotic *PTGS2* (also known as COX2) and its product PGE2 in SSc.	[116]
	WB from twin pairs discordant for SSc	Methylation of 153 and 266 distinct CpG sites was correlated with lcSSc and dcSSc, respectively.	[92]
	Fibroblasts from dcSSc patients, lcSSc patients, and HCs	2710 and 1021 CpG sites were differentially methylated in dcSSc and lcSSc, respectively. The majority of these CpG sites were hypomethylated in both dcSSc (61 %) and lcSSc (90 %). Hypomethylated genes such as *ADAM12*, *COL23A1*, *COL4A2*, *ITGA9*, *MYO1E*, *PAX9*, *RUNX2*, and *RUNX3* were overexpressed in fibroblasts of both lcSSc and dcSSc patients compared with HCs fibroblasts and may contribute to fibrosis events in SSc.	[117]
MS	WB from MS patients and HCs	*Alu* and *LINE-1* were hypermethylated in MS patients compared to HCs. The methylation levels of these genes were associated with the degree of EDSS score in MS patients.	[93]
	T cells from RRMS patients and HCs	Increased DNA methylation levels in the alternative promoter of the *VDR* gene lead to increased expression of the *VDR* in T cells of RRMS patients compared to HCs.	[94]
	PBMNs from MS patients and HCs	The decrease in methylation associated with an increase in *IL*-2RA expression was found in T cells from MS patients relative to HCs.	[95]
	Leukocytes of MS patients and HCs	Promoter hypermethylation and associated silencing of the *PTPN6* gene (also known as *SHP-1*), a negative regulator of pro-inflammatory signaling, may increase leukocyte-mediated inflammation in MS patients compared to HCs.	[118]
	cfDNA of plasma or serum from MS patients and HCs	The increased levels of fully unmethylated cfDNA fragments from the *MBP3* or *WM1* loci can distinguish genomic debris from oligodendrocyte cell death in MS patients from normal cellular debris in HCs.	[119]
	Cortical white matter from the brains of MS patients and HCs	The *PADI2* gene, which regulates the production of citrullinated MBP, showed decreased promoter methylation associated with the increased protein in the cortical white matter of MS patients compared to HCs. The aberrant expression of the PADI2 enzyme leads to myelin destabilization due to increased citrullinated MBP.	[120]

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Table 2 (*continued*)

Diseases	Cell/tissue type	Main findings	Ref
	NAWM from post-mortem brain FC specimens of MS patients and HCs	Hypermethylation and the decreased expression of oligodendrocyte survival regulatory genes, such as *BCL2L2* and *NDRG1*, were found in NAWM of MS–affected brains compared to HC brains. While the proteolytic genes *LGMN* and *CTSZ* were hypomethylated, their increased expression may increase microglial-mediated antigen presentation.	[121]
	Post-mortem hippocampus from myelinated and demyelinated MS patients	144 DMPs were detected in the demyelinated MS hippocampus compared to the myelinated MS hippocampus. The *AKNA* and *SFRP1* genes showed hypomethylation and increased expression, while *WDR81*, *NHLH2*, and *PLCH1* genes showed hypermethylation and associated silencing. Increased expression of *AKNA*, a gene involved in the regulation of CD40 and CD40 ligand interaction that induces the expression of TNF-α, as well as decreased expression of *WDR81*, a gene involved in nerve survival, may lead to neuronal damage.	[122]

ADAM12: ADAM metallopeptidase domain 12; AKNA: AT-hook transcription factor; BMPR2: bone morphogenetic protein receptor 2; BCL2L2: bcl-2-like protein 2; CHST12: carbohydrate sulfotransferase 12; CYP2E1: cytochrome p450 family 2 subfamily E member 1; CD40L: CD40 ligand; COL23A1: collagen type XXIII alpha 1 chain; COL4A2: collagen type IV alpha 2 chain; cfDNA: circulating cell-free DNA; *CTSZ*: cathepsin Z; DZ: dizygotic; DLE: discoid lupus erythematosus; DMCpGs: differentially methylated CpGs; DAGs: DMR-associated genes; *DUSP22*: dual specificity phosphatase 22; DMARDs: disease-modifying antirheumatic drugs; *DKK1*: dickkopf WNT signaling pathway inhibitor 1; dcSSc: diffuse cutaneous SSc; DMPs: differentially methylated positions; EA: European-American; EDSS: expanded disability status scale; FLSs: fibroblast-like synoviocytes; *FLI1*: friend leukemia integration 1 transcription factor; *FOXP3*: forkhead box P3; FC: frontal cortex; HCs: healthy controls; *IFN*: interferon; *IL-10*: interleukin 10; *IL-1R2*: interleukin 1 receptor type 2; *IFI44L*: interferon induced protein 44 like; *ITGAL*: integrin, alpha L; *IL-6R*: interleukin-6 receptor; *IL*-2RA: interleukin 2 receptor subunit alpha; *ITGA9*: integrin subunit alpha 9; lcSSc: limited cutaneous SSc; *LINE-1*: long interspersed nuclear element 1; *LGMN*: legumain; MS: multiple sclerosis; MZ: monozygotic; MTX: methotrexate; *MECP2*: methyl-CpG binding protein 2; MBD: methyl-CpG binding domain protein; MVECs: microvascular endothelial cells; *MYO1E*: myosin IE; MBP: myelin basic protein; NAWM: normal-appearing white matter; *NDRG1*: N-myc downstream regulated 1; *NHLH2*: nescient helix-loop-helix 2; PB: peripheral blood; PBMNs: peripheral blood mononuclear cells; pSS: primary Sjögren's syndrome; *PTPN11*: protein tyrosine phosphatase non-receptor type 11; *PTPN6*: protein tyrosine phosphatase non-receptor type 6; *PAX9*: paired box 9; *PADI2*: peptidyl arginine deiminase 2; *PTGS2*: prostaglandin-endoperoxide synthase 2; PLCH1: phospholipase C Eta 1; PGE2: prostaglandin E_2; OA: osteoarthritis; OASFs: osteoarthritis synovial fibroblasts; RA: rheumatoid arthritis; RASFs: rheumatoid arthritis synovial fibroblasts; *RUNX*: runt-related transcription factor; RRMS: relapsing-remitting MS; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; SLEDAI: systemic lupus erythematosus disease activity index; *TNFRSF25*: TNF receptor superfamily member 25; *TCIM*: transcriptional and immune response regulator; *SFRP1*: secreted frizzled related protein 1; TSS: transcription start site; TGF-β: transforming growth factor β; TNFSF7: tumor necrosis factor ligand superfamily member 7; TNF-α: tumor necrosis factor-α; TNFi: tumor necrosis factor inhibitor; *TIPARP*: TCDD inducible poly (ADP-ribose) polymerase; *VDR*: vitamin D receptor; WB: whole-blood; WM1: white matter 1; *WDR81*: WD repeat domain 81.

rate to methotrexate has also been reported; as the methylation levels are higher in RA patients, they may display lower responses to methotrexate [81].

4.2.3. Systemic sclerosis (SSc)

SSc is a multifaceted disease characterized by initial vascular injury and uncontrolled inflammatory reaction that leads to fibrosis in the skin and internal organs. Following early vascular injury, active endothelial cells produce endothelin-1 and chemokines, increasing the expression of adhesion molecules and ultimately leading to the recruitment of inflammatory cells. Activated platelets, dendritic cells, macrophages, T cells, and B cells secrete profibrotic growth factors and cytokines that in turn lead to the activation and differentiation of resident fibroblasts to myofibroblasts, which secrete the excessive extracellular matrix (ECM).

DNA methylation analysis has demonstrated that the cross-talk between $CD4^+$ T cells and fibroblasts in SSc patients can control the disease; while induction of DNA hypermethylation in fibroblasts is coupled with up-regulation of some DNA methylation regulators [82–86], DNA hypomethylation in $CD4^+$ T cells of SSc patients is responsible for orchestrating the pro-inflammatory responses [87–91]. Also, studying DNA methylation profiles may be an effective method to distinguish two subtypes of SSc: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). Thus far, 153 and 266 differentially methylation sites have been identified for lcSSc and dcSSc, respectively [92]. A schematic representation of the role of DNA methylation alterations in the pathogenesis of SSc is demonstrated in Fig. 5.

4.2.4. Multiple sclerosis (MS)

MS is a chronic, inflammatory, autoimmune, demyelinating, and neurodegenerative disease of the CNS. DNA methylation signatures can be used as a marker to predict the risk of MS development. For example, patients with evidence of hypermethylation at the genes of *Alu* and *LINE1* may develop a severe form of the disease [93]. Or, the methylation of vitamin D receptor (*VDR*) and *IL2RA* can also increase the risk of MS development [94,95]. The correlation between the incidence of methylation in *HLADRB1* in $CD4^+$ T cells and the severity of the disease has also been reported [96]. Moreover, the reduction in 5hmC in peripheral blood mononuclear cells (PBMCs) is associated with the downregulation of *TET2* expression [97]. Fig. 6 provides a schematic representation of the role of DNA methylation alterations in the pathogenesis of MS. Table 2 summarizes the results of various studies that highlighted the role of DNA methylation markers in autoimmune diseases.

Table 3
Potential DNA methylation markers in metabolic disorders.

Diseases	Cell/tissue type	Main findings	Ref
T2D	Pancreatic islets from non-diabetics and diabetics	1649 CpG sites annotated to 843 genes, including *INS*, *PDX1*, *ADCY5*, *FTO*, *IRS1*, *KCNQ1*, and *TCF7L2,* were differently methylated in islets from T2D patients relative to HCs. 102 of these genes, including *CDKN1A*, *PDE7B*, *SEPTIN9*, and *EXOC3L2* exhibited differential expression in T2D islets.	[123]
	Muscle and SAT from MZ pairs discordant for T2D	Hypermethylation in promoter regions of known T2D-related genes, such as *PPARGC1A* in muscle and *HNF4A* in adipose tissue, were detected in the MZ twins discordant for T2D.	[124]
	SAT from MZ pairs discordant for T2D and independent case-control cohorts	15,627 CpG sites annotated to 7046 genes, including *PPARG*, *KCNQ1*, *TCF7L2*, and *IRS1,* were differently methylated in adipose tissue from T2D patients relative to HCs, and 1410 of these sites did also exhibit differential DNA methylation in the MZ twins discordant for T2D.	[125]
	Liver biopsies from T2D patients and nondiabetic subjects	251 CpG sites were differently methylated in the liver from T2D patients relative to HCs, and 94 % of the CPG sites displayed decreased DNA methylation that may be explained by reduced circulating folate levels in the human diabetic liver.	[126]
	Liver biopsies from obese women with T2D and obese women with normal glucose levels	The hypomethylation associated with overexpression of *PDGFA* in the liver of obese patients with T2D was correlated with increased insulin resistance, hyperinsulinemia, and increased T2D risk.	[127]
	WB and skeletal muscle from lean healthy, obese non-diabetic, and T2D volunteers before and after exercise	In patients with T2D, the decreased methylation levels of *PPARGC1A* (also known as *PGC-1α*) promoter after exercise may be used potentially as a prognostic marker for monitoring lifestyle intervention.	[128]
	Adipose tissue from healthy men, with a previous low level of physical activity, before and after exercise intervention	17,975 CpG sites annotated to 7663 genes, 18 obesity, and 21 T2D candidate genes (such as *TCF7L2* and *KCNQ1*) showed altered levels of DNA methylation in adipose tissue after the exercise intervention.	[135]
	Blood DNA of cases that were all non-diabetic at baseline	DNA methylation at the *ABCG1* locus was associated with a 9 % increased risk, while DNA methylation at the *PHOSPHO1* locus was associated with a 15 % decreased risk of future T2D.	[136]
	PB leukocytes from T2D cases and HCs	The promoter region of 1091 genes hypermethylated in the blood DNA of T2D patients compared to HCs. Promoter hypermethylation and associated silencing of the *NT5C2* gene were strongly correlated with increased susceptibility to T2D.	[137]
Obesity	Adipose tissue and WB samples	Increased methylation at the *HIF3A* locus in adipose tissue and blood cells was associated with increased BMI.	[131]
	Adipose tissue and WB samples	DNA methylation and expression of 2825 genes (e.g., *FTO*, *MTCH2*, *ITIH5*, *CCL18*, *IRS1*, and *SPP1*) were associated with BMI.	[132]
	PB of obese children and normal-weight individuals	Hypermethylation in intron 2/exon 3 of *POMC* might have an association with childhood obesity.	[133]
	WB from obese cases and normal weight controls	187 CpG sites were significantly associated with BMI. 62 of 187 methylation markers were associated with the incidence of T2D and can be used as a biomarker to predict the risk of future T2D development in obese patients.	[134]
	$CD4^+$ T cells of participants	Four CpG sites annotated to *CPT1A*, *PHGDH*, *CD38,* and *LINC00263* were associated with BMI and WC. The methylation level of a CpG site near the TSS of *CD38* was positively associated with BMI and WC, while a CpG site in the first intron of *CPT1A* was inversely associated with BMI and WC.	[138]
	WB and $CD4^+$ T cells	83 CpG sites were significantly associated with BMI, and a CpG site in the intron of *SREBF1* gene was strongly associated with BMI, adiposity-related traits, and coronary artery disease.	[139]
	PB leukocytes and neutrophils of obese and lean individuals	54 CpG sites were significantly associated with obesity, and the decreased DNA methylation level and increased expressions of *SOCS3*, *CISH*, *PIM3*, and *KLF4* genes were associated with obesity.	[140]

ADCY5: adenylate cyclase 5; *ABCG1*: ATP binding cassette subfamily G member 1; BMI: body mass index; *CDKN1A*: cyclin dependent kinase inhibitor 1A; *CCL18*: C–C motif chemokine ligand 18; *CISH*: cytokine inducible SH2 containing protein; *EXOC3L2*: exocyst complex component 3 like 2; FTO: FTO alpha-ketoglutarate dependent dioxygenase; HCs: healthy controls; *HNF4A*: hepatocyte nuclear factor 4 alpha; *HIF3A*: hypoxia inducible factor 3 subunit; alpha; *IRS*: insulin receptor substrate; *INS*: insulin; *ITIH5*: inter-alpha-trypsin inhibitor heavy chain 5; *KCNQ1*: potassium voltage-gated channel subfamily Q member 1; *KLF4*: kruppel like factor 4; LINC00263: long intergenic non-coding RNA 00263; MZ: monozygotic; *MTCH2*: mitochondrial carrier 2; *NT5C2*: 5′-nucleotidase, cytosolic II; PB: peripheral blood; *PDX1*: pancreatic and duodenal homeobox 1; *PDE7B*: phosphodiesterase 7B; *PPARGC1A*: PPARG coactivator 1 alpha; *PPARG*: peroxisome proliferator activated receptor gamma; *PDGFA*: platelet derived growth factor subunit A; *PHOSPHO1*: phosphoethanolamine/phosphocholine phosphatase 1; *PHGDH*: phosphoglycerate dehydrogenase; *PIM3*: pim-3 proto-oncogene, serine/threonine kinase; SAT: subcutaneous adipose tissue; *SEPTIN9*: septin 9; *SPP1*: secreted phosphoprotein 1; *SREBF1*: sterol regulatory element binding transcription factor 1; *SOCS3*: suppressor of cytokine signaling 3; TSS: transcription start site; T2D: type 2 diabete; *TCF7L2*: transcription factor 7 like 2; WB: whole-blood; WC: waist circumference.

Table 4
Aberrations of the DNA methylation machinery in human cancers.

	Cancer type	Aberrations	Outcome of aberrations	Ref
DNA methyltransferases; writers				
DNMT1	CRC	Rare inactivating mutations	–	[171]
	PA	Overexpression	Correlated with aggressive disease and high-methylation tumors.	[172]
	GC	Overexpression	Regional DNA hypermethylation and gene silencing; Correlated to the development of poorly differentiated GC.	[173]
	LC	Overexpression	Correlated with aberrant TP53/SP1 pathway and poor prognosis.	[174]
	CML	Overexpression	Increased expression during progression to the acute phase.	[158]
	AML	Overexpression	Regional DNA hypermethylation and gene silencing.	[158]
DNMT3A	PA	Overexpression	Correlated with aggressive disease and high-methylation tumors.	[172]
	VSCC	Overexpression	Correlated with an increased risk of LVR.	[175]
	GC	Allelic loss	Regional DNA hypomethylation.	[176]
	LC	Allelic loss	Regional DNA hypomethylation.	[176]
	CML	Overexpression	Increased expression during progression to the acute phase.	[158]
	AML	Overexpression	Regional DNA hypermethylation and gene silencing.	[158]
	AML	Inactivating mutation	DNA hypomethylation by DNMT3A R882 mutation correlated with a poor prognosis.	[143–145]
	MDS	Inactivating mutation	Causes regional DNA hypomethylation, and is correlated with worse OS and more rapid progression to AML.	[146]
	TCL	Inactivating mutation	Regional DNA hypomethylation.	[147–150]
	T-ALL	Inactivating mutation	Causes regional DNA hypomethylation, and correlated with poor prognosis in adult T-ALL.	[151]
	NHL	Allelic loss	Regional DNA hypomethylation.	[176]
DNMT3B	HCC	Overexpression	Increased expression of DNMT3B/OCT4 by the IL6/STAT3 pathway triggers sorafenib resistance and early recurrence.	[177]
	HCC	Overexpression of *DNMT3B4*	Correlated with DNA hypomethylation in pericentromeric satellite regions and genome instability.	[162]
	EC	Overexpression	Regional DNA hypermethylation.	[178]
	CML	Overexpression	Increased expression during progression to the acute phase.	[158]
	AML	Overexpression	Regional DNA hypermethylation and gene silencing.	[158]
Methyl-CpG binding proteins (MBDs); Reader				
MECP2	BrC	Overexpression	Correlated with higher levels of ER in both neoplastic and non-neoplastic tissues.	[179]
MBD1	LC	SNPs (rs125555, rs140689)	Correlated with increased LC risk.	[180]
	PC	Overexpression	Promotes PC invasion and metastasis by epigenetic down-regulation of E-cadherin.	[181]
MBD2	PrC	Overexpression	Associated with aberrant hypermethylation of the GSTP1.	[163]
MBD3	EC	Deletion	Frequent deletion of a small segment of chromosome 19 containing MBD3.	[182]
MBD4	CRC and GC	Frameshift mutations	Correlated with microsatellite instability.	[165,166]
	HCC	Downregulated	Significantly correlated with poorer tumor differentiation and involvement of portal vein.	[183]
DNA-demethylating enzymes; Erasers				
TET1	AML	Rare LOF mutations	–	[184]
TET2	HM	Frequent LOF mutations	Causes promoter DNA hypermethylation and gene silencing, and is correlated with poor prognosis in AML and lymphomas (AITL and PTCL-NOS).	[147–149, 152–155]
Metabolic enzymes (enzymes of the tricarboxylic acid (TCA) cycle)				
IDH1	LGG, CS, CCCs, TC, and HM	Frequent oncogenic mutation*	IDH1 mutant produces 2-HG, which inhibits demethylases and triggers hypermethylation of DNA and histones.	[157,185–189]
IDH2	LGG, CS, and HM	Frequent oncogenic mutation**	*IDH2* Mutant produces 2-HG, which inhibits demethylases and triggers hypermethylation of DNA and histones.	[149], [157], [185–188]
FH	UF and RC	Inactivating mutations	Accumulation of fumarate inhibits demethylases and triggers hypermethylation of DNA and histones.	[190]
SDH	HPGL/PCC and GIST	Inactivating mutations	Accumulation of succinate inhibits demethylases and triggers hypermethylation of DNA and histones.	[191,192]

*R132H being the most common; ** mutations at R172 and R140 being most common; AML: acute myeloid leukemia; AITL: angioimmunoblastic T-cell lymphoma; BrC: breast cancer; CRC: colorectal cancer; CML: chronic myelogenous leukemia; CMML: chronic myelomonocytic leukemia; CS: chondrosarcoma; CCCs: cholangiocarcinomas; DNMT: DNA (cytosine-5)-methyltransferase; EC: endometrial cancer; FH: fumarate Hydratase; GC: gastric cancer; GIST: gastrointestinal stromal tumors; GSTP1: glutathione S-transferase pi 1; HCC: hepatocellular carcinoma; HM: hematological malignancies; 2-HG: 2-hydroxyglutarate; IDH: isocitrate dehydrogenase; IL6/STAT3: interleukin-6/signal transducer and activator of transcription 3; LC: lung cancer; LGG: low-grade gliomas; LOF: loss-of-function; LVR: local vulvar recurrence; MECP2: methyl CpG binding protein 2; MBD: methyl-CpG binding domain; MDS: myelodysplastic syndrome; MPNs: myeloproliferative neoplasms; NHL: non-hodgkin lymphoma; OS: overall survival; OCT4: octamer-binding transcription factor 4; ER: oestrogen receptor; PA: pituitary adenoma; PC: pancreatic cancer; PrC: prostate cancer; HPGL/PCC: paraganglioma/phaeochromocytoma syndrome; PTCL-NOS: peripheral T-cell lymphoma, not otherwise specified; RC: renal cancer; SNPs: single nucleotide polymorphisms; SDH: succinate dehydrogenase; TET: tet methylcytosine dioxygenase; TCL: T-cell lymphoma; T-ALL: T-cell acute lymphoblastic leukemia; TC: thyroid cancer; UF: uterine fibroid; VSCC: vulvar squamous cell carcinoma.

Table 5
Potential DNA methylation markers in human cancers.

	Sample type	Biomarkers	Indication	Status	Ref
Markers for diagnosis/detection					
BC	Voided urine (Patients diagnosed with hematuria)	*TWIST1*, *ONECUT2*, and *OTX1* methylation	Diagnosis (NIBC detection)	CE-IVD device (AssureMDX)	[193]
BC	Urine samples	Methylation levels of *TRNA-Cys, SIM2, and NKX1-1* and 2 internal control loci	Diagnosis	Bladder CARE™ (CE-marked IVD) test	[194]
BC	Urine samples	*ZNF671, OTX1*, and *IRF8* methylation	Diagnosis	Preclinical	[195]
BrC	Plasma cfDNA	Differentially methylated regions (DMRs) of *SPAG6, LINC10606* and *TBCD/ZNF750* genes	Diagnosis (early detection of triple-negative breast cancer)	Preclinical	[196]
BrC	PBMCs	Four selected PBMCs DNA methylation markers	Diagnosis	Preclinical	[197]
Small BrC	Tissue samples	*GCM2*, *ITPRIPL1*, *CACNA1E*, and *DLGAP2* methylation	Diagnosis	Preclinical	[198]
CRC	Stool (Patients who are candidates for screening)	*BMP3*, and *NDRG4* methylation	Early detection of CRC	FDA-approved (ColoGuard)	[168]
CRC	Stool (Patients unwilling or unable to undergo a more invasive exam)	*VIM* methylation	Early detection of CRC	CLIA level (ColoSure)	[199]
CRC	Plasma (Patients, 50 years or older, with average risk for CRC, who decline other CRC screening)	*SEPTIN9* methylation	Early detection of CRC	FDA approved (Epi proColon); CLIA level (ColoVantage)	[169, 170, 200]
CRC	Blood samples	*MYO1G* methylation	Diagnosis (Monitoring disease)	Preclinical	[201]
CRC	Stool	Methylated *COL4A1, COL4A2, TLX2, and ITGA4*	Diagnosis	Preclinical	[202]
CRC	Plasma samples	Methylated SFRP1, SFRP2, SDC2, and PRIMA1	Diagnosis	Preclinical	[203]
CC	A cervical smear of women who are HPV^+ with abnormal cytology findings	*ASTN1*, *DLX1*, *ITGA4*, *RXFP3*, *SOX17*, and *ZNF671* methylation	Triage of unclear CC screening tests	CE-IVD (GynTect®)	[204]
CC	Cervical scrape and vaginal samples of HPV^+ women	*FAM19A4*, and *hsa-mir124-2* methylation	Triage of unclear CC screening tests	CE-IVD (QIAsure)	[205]
CC	Liquid-based cytology (LBC) samples	Methylation detection of the promoter region of *EPB41L3* and five late regions of HPV types 16, 18, 31, and 33 by pyrosequencing	Predicts incident high-grade CIN and ICC among high-risk HPV^+ women	Preclinical (S5 DNA methylation classifier test)	[206]
CC	Cervical brush samples	*PCDHGB7* hypermethylation-based Cervical cancer Methylation (CerMe) detection	CerMe detection efficiently differentiated CIN grade 2 or worse ($CIN2^+$) from CIN grade 1 or normal ($CIN1^-$) women	Preclinical	[207]
CC	Histological cervical specimens	*ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671*	ZNF671 methylation has diagnostic value for the diagnosis of CIN2+ and CIN3+.	Preclinical (GynTect® assay)	[208]
GC	Circulating cell-free DNA	5-hydroxymethylcytosine of *FBXL7, PDE3A, TPO, SNTG2, and STXBP5*	Diagnosis	Preclinical	[209]
GC	Plasma cfDNA	Methylated *RNF180*, and *SEPTIN9*	Diagnosis	Preclinical	[210]
HCC	Plasma cfDNA	*SEPTIN9* methylation	Detection of HCC in patients with cirrhosis	CE-IVD device (HCCBloodTest)	[211]
HCC	Plasma cfDNA	A single *F12* gene CpG site	Diagnosis	Preclinical	

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Table 5 (*continued*)

	Sample type	Biomarkers	Indication	Status	Ref
HPV-HNC	Saliva samples	HPV late genes and long control region (LCR)	Higher levels of DNA methylation in HPV late genes is a potential supplementary biomarker for salivary HPV detection-based HPV-HNC screening.	Preclinical	[212]
LC	Plasma (Increased risk patients defined by life history, symptoms, and radiological findings in the lung)	*SHOX2* and *PTGER4* methylation	Detection of LC in patients at increased risk for the disease	CE-IVD device (Epi proLung)	[213]
LC	Plasma cfDNA	Circulating tumor DNA methylation model by Next-generation sequencing (NGS)	Accurate diagnosis of early stage lung cancer from pulmonary nodule patients	Clinical trial (NCT03651986); PulmoSeek model	[214]
LC	Plasma cfDNA	Circulating tumor DNA methylation model by Next-generation sequencing (NGS)	Early diagnosis of lung cancer from pulmonary nodule patients	Clinical trials (NCT03181490, NCT03651986); PulmoSeek plus model (CE Marked)	[215]
NSCLC SCLC	Plasma cfDNA	Six-marker panel methylation-based plasma test	Early detection	Clinical trial (NCT02373917); Lung EpiCheck (Nucleix)	[216]
LC	Broncho-exfoliated cells (BEC)	*SHOX2* and *RASSF1A* methylation	Diagnosis	Preclinical (LungMe® kit)	[217]
LC	Bronchial washing fluid	*PCDHGA12* and *CDO1* methylation	Diagnosis	Preclinical	[218]
OC	Plasma cfDNA	*ZNF154, C2CD4D,* and *WNT6*	Ovarian cancer screening in high-risk populations	Preclinical	[219]
EOC	CfDNA liquid biopsy	A cfDNA Methylation Liquid Biopsy test (OvaPrint)	Diagnosis (Risk assessment of High-grade serous ovarian carcinoma in symptomatic women)	Preclinical	[220]
PCa	Prostate biopsy from men with established risk factors	*GSTP1*, *APC*, and *RASSF1* methylation	Detection of occult PCa in men with previous histopathologically negative biopsies who were considered to be at risk for harboring cancer	CE-IVD device (ConfirmMDX)	[221]
PCa	Urine extracellular vesicles (EVs) DNA	*RASSF1* methylation	Diagnosis	Preclinical	[222]
PCa	Plasma cfDNA	A targeted DNA methylation sequencing panel	Detection of Neuroendocrine PCa	Preclinical	[223]
PCa	Prostate tissue	*GSTP1, APC, TNFRFS10c*, *RASSF1A*, and *RUNX3* methylation	Diagnosis (APC was associated with an increased risk of biochemical recurrence.)	Preclinical	[224]
Markers for prognosis					
BC	PB samples	DNA methylation clocks	Age acceleration was associated with worse OS	Preclinical	[225]
CRC	Plasma samples	High methylated *SEPTIN9* levels	Shorter OS and PFS	Preclinical	[226]
CRC	Primary tumor biopsy samples	Hypermethylation of *BMP2*	Worse OS	Preclinical	[227]
CRC	Primary tumor biopsy samples	Methylation level of *POU3F3*, *SYN2*, and *TMEM178A*	Significantly related to poorer survival in the high-risk tumor mutation burden (TMB) samples	Preclinical	[228]
CRC	Circulating tumour DNA (ctDNA) methylation haplotype patterns	ctDNA methylation haplotype patterns using the ColonES assay	CRC patients with high preoperative ctDNA methylation levels have worse relapse-free survival (RFS) than patients with low ctDNA methylation levels	Preclinical	[229]
CRC	Tumor biopsy samples	Hypermethylation of 3 site (cg02604524, cg07628404, and cg27364741)	Predicts poor survival	Preclinical	[230]
CC	Plasma samples	*SEPTIN9* methylation	Predicts pelvic nodal metastasis	Preclinical	[231]
GBM	Tomur samples	*MGMT* methylation	Higher PFS and OS	Phase II and III clinical trials	[232]

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Table 5 (*continued*)

	Sample type	Biomarkers	Indication	Status	Ref
GBM	Tissue samples	Hypermethylation of specific LTBR CpG sites	Predicts better OS	Preclinical	[233]
HCC	Tissue samples	Methylation level of cg01798157 in the *BTG2* gene	Shorter OS	Preclinical	[234]
HCC	Liquid biopsy	*SEPTIN9* methylation	Poor OS	Preclinical	[235]
OC	Primary OC samples with tumor protein 53 (TP53) wild-type	*Five aberrant methylated differentially expressed genes (DEGs)*	Predict the risk of death	Preclinical	[236]
PC	Pancreatic cancer tissues and adjacent non-cancer tissues	*CHFR* promoter methylation	Significantly associated with lymph node metastasis	Preclinical	[237]
PCa	Biopsy tissues	Methylation levels of *ADM, AEN, CCND1, CDC2, EPHX3, RARB,* and *VCAM1*	Predicts risk of metastasis in untreated localized PCa patients	Preclinical	[238]
PCa	Biopsy tissues	Hypermethylation of *CCND2* and *GSTP1*	Associated with an increased risk of tumor progression	Preclinical	[239]
Markers for spatial localization					
CUP	Fresh frozen or FFPE tumor biopsy	The algorithm established from Illumina 450K/EPIC methylation arrays	Predict cancer tissue of origin to enable the direction of tumor type-specific therapy	CE-IVD device (EPICUP)	[167]
Multiple types of tumors	Plasma cfDNA (from cancer and non-cancer participants) and FFPE tumor biopsies	A panel of >100,000 informative methylation regions (whole-genome bisulfite sequencing)	Multi-cancer early detection, as well as TOO prediction	Clinical trial	[240]
Multiple types of tumors	Plasma (cfDNA) and primary tumor biopsy samples	A panel of 42,374 CpG clusters was established using TCGA tumor data and healthy plasma data (CancerLOCATOR)	Non-invasive cancer diagnosis and TOO prediction	Preclinical	[241]
Multiple types of tumors	Tumor tissue, adjacent normal tissue, and normal WB samples	Selected 3139 CpG sites that were hypermethylated in more than 10 % of tumors within any of the 33 cancer types (using HM27/HM450K array)	Diagnosis and prediction of the cell of origin (Classification of tumors using DNA hypermethylation, mRNA–miRNA expression levels, and reverse-phase protein data)	Preclinical	[242]
Markers for drug response					
BC	Pretreatment cfDNA	The levels of *MIR145* core promoter	Reduced pretreatment cfDNA methylation of *MIR145* core promoter predicts response of therapy	Preclinical	[243]
BC	Plasma cfDNA	Methylation-based response score (mR-score)	Predicts response to neoadjuvant chemotherapy	Preclinical	[244]
BrC	FFPE tissue samples from LN^+, ER^+ and $HER2^-$ HRBC patients	Higher methylation levels of *PITX2*	Predicts resistance to anthracycline-based chemotherapy	CE-IVD (Therascreen®PITX2 RGQ)	[245]
BrC, OC	Primary tumor biopsy samples	*BRCA1* methylation	Predicts sensitivity to PARP inhibitor	Phase II clinical trial	[246]
EOC	Tissue sample	*DDR2* hypomethylation	Predicts resistance to chemotherapy	Preclinical	[247]
GBM	Primary tumor biopsy and PB samples	*MGMT* methylation	Predicts sensitivity to temozolomide	CE-IVD Therascreen MGMT Pyro kit	[248]
GBM	Primary and relapsed samples	Mean higher global DNA methylation	Associated with better radiotherapy response and less aggressive phenotype	Preclinical	[249]
CRC	Primary tumor biopsy samples	*SRBC* methylation	Predicts resistance to oxaliplatin	Preclinical	[250]
CRC	Primary tumor biopsy samples	*DERL3* methylation	Predicts sensitivity to glycolysis inhibitors	Preclinical	[251]
CRC	FFPE tissue samples	*LINE-1* hypomethylation	Predicts sensitivity to 5-FU	Preclinical	[252]
CRC	Tissue samples	*TFAP2E* methylation	Predicts resistance to chemotherapy	Preclinical	[253]
mCRC	Metastatic tumor biopsy samples	*CHFR* methylation	Predicts sensitivity to Irinotecan	Preclinical	[254]

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Table 5 (*continued*)

	Sample type	Biomarkers	Indication	Status	Ref
mCRC	Plasma cfDNA	*NPY* methylation	Predicts respose to therapy	Preclinical	[255]
GC, CC	Primary tumor biopsy samples	*TP53TG1* methylation	Predicts resistance to DNA-damaging drugs	Preclinical	[256]
OC, NSCLC	Primary tumor biopsy samples	*SLFN11*methylation	Predicts resistance to platinum drugs	Preclinical	[257]
NSCLC	Tumor biopsy samples	Unmethylated *FOXP1*	Predicts sensitivity to PD-1 blockade	Preclinical	[258]
Markers for monitoring residual disease or predicting cancer recurrence					
BC	Voided urine	Hypermethylated *SOX1* and *IRAK3*; hypomethylated *LINE1*	Predicts bladder cancer recurrence	CLIA LDT (Bladder CARE™)	[259]
NMIBC	Voided urine	Score over 15 methylation markers	Monitoring for tumor recurrence in patients previously diagnosed with bladder cancer	CE-IVD (BladderEpiCheck®)	[260, 261]
CRC	Plasma	*IKZF1* and *BCAT1* methylation	Early detection of CRC recurrence, and detection of residual disease post-surgical resection	CLIA LDT (COLVERA™)	[262]
CRC	Plasma cfDNA	*SEPTIN9* methylation	Serial detecting of mSEPTIN9 could be a potential biomarker for minimal residual disease (MRD) in CRC patients.	Preclinical	[263]
CRC	Tumor biopsy samples	Eight-CpG DNA methylation signature	Predicts disease recurrence in patients with stage II CRC	Preclinical	[264]
HNSCC	Plasma cfDNA at the time of post-surgically (days 2–30) from patients with HNSCC	*SEPTIN9* methylation	SEPT9 ccfDNA methylation could be a potential biomarker for detecting minimal residual disease (MRD) in HNSCC patients	Preclinical	[265]

ASTN1: astrotactin 1; *APC*: adenomatous polyposis coli; BC: bladder cancer; BrC: breast cancer; *BMP3*: bone morphogenetic protein 3; *BRCA1*: breast cancer 1; *BCAT1*: branched chain amino acid transaminase 1; CRC: colorectal cancer; CC: cervix cancer; CIN: cervical intraepithelial neoplasia; CUP: cancer of unknown primary; cfDNA: circulating cell-free DNA; *CHFR*: checkpoint with forkhead and ring finger domains; CE-IVD: european conformity–*in vitro* diagnostic medical device; CLIA: clinical laboratory improvement amendments; *DLX1*: distal-less homeobox 1; *DERL3*: derlin 3; ER^+: estrogen receptor-positive; FFPE: formalin-fixed paraffin embedded; *FAM19A4*: family with sequence similarity 19 (chemokine (C–C motif)-like) member A4 (also known as *TAFA4*); *FOXP1*: forkhead box P1; FDA: US food and drug administration; 5-FU: 5-fluorouracil; GBM: glioblastomas; GC: gastric cancer; *GSTP1*: glutathione S-transferase pi 1; HCC: hepatocellular carcinoma; HPV-HNC: HPV-driven HNC; HRBC: high-risk breast cancer; HPV^+: human papillomavirus positive; $HER2^-$: human epidermal growth factor receptor 2-negative; HM27/HM450K array: humanmethylation27/humanmethylation 450 beadchip array; HNSCC: head and neck squamous cell carcinoma; *ITGA4*: integrin subunit alpha 4; *IRAK3*: interleukin 1 receptor associated kinase 3; *IKZF1*: IKAROS family zinc finger 1; ICC: invasive cervical cancer; LC: lung cancer; LN^+: lymph node-positive; *LINE-1*: long interspersed nuclear element 1; mCRC: metastatic CRC; *MGMT*: O6-methylguanine–DNA methyltransferase; NSCLC: non-small-cell lung cancer; NMIBC: Non-muscle-invasive bladder cancer; *NDRG4*: NDRG family member 4; NIBC: noninvasive bladder cancer; OC: ovarian cancer; *ONECUT2*: one cut homeobox 2; *OTX1*: orthodenticle homeobox 1; PCa: prostate cancer; PC: pancreatic cancer; PB: peripheral blood; *PTGER4*: prostaglandin E receptor 4; *PITX2*: paired like homeodomain 2; *PD-1*: programmed cell death protein 1 (also known as *PDCD1*); *RXFP3*: relaxin family peptide receptor 3; *RASSF1*: ras association domain family member 1; *PARP*: poly (adenosine diphosphate) ribose polymerase; SCLC: small cell lung cancer; *SEPTIN9*: septin 9; *SOX17*: SRY-box transcription factor 17; *SHOX2*: short stature homeobox 2; *SRBC*: also known as *CD2*; *SLFN11*: schlafen 11; *SOX1*: SRY-box transcription factor 1; *TWIST1*: twist family BHLH transcription factor 1; *TFAP2E*: transcription factor AP-2 epsilon; *TP53TG1*: TP53 target 1; TOO: tissue of origin; *VIM*: vimentin WB: whole blood; *ZNF671*: zinc finger protein 671.

Table 6
DNA methylation-targeting drugs that are approved or in clinical trials.

Drug	Diseases	Phase	Clinical intervention	Ref/NCT
DNA methyltransferase (DNMT) inhibitors				
DAC	AML and MDS	EMA and FDA approval	There is a significant improvement in survival or PFS compared to supportive care or conventional therapy in AML and MDS.	[266]
	Solid tumors	Phase I/II	Although single-agent efficacy in solid tumors was not convincing, combination therapy has resulted in promising responses.	[274,275]
5-AC	AML, CMML, MDS	EMA and FDA approval	There is a significant improvement in survival or PFS compared to supportive care or conventional therapy in AML, CMML, and MDS.	[266]
	Solid tumors	Phase I/II	Although single-agent efficacy in solid tumors was not convincing, combination therapy has resulted in promising responses.	[274,276]
ASTX727	CMML and MDS	Phase I/II	The safety and efficacy of ASTX727 were consistent with those from IV DAC in CMML and MDS patients.	[277]
	Patients with MPNST	Phase II	25 patients were enrolled to evaluate whether ASTX727 could be an effective treatment for MPNST patients with a PRC2 mutation.	NCT04872543
	SCCHN	Phase I/II	25 patients were enrolled to evaluate the safety and efficacy of oral ASTX727 and MEDI4736 combination in recurrent and/or metastatic SCCHN who have progressed during or after Immunotherapy.	NCT03019003
CC-486	AML, MDS	Phase I/II	CC-486 maintenance therapy after alloHSCT was correlated with a relatively low rate of relapse (21 %) during treatment.	[278]
	AML	Phase III	360 patients were enrolled to evaluate the safety and efficacy of oral ABT-199 and oral or injectable 5-AC vs best supportive care as maintenance therapy in patients with AML.	NCT04102020
	R/R iBCL	Phase I	24 patients were enrolled to evaluate the side effects of CC-486 and how well it works in combination with GA-101 and CC-5013 in R/R iBCL.	NCT04578600
	75 years or older patients with newly diagnosed DLBCL	Phase III	422 patients were enrolled to evaluate the side effects and activity of CC-486 in combination with the standard drug therapy (R-miniCHOP) vs R-miniCHOP alone in patients 75 years or older with newly diagnosed DLBCL.	NCT04799275
	Advanced microsatellite stable CRC; Platinum-resistant OC; ER^{+}/$HER2^{-}$ BrC	Phase II	The combination therapy of CC-486 and MEDI4736 did not show robust PD or clinical activity in advanced solid tumors.	[279] NCT02811497
	Patients with mM	Phase I	71 patients were enrolled to evaluate the efficacy and safety of CC-486 in combination with MK-3475 in patients with melanoma.	NCT02816021
SGI-110	AML	Phase III	302 participants were enrolled to evaluate the safety and efficacy of SGI-110 vs choice treatment in adults with previously treated AML.	NCT02920008
	mCRC	Phase I/II	The study is investigating if SGI-110 can reverse chemoresistance to IRI in 18 mCRC patients that have developed resistance to IRI.	NCT01896856
	mCRC	Phase I	SGI-110 + GVAX was tolerable but demonstrated no significant clinical activity in mCRC patients	[280]
	Platinum-resistant, recurrent OC	Phase II	Although this trial did not demonstrate superiority for PFS of SGI-110 and carboplatin (G + C) versus treatment of choice, the 6-month PFS increased in G + C treated patients.	[281] NCT01696032
	R/R GCT	Phase I	14 patients were enrolled to evaluate the efficacy and safety of SGI-110 in combination with cisplatin in subjects with R/R GCT.	NCT02429466
Isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) inhibitors				
AG-221	R/R AML patients with *IDH2* mutation	FDA approval	There is a significant increase in ORR (40 %) and CRR (19 %) in R/R AML patients with an *IDH2* mutation.	[271]
AG-120	R/R AML with *IDH1*-mutated	FDA approval	There is a significant increase in ORR (41.6) and CRR (21.6 %) in R/R AML patients with *IDH1* mutation	[272]
AGG-881	LGG with *IDH1* or *IDH2* mutation	Phase I	Vorasidenib showed good brain penetrance and a decrease in 2-HG levels by approximately 93 % in LGG patients.	[273]
	R/R Grade 2 glioma with *IDH1* or *IDH2* mutation.	Phase III	340 patients were enrolled to evaluate the efficacy of AGG-881 compared to placebo in patients with residual or relapsed Grade 2 glioma with *IDH1* or *IDH2* mutation.	NCT04164901
FT-2102	AML or MDS with an *IDH1* mutation	Phase I/II	500 patients were enrolled to evaluate the safety, efficacy, PK, and PD of FT-2102 as a single agent or in combination with 5-AC or Ara-C.	NCT02719574
LY3410738	AML, CMML, MDS, and MPNs patients with *IDH1* or *IDH2* mutation	Phase I	220 patients were enrolled to evaluate the safety and clinical activity of oral LY3410738 in patients with advanced HMs with *IDH1* or *IDH2* mutations.	NCT04603001
LY3410738	Advanced solid tumors with *IDH1* mutations	Phase I	180 patients were enrolled to evaluate the safety and efficacy of oral LY3410738 in advanced solid tumors with *IDH1* R132-mutation.	NCT04521686
PEPIDH1M	Recurrent Grade II Glioma with *IDH1* mutation	Phase I	24 patients were enrolled to evaluate the safety of the PEPIDH1M vaccine in combination with standard chemotherapy (TMZ).	NCT02193347

DAC (decitabine; also known as 5-aza-2-deoxycytidine, dacogen; is a DNMT inhibitor); 5-AC (azacitidine; also known as vidaza, azadine, onureg; is a DNMT inhibitor); ASTX727 (oral cedazuridine/decitabine; is an oral DNMT inhibitor); CC-486 (oral azacitidine; is an oral DNMT inhibitor); SGI-110 (guadecitabine; is an DNMT inhibitor); AG-221 (enasidenib; is an IDH2 inhibitor); AG-120 (ivosidenib; is an IDH1 inhibitor); AGG-881 (vorasidenib; is a dual IDH1/IDH2 inhibitor); FT-2102 (olutasidenib; is an oral IDH1 inhibitor); LY3410738 (is an oral IDH1/IDH2 inhibitor); PEPIDH1M (is an

IDH1 peptide vaccine); MEDI4736 (durvalumab; is a check point inhibitor); ABT-199 (venetoclax; is a B-cell lymphoma 2 (BCL2) inhibitor); GA-101 (obinutuzumab; is an anti-CD20 monoclonal antibody R7159); CC-5013 (lenalidomide; is a TNF-α secretion inhibitor); MK-3475 (pembrolizumab; is an anti–PD-1 monoclonal antibody); IRI (irinotecan, is a topoisomerase I inhibitor); GVAX (is an allogeneic colon cancer cell vaccine); Carboplatin (is an alkylating antineoplastic agent); Cisplatin (is a an cytotoxic chemotherapy drug); Ara-C (cytosine arabinoside; also known as cytarabine; is a an cytotoxic chemotherapy drug): TMZ (temozolomide; is an alkylating antineoplastic agent); AML: acute myeloid leukemia; Allo-HSCT: allogeneic hematopoietic stem cell transplantation; BrC: breast cancer; CMML: chronic myelomonocytic leukemia; CRR: complete remission rate; DLBCL: diffuse large B-cell lymphoma; ER+: estrogen receptor positive; EMA: european medicines agency; FDA: US food and drug administration; HER2-: human epidermal growth factor receptor 2-negative; 2-HG: 2-hydroxyglutarate; HMs: hematologic malignancies; IDH: isocitrate dehydrogenase; IV: intravenous; LGG: low grade glioma MDS: myelodysplastic syndrome; MPNST: malignant peripheral nerve sheath tumors; mM: metastatic melanoma; mCRC: metastatic colorectal cancer; MPNs: myeloproliferative neoplasms; OC: ovarian cancer; ORR: overall response rate; PFS: progression-free survival; PRC2: polycomb repressive complex 2; PD: pharmacodynamic; PK: pharmacokinetic; R/R iBCL: relapsed/refractory indolent B-Cell lymphoma; R/R GCT: relapsed/refractory germ cell tumor; R/R AML: relapsed or refractory AML; RR: relapsed or refractory; R-miniCHOP: reduced dose rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone; SCCHN: squamous cell carcinoma of the head and neck.

4.3. DNA methylation in metabolic disorders

4.3.1. Type 2 diabetic Mellitus

The signs of DNA methylation patterns in 1649 CpG sites of about 843 genes involved in the pathogenesis of Type 2 diabetic (T2D), such as those encoding *insulin* and *PDX1* in the pancreatic islets, clearly suggest that epigenetic alterations could impair insulin release. Overexpression of *CDKN1A*, *PDE7B*, and *SEPTIN9* in clonal β-cells of pancreatic islets due to induction of DNA hypomethylation not only halts the proliferation of these cells but also reduces their sensitivity to glucose fluctuations, leading to the lack of insulin production [123]. Notably, DNA methylation alterations in T2D patients are not restricted to the genes expressed in pancreatic tissue, as the Illumina sequencing arrays identified methylation sites in more than thousands of genes expressed in other tissues, such as human adipose tissue, liver, and skeletal muscle [124–127]. Identifying these sites sheds light on the mechanisms by which we can control the development of T2D with some common behaviors such as physical exercise or energy-dense diets with high-fat content. In the skeletal muscle of patients with T2D, for instance, it has been reported that the methylation pattern of the gene encoding *PPARGC1A* is altered in response to exercise [128]. The overexpression of *PPARGC1A*, a primary regulator of liver gluconeogenesis, can alleviate the symptoms in T2D patients [129]. The existence of such non-pancreatic methylation sites can connect T2D to other inflammatory difficulties. Indeed, alteration in the methylation of these sites upregulates the expression of pro-inflammatory mediators within the target cell, which may increase the risk of cardiovascular and reproductive diseases or the incidence of cancer [130].

4.3.2. Obesity

The genome-wide DNA methylation analyses showed the presence of several methylation sites in obese patients, especially in the gene encoding *HIF3A* [131,132]. Over 1000 CpG DNA methylation signatures have also been identified in *FTO*, *CCL18*, *MTCH2*, *SPP1*, and *IRS* genes in subcutaneous adipose tissue (SAT). Interestingly, the methylation of these CpG sites has a tight correlation with the higher body mass index (BMI) [132]. Some of these methylation sites were similar to those previously described in T2D; highlighting that these metabolic diseases might influence each other [132]. Kuehnen et al. proposed that the hypermethylation in intron 2/exon 3 of *POMC* may be associated with childhood obesity [133]. Also, such methylation sites can be used as biomarkers to predict the risk of future T2D development in obese patients [134]. Table 3 summarizes the results of various studies highlighting the role of DNA methylation markers in metabolic disorders.

4.4. DNA methylation in cancer

DNA methylation alterations are one of the principal mechanisms that cancer cells recruit to prolong their survival, especially in the presence of anti-cancer signaling. DNA methylation can diminish the expression of a wide range of tumor suppressor proteins, foremost TP53, which is known as a guardian of the genome. Moreover, loss of CpG methylation can make the genome unstable and make it more vulnerable to genetic abnormalities [141]. As epigenetic alteration is an age-related phenomenon, it has been widely believed that changes in DNA methylation patterns happen during the aging of normal cells, making them transform into malignant counterparts. The confirmation that the degree of DNA methylation of one tissue differs in different cancer patients based on age [142].

4.4.1. DNA methylation in hematological malignancies

Hematologic malignancy was the first cancer in which the influence of DNA methylation alteration in the control of cell differentiation, proliferation, and survival has been described. Interestingly, the signs of abnormalities in DNA methylation machinery are evident in all types of hematologic malignancies from acute leukemia to chronic neoplasms such as myeloproliferative neoplasms (MPNs) and myelodysplastic syndrome (MDS). For example, the mutation in *DNMT3A* with the ability to reduce the catalytic activity of this DNA methyltransferase is a common feature in early colons of AML, MDS, T cell lymphoma, and adult T acute lymphoblastic leukemia (ALL) [143–151]. Notably, the importance of *DNMT3A* mutation is to the degree that endows it the prognostic value in hematologic malignancies [144,151]. Interestingly, more than half of *DNMT3A* mutations in AML patients occur at amino acid R882 in the catalytic domain of the enzyme. It seems that R882 mutations have a dominant-negative effect on the formation of wild-type DNMT3A tetramers, leading to reduced catalytic activity and consequent focal hypomethylation compared to the hypermethylation pattern observed with wild-type DNMT3A R882 in AML [145]. *DNMT3A* mutations are generally present in the ancestral or founder

preleukemic clone, and they may create an environment in which additional mutations trigger malignant clones.

Co-occurring with *DNMT3A* mutations, *TET2* mutations have also been reported in AML, MDS, T cell lymphoma, and MPNs [147–149,152–155]. The collaboration between *TET2* and *DNMT3A* mutations could block the differentiation of hematopoietic stem cells (HSCs) and increase their self-renewal capacity by altering the methylation pattern of several genes [156]. *TET2* LoF mutations and *isocitrate dehydrogenases (IDH)* GoF mutations are mutually exclusive in AML, suggesting that lesions in these genes may be biologically redundant [157]. *DNMT1* and *DNMT3B* are other DNA methylation writers whose expressions are manipulated in hematologic malignancies. In chronic myeloid leukemia (CML), *DNMT1*, *DNMT3A*, and *DNMT3B* overexpression contribute to the progression of the chronic phase into the acute phase [158]. In agreement, the up-regulation of these enzymes in AML can support the survival and proliferation of leukemic cells [158].

In addition to the mutation in DNA methylation machinery, the aberrant DNA methylation in the promoter of the tumor suppressor genes (TSGs) plays a critical role in the pathogenesis and progression of blood malignancies. Recent studies have reported that there is a good correlation between the DNA methylation status of the gene prompters and the residual disease in patients with neuroblastoma, natural killer cell lymphoma, diffuse large B cell lymphoma (DLBCL), and ALL. Residual methylation of gene promoters might be potential biomarkers for monitoring minimal residual disease (MRD) in patients with blood malignancies [159].

4.4.2. DNA methylation in solid tumors

The incidence rate of mutations in DNA methylation machinery in solid tumors is lower compared to hematologic malignancies. For example, as *DNMT3A* mutations have been detected in the majority of cases of hematologic malignancies, this abnormality is only detectable in less than 2 % of solid tumors based on the last reports of the Cancer Genome Atlas (TCGA) [160,161]. Aberrant alternative splicing in the *DNMT3B* gene, which gives rise to DNMT3B4, is mainly detectable in hepatocellular carcinoma. The non-functional DNMT3B4 –which lacks DNMT motifs– induces hypomethylation in pericentromeric satellite regions, increasing the sensitivity of the genome to additional mutations [162]. Mutations within MBD domains of DNA methylation readers are other abnormalities of the DNA methylation machinery in different solid tumors. Notably, the function of mutant MBD proteins is distinct in each type of cancer. In this vein, MBD2 is the best example. *MBD2* overexpression in prostate cancer is associated with aberrant hypermethylation of CpG islands of a tumor suppressor protein named *GSTP1* [163]. In colorectal cancer, MBD2 recognizes methylated sites in promotors of *p16* and *p14* and makes an anchor for the binding of histone deacetylases (HDACs) to promote gene silencing [164]. Therefore, in cancer cells, MBD2 is not only a reader of DNA methylation machinery but also can promote a rewriter, as it can make a complex with several DNMTs to change the expression of a broad group of tumor suppressor genes [163]. Another MBD protein whose mutation seems to play a fundamental role in tumorigenesis is MBD4, a methylation reader that reduces the chance of DNA repair in malignant cells. The incidence of *MBD4* mutations in cancer with microsatellite instability, such as colorectal and gastric cancer, is higher [165,166]. The frequency of mutations in other genes encoding the components of DNA methylation machinery is very infrequent in solid tumors.

Although occurring on a rare scale, DNA methylation-based biomarkers have found solid feet in the early diagnosis of many solid tumors due to easy detection, long stability, presence in various body fluids, and non-invasiveness detection, and, more importantly, cell-type specificity. These alterations can also be used as prognostic factors. Of particular interest, analysis of the DNA methylation pattern can be useable for establishing the type of those cancers with unknown sources or carcinoma of unknown primary (CUP) [167]. Thus far, two tests have succeeded to received FDA approval for analyzing the alteration of DNA methylation: Cologuard, also known as *NDRG4* or *BMP3*, is developed to analyze DNA methylation patterns in stool samples [168], and Epi proColon (*SEPTIN9*) analyzes the DNA methylation in blood samples of patients [169,170]. Therefore, studying DNA methylation alterations seem to be fertile ground for revolutionizing the molecular landscape of solid tumors. To provide a better overview, we summarized the aberrations of the DNA methylation machinery and its outcomes in different cancer types spanning from hematologic malignancies to various solid tumors in Table 4. The promising applications of DNA methylation markers in solid tumors are also represented in Table 5.

5. The DNA methylation machinery in the perspective of pharmaceutic approaches

The development of technologies, including the Infinium humanmethylation450K (HM450K), 850k EPIC array, whole-genome bisulfite sequencing (WGBS), methylated DNA immunoprecipitation (MeDIP), and chromatin immunoprecipitation sequencing (ChIP-Seq) all together opened the eyes of researchers to the value of DNA methylation alterations in the better understanding of diseases with different types and origins. These molecular efforts soon translated into pharmaceutical attempts to develop several DNA methylation inhibitors for treating various diseases, especially cancers [266]. However, most of these efforts failed to move into the clinics due to the high toxicity, cell cycle dependency, and need for continuous administration [266,267]. Moreover, most of these agents cause random DNA hypomethylation in genes with devastating outcomes in some cases [268]. There are always some expectations, such as what happened for two nucleoside DNMT inhibitors named 5-AC and decitabine (DAC), which gained European Union approval for the treatment of newly diagnosed or secondary AML who are ineligible for transplantation [266]. Of course, it should not be forgotten that neither 5-AC nor DAC exerts favorable anti-cancer effects on solid tumors due to the slower pace of cell cycle progression [269]. Nevertheless, efforts are ongoing to identify suitable non-nucleoside DNMT inhibitors to overcome these limitations. The promising results of a recent study have introduced a first-in-class, potent, non-nucleoside, reversible, selective inhibitor of DNMT1, GSK3685032, with improved tolerability and efficacy in AML models. Hence, due to the higher *in vivo* tolerability compared with DAC, GSK3685032 induces robust loss of DNA methylation associated with transcriptional activation, resulting in tumor regression and improved survival in mouse models of AML [270].

Other DNA methylation-based agents that have received FDA approval are enasidenib (AG-221) and ivosidenib (AG-120), which

target IDH2 and IDH1, respectively [271,272]. Both agents can significantly prevent the production of 2-Hydroxyglutarate (2-HG) from mutant IDH1/IDH2 and thereby reactivate TET2 in malignant cells [271,272]. But, despite efficacy, the lower incidence of *IDH1/IDH2* mutations in human cancers practically reduces the usefulness of the agents. Glioblastoma is the only cancer with a higher rate of *IDH1/IDH2* mutations; however, in this type of cancer, the blood-brain barrier (BBB) prevents the penetration of enasidenib and ivosidenib into the malignant cells. Recently, the results of phase 1 clinical study declared the success of Vorasidenib (AGG-881), a dual IDH1/IDH2 inhibitor, in penetrating BBB of glioma patients, suggesting perhaps this agent may bring a ray of hope for glioma patients with *IDH1/IDH2* mutations [273]. Table 6 provides further information about drugs that target DNA methylation.

6. Conclusion and future perspectives

Despite numerous studies trying to shed light on the importance of DNA methylation machinery in the development of several diseases, the translation of this knowledge into a clinical perspective is in a nascent stage. It may be due to the complexity of DNA methylation alterations that make it impossible to find out whether these alterations are a cause or consequence of disease [76,134,282]. A non-causal DNA methylation marker could be useable for diagnosis, prediction of treatment response, and monitoring residual disease, but it would not be useable as a potential therapeutic target for mitigating the disease phenotype. Another problem in studying DNA methylation alterations is the tissue- or cell-specific feature of DNA methylation and that it changes over time. Furthermore, most cell populations contain heterogeneous cellular compositions that have different methylation patterns. This challenge is most important in diseases such as cancer, which have a mixed population of normal and tumor cells, or in immune diseases, where the composition of blood cells varies widely among patients. To address this issue, two strategies have been developed to decode DNA methylation patterns according to the cell origin. While one of these strategies can resolute DNA modifications (5 mC or 5hmC) in the single-cell scale [283], the other can generalize these modifications to variable cellular composition by comparison with a reference epigenome or by using reference-free algorithms [284].

Despite all the mentioned challenges, increasing reports reveal a strong association between DNA methylation profiles and clinical outcomes in various diseases, making DNA methylation profiles valuable as biomarkers for diagnosis, prognosis, or response to therapy. Detection of DNA methylation changes in non-invasive liquid biopsy samples, such as blood cells, plasma cell-free DNA, saliva, stool, or urine, is potentially used as a useful diagnostic and prognostic biomarker in clinical practice. However, the sensitivity and reproducibility of liquid biopsy-based techniques still need to be improved, especially when working with samples with suboptimal DNA quality or low cell numbers.

Nevertheless, DNA methylation alterations are well-recognized drivers that initiate and prolong human disease. Fortunately, the fact that DNA methylation alterations are reversible converts them into valuable therapeutic targets. Current FDA-approved DNA methylation-based drugs are used to treat cancer alone or in combination with other therapies, but this approach appears to affect DNA methylation signatures at many genomic sites indiscriminately. The CRISPR technology can direct the enzymatic DNA methylation machinery to specific sites in the genome, making characteristic changes in the DNA methylation pattern to control gene expression. CRISPR, sequencing, and cloning technologies can all come to give value to DNA methylation alterations in the new era of molecular biology. In conclusion, the study of DNA methylation alterations is a phenomenal intersection that each of its ways can reveal the mysteries of various diseases, introduce new diagnostic and prognostic biomarkers, and propose a new patient-tailored therapeutic approach for diseases.

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Declaration of competing interest

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Abbreviations

2-HG 2-Hydroxyglutarate
5-AC 5-azacitidine
5-carboxyl 5-caC 5-carboxyl
5-fc 5-formyl
5hmC 5-hydroxymethylcytosine
5 mC 5-methylcytosine
ADAC-DN Autosomal dominant cerebellar ataxia with deafness and narcolepsy
ADD ATRX- DNMT3- DNMT3L
ALL Acute lymphoblastic leukemia
AML Acute myeloid leukemia
ARTs Assisted reproductive technologies
AS Angelman syndrome
Aβ Amyloid beta
BAH Bromo adjacent homology
BBB Blood-brain barrier
BER Base excision repair
BMI Body mass index
BWS Beckwith–Wiedemann syndrome
ChIP-Seq Chromatin immunoprecipitation sequencing
CML Chronic myeloid leukemia
c-NHEJ Classical non-homologous end joining
CREB1 cAMP-responsive element-binding protein 1
CSR Class switch-recombination
CUP Carcinoma of unknown primary
DAC Decitabine
dcSSc Diffuse cutaneous SSc
DNMTs DNA methyltransferases
GoF Gain-of-function
H3K4 Histone H3 lysine 4
HDACs Histone deacetylases
HEK Human embryonic kidney
HM450K Infinium humanmethylation450K
HSCs Hematopoietic stem cells
HSNA1E Hereditary sensory autonomic neuropathy 1E
ICF Immunodeficiency, centromeric instability, and facial anomalies syndrome
ICRs Imprinting control regions
IDHs Isocitrate dehydrogenases
IGF2 Insulin-like growth factor 2
lcSSc Limited cutaneous SSc
LoF Loss-of-function
MBD Methyl-CpG-binding domain
MDS Myelodysplastic syndrome
MeDIP Methylated DNA immunoprecipitation
MEFs Mouse embryonic fibroblasts
mESCs Mouse embryonic stem cells
MPNs Myeloproliferative neoplasms
MS Multiple sclerosis
MTase Highly conserved DNMT
MRD Minimal residual disease
PB Peripheral blood
PBMCs Peripheral blood mononuclear cells
PHD Plant homeodomain
PTMs Post-translation modifications
PWS Prader–Willi syndrome

PWWP	Pro-Trip-Trip-Pro
RA	Rheumatoid arthritis
RFTS	Replication foci targeting sequence
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SAT	Subcutaneous adipose tissue
SLE	Systemic lupus erythematosus
SNP	Single-nucleotide polymorphism
SNRPN	Small nuclear ribonucleoprotein polypeptide N
SRA	SET- and RING-associated
SRS	Silver–Russell syndrome
SSc	Systemic sclerosis
T2D	Type 2 diabetic
TCGA	The Cancer Genome Atlas
TDG	Thymine glycosylase
TET	Ten-eleven translocation
TFs	Transcription factors
Th2	T helper 2
TRD	Transcriptional repression domain
tRNAs	Transfer RNAs
TTD	Tandem Tudor domain
UBL	Ubiquitin-like
UPD	Uniparental disomy
WGBS	Whole-genome bisulfite sequencing
ZnF	Zinc-finger
α-KG	Alpha-ketoglutarate

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