DNA (cytosine-5)-methyltransferase 1 - [Isoform 1]

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Target Validation

Experimental models

The murine equivalent of the human DNMT1 (DNA [cytosine-5]-methyltransferase 1; Swiss-Prot accession number: P26358) gene was deleted in ES cells via gene targeting [1]. *Dnmt1-/-* cells possessed dramatically decreased genomic methylation and were viable; however, the mutation caused a homozygous lethal phenotype when introduced into the germline (see Table of experimental models for DNMT1) [1]. Cre/*loxP*-mediated deletion of *Dnmt1* in mice has given rise to several lines of conditional mutants in the nervous system and the immune system [2,3,4]. Mice carrying a hypomorphic *Dnmt1* allele showed reduced DNMT1 expression (10% that of wild-type levels) and substantial genome-wide hypomethylation in all tissues [5]. The hypomorphic mutants developed aggressive T-cell lymphomas, supporting a causal role for DNA hypomethylation in tumor formation [5]. When the *Dnmt1* hypomorphic allele was introduced to the *Apc*$^{Min/+}$ intestinal murine model, a complete suppression of multiple intestinal metaplasia cancerous polyp formation was observed along with reduced CpG island methylation in the intestine, suggesting that *Dnmt1* is a genetic suppressor of intestinal polyp formation [6]. Using the same model, the overall inhibition of intestinal tumorigenesis in hypomethylated *Apc*$^{Min/+}$ mice was accompanied by microscopic liver tumors; thus, DNA hypomethylation could suppress late stages of intestinal tumorigenesis, but promote early liver lesions [7]. In the human colon cancer cell line HCT116, genetic deletion of DNMT1 did not lead to loss of genomic methylation or reactivation of tumor suppressor genes [8]; however, it has been recently shown that this model was incorrectly targeted, resulting in a catalytically active truncated protein [10,9]. DNMT1 protein has been knocked down via siRNA or oligonucleotide antisense (MG98, see *Function and Localization*: *In disease*) degradation of DNMT1 mRNA in HCT116 cells [11]. The effect of depleting DNMT1 from human cancer cell lines using this approach is the basis for MG98 clinical trials. Moreover, the conditional deletion of DNMT1 leads to mitotic catastrophe and ATM/ATR-mediated cell death of HCT116 cells [12]. In HCT116 cells, the conditional deletion model [12] differs from the siRNA knockdown model [11] in that the siRNA is an incomplete knockdown with a less severe phenotype, while the genetic deletion of DNMT1 leads to cell cycle arrest and cell death.

Drugs and Biologicals

Current status

Drugs

DNMT1 (DNA [cytosine-5]-methyltransferase 1) inhibitors, specifically cytidine analogs, are potent anticancer reagents in cell culture models in a variety of human cancers [13]. Hypermethylation of tumor suppressor genes is a common mechanism of gene silencing observed in cancer [14]. In seminal experiments, the inhibition of DNMT1 by either antisense knockdown, cytidine analogs [15,16] or genetic approaches [17] was shown to inhibit adrenocortical Y1 tumors [16] and intestinal cancer [17] in murine models. These studies, along with testing of cytidine analog inhibitors in a variety of human cancer cell cultures [13], have paved the way for inhibition of DNA methyltransferases in clinical trials. The following cytidine analogs have been clinically tested for over 25 years: 5-azacytidine (5-aza-CR; Vidaza®, Pharmion), 5-aza-2'-deoxycytidine (5-aza-CdR; decitabine; Dacogen®, Supergen and MGI Pharma), and dihydro-5-azacytidine (DHAC). The first two are approved by the FDA for the treatment of myelodysplastic syndrome (MDS), but show little activity in treating solid tumors (see *DNA methyltransferase inhibitors in current clinical trials*). DHAC is no longer used in clinical trials due to lack of efficacy after Phase II clinical trials treating lung cancer [18], mesothelioma [19,20] or melanoma [21].

It is important to note that one caveat of introducing demethylating drugs is global DNA hypomethylation, leading to the reactivation of previously silenced pro-metastatic genes [22] in breast cancer cells. Whether demethylating drugs could alter the long-term properties of cancer cells and lead to undesirable outcomes *in vivo* still remains to be seen.

DNA methyltransferase inhibitors in current clinical trials

Cytidine analogs

5-Aza-CR is a chemical analog of the cytosine residue. 5

-Aza-CR is activated by uridine-cytidine kinase [23,24] and is incorporated in place of cytosine into DNA of replicating cells, as well as RNA during transcription [25,26]. Once incorporated into DNA strands, 5-aza-CR works to inhibit all DNA methyltransferases including DNMT1 by covalently binding to the methyltransferase catalytic domain of the DNA methyltransferase and trapping the enzyme on the DNA strand, thus effectively reducing active DNA methyltransferase and causing genomic demethylation with subsequent rounds of replication [25,27,28]. Vidaza® is the first FDA-approved drug for the treatment of MDS (FDA application 050794) [29]. Unfortunately, there are drawbacks to the usage of 5-Aza-CR to treat cancer in patients. 5-Aza-CR is unstable in aqueous solution and is highly toxic to cells [30,31]. Even when administered at low dosages, 5-Aza-CR's side-effects include nausea, fatigue, neutropenia, thrombocytopenia, vomiting and fevers [32]. 5-Aza-CR also has a higher preference for RNA incorporation *in vivo* [32].

5-Aza-CdR is enzymatically altered by DCK (dCK) and replaces cytosines in the DNA of replicating cells [23]. However, it is not incorporated into RNA and does not alter cellular transcriptional machinery [33]. As 5-aza-CdR's mode of action is as a cytosine analog incorporated into DNA during replication, it also traps DNA methyltransferases to DNA via covalent binding to the methyltransferase catalytic domain of the DNA methyltransferase. Cellular toxicity remains a major setback for widespread clinical use. In cell culture systems, 5-aza-CdR induces cell death via P53-mediated apoptosis due to the activation of ATM/ATR cell cycle checkpoint pathways in response to genomic damage [34,35]. Dacogen® received FDA approval in 2006 as a treatment for MDS [36]. Patients treated with 5-aza-CdR experience the same side-effects listed for 5-aza-CR usage [32]. Furthermore, cytosine analogs result in a permanent alteration of the genome and can be mutagenic in hypomethylated daughter cells [37,38]. Also, demethylation of the genome in satellite repeat regions can lead to chromosomal instability and cell death [5]. As of 01 July 2008, Eisai, the parent of MGI Pharma, released the results of a Phase III study (NCT00043134; [39]) showing no statistically significant advantage of 5-aza-CdR treatment on median overall survival in elderly MDS patients [40]. Although disappointing, over 30 clinical trials using 5-aza-CdR, alone and in combination with other therapies, in a variety of cancers are ongoing.

Non-cytidine analogs

Procainamide (Procanbid®, King Pharmaceuticals) is an approved anti-arhythmic and local anesthetic. Procainamide has demethylating activity by preferentially binding CpG-rich DNA sequence and sterically hindering DNMT1 binding to CpG dinucleotides; thus, it does not directly bind to DNMT1 [41]. Procainamide does not inhibit the catalytic activity of DNMT1, but acts as a partial competitor for the methyl donor group*S*-adenosylmethionine [42]. Since procainamide has multiple clinical uses, it might not be best suited for treating DNA methyltransferase-specific disorders; furthermore, high concentrations are necessary to see demethylating activity in human PCa cells *in vitro* [43]. Another caveat is a recent study in which no demethylating effects of procainamide were detected in a variety of human cancer cell lines [44].

Anti-sense inhibitors

MG98 (MGI Pharma and MethylGene), an antisense oligonucleotide, specifically targets and degrades DNMT1 mRNA in cells, leading to over 80% reduction of DNMT1 protein, subsequent genomic demethylation and reactivation of tumor suppressor genes in colon cancer cells *in vitro* [11,45]. In tumor xenograft animal experiments, tumor cell proliferation slowed and regression was observed after MG98 administration [46]. Based on these experiments, clinical Phase I trials (NCT00003890; [47]) of intravenous infusion of MG98 commenced in patients displaying multiple solid tumors of different origin [48]. MG98 caused reactive side effects such as fatigue, fever, chills, rigor and confusion [48].

DNA methyltransferase inhibitors in preclinical trials

Cytidine analogs

Zebularine (1-[β-D-ribofuranosyl]-1,2-dihydropyrimidin -2-one; NSC 309132, Developmental Therapeutics Program, National Cancer Institute, USA) is a chemically stable analog of 5-aza-CR with the same mechanism of action [49]. Zebularine is first processed by uridine-cytidine kinases, which allows for the analog to be incorporated into DNA and RNA by replacing cytosine [50]. Like 5-aza-CR and 5-aza-CdR, DNA methyltransferases are covalently bound to zebularine-incorporated DNA strands via the methyltransferase domain, with an apparent preference for DNMT1 [50]. Upon zebularine treatment, genomic demethylation and reactivation of tumor suppressor genes was seen *in vitro* [49,51,52]. Due to its stability, it can be orally administered and shows less cellular toxicity in animal cancer models [53]. Although zebularine decreases the unwanted toxicity and instability of other cytidine analogs, its mechanism of action is the same and is known to be cytotoxic [31]. Furthermore, to achieve demethylation levels similar to 5-aza-CdR, zebularine must be administered at 100-fold higher concentration *in vitro* [51]. In a preclinical pharmacokinetic study of

zebularine, zebularine metabolism by the liver leads to poor systemic bioavailability in monkey, dog, rat and mouse [54,55]. For these reasons, there are no current clinical trials using zebularine and research is still at preclinical stages.

Non-cytidine analogs

Hydralazine (1-hydrazinophthalazine monohydrochloride; apresoline, Novartis Pharmaceuticals), a cardiovascular hypertensive drug, is a weak non-nucleoside inhibitor of DNA methyltransferases through interaction of its nitrogen atoms and the catalytic site of DNA methyltransferases. Hydralazine could cause genomic demethylation and tumor suppressor gene reactivation in breast cancer cell lines [56]. However, when testing suppression of DNA methylation by hydralazine in direct comparison to 5-aza-CR in a variety of cancer cell lines, hydralazine failed to reactivate tumor suppressor genes or reduce DNA methylation levels [44]. Thus, the role of hydralazine as a DNA methyltransferase inhibitor is still controversial.

RG108 (2-[1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl]-3-[1H-indol-3-yl] propanoic acid, first synthesized in Frank Lyko's laboratory [German Cancer Research Center Division of Epigenetics, Heidelberg, Germany], now produced by Sigma Aldrich), a novel small molecule, effectively inhibited DNMT1 activity (IC_{50}=115nM) with subsequent demethylation and reactivation of tumor suppressor genes in the human cancer cell lines NALM-6 (acute lymphoblastic leukemia B-cell line) and HCT116 (colon carcinoma cell line) [57]. The inhibitor sterically blocks the catalytic binding site of the human DNMT1 enzyme to induce DNA demethylation [57]. RG108 does not direct demethylation of minor satellite repeats, indicating that RG108 administration will not produce cellular genomic instability and potential mutagenesis [57].

Patents

Please see Table 1 for a list of relevant DNMT1 patents.

Ligands and antibodies

Examples of DNMT1 antibodies (85 listed on Biocompare; keyword DNMT1) are given in Table 2.

Therapeutic antibodies

As of 18 November 2008, we are not aware of any reports of immunodepletion or immunosuppressive approaches to target DNMT1 in disease.

Next frontiers

Due to the toxicity of cytidine analogs, researchers are looking into novel small molecule inhibitors directly targeting either the catalytic region of DNMT1 or the target recognition domain (TRD). However, this research is hindered by the lack of known three-dimensional structures for full-length human DNMT1 (as of 18 November 2008, we are not aware of a three-dimensional structure of human DNMT1 on PDB). Although MG98 directly targets DNMT1 [11], the high doses necessary to knockdown DNMT1 protein produced multiple side-effects outweighing any potential benefits for patients [48].

Function and Localization

In homeostasis

Function of DNMT1 in gene silencing

Human DNMT1 (DNA [cytosine-5]-methyltransferase 1) is a nuclear protein present in somatic cells and is highly expressed in fetal tissue, moderately expressed in adult brain, heart, thymus and kidney, and weakly expressed in adult skeletal muscle, colon, spleen, liver and lung [58]. When normal mammalian somatic cells are not undergoing mitosis, DNMT1 exhibits a diffusible nucleoplasmic distribution in non-S phase cells and once S phase begins, DNMT1 is targeted to replication foci via N-terminal domains such as the replication foci-directing domain (RFDD) and the PCNA (cyclin)-binding domain (see Figure 1) [59,60]. Furthermore, DNMT1 can form a complex with the co-repressor protein DMAP1 and histone-modifying enzymes at the replication foci [61,62]. The concept of DNA methylation regulating tissue-specific gene expression was proposed by two independent groups in 1975 [63,64], yet the enzyme that catalyzes this process in mammals was not identified until the late 1980s [65]. The mammalian DNMT1 genomic sequence was found based on homology to bacterial type II restriction methyltransferase [66]. DNMT1 catalyzes the addition of a methyl group from the donor *S*-adenosylmethionine onto the 5' carbon of cytosine residues located in CpG dinucleotides [67,68,69]. *In vitro* kinase assays revealed that the mammalian DNMT1 preferentially targets hemimethylated DNA, thus maintaining patterns of DNA methylation during replication [70,71,72]. DNM3A (DNMT3a) and DNM3B (DNMT3b) were cloned from the murine genome with homology to DNMT1, and these enzymes are responsible for establishing *de novo* methylation patterns during development [73,74]. DNA methyltransferase-catalyzed DNA methylation is one of the best-characterized epigenetic events, which are classified as heritable

changes in DNA structure that do not alter DNA base sequence [75].

DNA methylation can induce gene silencing in a variety of ways. First, the addition of a methyl group to CpG dinucleotides in the proximal promoters of genes can sterically hinder the binding of transactivating factors [76]. Second, methylated CpG dinucleotides are binding sites for repressive proteins that, when recruited to methylated DNA, either directly repress transcription or recruit chromatin remodeling proteins such as histone deacetylases to silence gene transcription [77,78,79,80,81]. Furthermore, DNMT1 has been shown to be a transcriptional repressor in the absence of enzymatic activity by binding to RB (pRb) protein and inhibiting E2F transcription targets [82,83].

DNMT1 isoforms

DNMT1 has different translational start sites [84] and exists in different splice variants [85]. The predominant isoform in human somatic cells comprises 1616 amino acid residues [86]. A shorter form of DNMT1 called DNMT1o is found specifically in growing oocytes and is also expressed during pre-implantation (see Figure 1) [85,87]. DNMT1o lacks the N-terminal 114 amino acid residues and possesses increased stability against degradation in oocytes [88]. Another splice isoform is DNMT1b, whose transcript contains the in-frame addition of 48 nucleotides between exons 4 and 5 [89,90]. Since the amount of DNMT1b is less than 5% the level of the predominant DNMT1 in somatic cells, it is unclear what role DNMT1b plays in somatic cells [89].

DNMT1: role in embryonic development, imprinting and genome stability

The role of DNMT1 in mammalian cells has been thoroughly investigated in mice or murine ES cells. The loss of functional *Dnmt1* alleles produces several major changes including severe demethylation of the genome, a modest increase in mutation rates in ES cells and defects in the mismatch repair system [91,92,93]. *Dnmt1* $^{-/-}$ mouse ES cells grow normally in the undifferentiated state, but enter apoptosis when forced to differentiate [1]. Apoptosis is also the cause of death of embryos lacking *Dnmt1* during mid-gestation [1]. Furthermore, inactivation of all X chromosomes is observed in mutant embryos due to the demethylation of the Xist promoter and its subsequent reactivation [94]. Bi-allelic expression of imprinted genes is also observed in *Dnmt1* $^{-/-}$ embryos [95]. Finally, *Dnmt1* is required for the repression of retrotransposons in mammalian somatic cells, thus enforcing genomic stability [96,97].

In disease

Cancer

Under the theory of Knudson's two-hit model [98], complete loss of function of a tumor suppressor gene requires loss of function on both alleles. Along with traditional DNA sequence mutations in tumor suppressor genes, aberrant DNA hypermethylation directed by DNMT1 and/or DNM3B [99] of the promoter of the wild-type allele can render a heterozygous genotype with complete loss of function. Inactivation of genes in a wide variety of cancer cell lines is frequently associated with hypermethylation of CpG islands in promoters of tumor suppressor genes [100] and can be grouped into the following categories: cell cycle regulation and apoptosis (*CDKN2A* [*p14ARF*], *CDKN2B* [*p15INK4b*], *CDKN2A* [*p16INK4a*] *APC*, *HIC1*), DNA repair genes (*MLH1*, *GSTP1*, *MGMT*, *BRCA1*) and metastatic genes (*CDH1*, *TIMP3*, *DAPK1*, *TP73* [*p73*], *THBS1* [*TSP*], *VHL*) [101,102,103,104]. However, the underlying mechanisms leading to aberrant DNA methylation patterns in cancer cells have yet to be elucidated. This DNA methylation work in animal cancer models and human cell lines has laid the framework for clinical trials investigating the role of DNMT1 inhibitors in a variety of cancer sub-types, such as MDS, AML/CML, breast cancer, renal cell carcinoma, and prostate cancer.

MDS

5-Aza-CR and 5-aza-CdR are the two most widely used DNA methyltransferase inhibitors in clinical practice, with both 5-aza-CR and 5-aza-CdR approved by the FDA for the treatment of MDS [105,36]. According to Dacogen® literature, clinical injection could improve MDS symptoms by altering bone marrow function to increase hematological cell counts and improve overall patient health. By altering DNA methylation abnormalities observed in MDS, clinical injections of 5-aza-CR and 5-aza-CdR have shown promising results. Low-dose subcutaneous injections of 5-aza-CR in high-risk MDS patients yielded successful response rates, with a prolongation of transformation into the more advanced AML [29]. With a low-dosage administration schedule, 5-aza-CdR has also shown promising survival advantages in patients with high-risk MDS and a reduction in transformation into advanced AML [106,107]. Currently, there are multiple ongoing MDS clinical studies to evaluate optimal dosage as well as the effects of either 5-aza-CR or 5-aza-CdR in combination with other chemotherapeutics.

AML/CML

Aggressive forms of MDS can transform into AML. AML patients treated with 5-aza-CR showed marked remission and prolonged survival rates, providing improved clinical benefits to patients with this disease [108]. 5-Aza-CdR treatment of AML also shows improved response rates when given in low dosages [109], and currently Phase I /II trials are ongoing to optimize both 5-aza-CR (27 trials, e.g. NCT00569010; [110]) and 5-aza-CdR (18 trials, e.g. NCT00760084; [111]) treatment in patients diagnosed with AML. CML results from a chromosomal translocation (Philadelphia chromosome) resulting in the functional fusion protein BCR–ABL, a tyrosine kinase [112]. A recent Phase II clinical trial (NCT00054431) revealed that 5-aza-CdR administered along with imatinib, a tyrosine kinase inhibitor, increased the percentage of favorable responses in imatinib-resistant CML patients [113]. Clinical trials are ongoing to investigate the effects of DNA methyltransferase inhibitors for treatment of patients with CML as well as other leukemias.

Breast cancer

A non-randomized proof-of-principle study involved the administration of hydralazine in combination with valproate, a histone deacetylase inhibitor, along with chemotherapy to 16 patients. Treatment was well tolerated and appeared to increase the efficacy of patients' chemotherapies, for no patients' symptoms progressed [114]. A Phase I/II clinical trial is ongoing to determine the maximal tolerated dose of hydralazine used in conjunction with chemotherapy in women with breast cancer, with a following Phase II trial to evaluate the efficacy of hydralazine in producing a demethylation effect (NCT00575978; [115]).

A Phase I trial is ongoing to study the side-effects and best dose of 5-aza-CdR in treating patients with advanced solid tumors (including breast cancer) that have not responded to previous treatment (NCT00030615; [116]).

Renal cell carcinoma/multiple solid tumors

In preclinical experiments, colon cancer cell lines treated with the antisense inhibitor of DNMT1, RG108, showed subsequent genomic demethylation and reactivation of tumor suppressor genes [11,45]. MG98 (MGI Pharma and MethylGene), an antisense oligonucleotide, specifically targets and degrades DNMT1 mRNA in cells, leading to over 80% reduction of DNMT1 protein, subsequent genomic demethylation and reactivation of tumor suppressor genes in colon cancer cells *in vitro* [11,45]. In tumor xenograft animal experiments, tumor cell proliferation slowed and regression was observed after MG98 administration [46], thus providing good evidence that abnormal upregulation of DNMT1 might play a direct role in cancer pathogenesis. Based on these experiments, clinical Phase I trials (NCT00003890; [47]) of intravenous infusion of MG98 commenced in patients displaying multiple solid tumors of different origin [48]. MG98 caused reactive side-effects such as fatigue, fever, chills, rigor and confusion [48].

Prostate cancer

In human prostate cancer cell culture models, investigators have shown that DNMT1 activity and transcriptional levels are significantly higher in cancerous cells versus benign prostate cancer cells [117]. In mouse models of prostate cancer, treatment with the DNA methyltransferase inhibitor 5-aza-CdR prevented prostate cancer tumor formation and no occurrence of hypermethylation of the repair gene *Mgmt* was observed [118]. Furthermore, genetic analysis of induced murine prostate tumors revealed a 2.4% hypermethylation change out of 1200 loci investigated, thus providing evidence for a functional role of DNA methylation in prostate cancer development [119]. A clinical Phase II trial is ongoing to determine what effects 5-aza-CR treatment has in prostate cancer patients (5-aza-CR is only approved by the FDA for MDS) when combined with hormone replacement therapy (NCT00384839; [120]).

Cancer subtypes

There are several ongoing clinical trials to assess the role of DNA methyltransferase inhibitors in a variety of cancer subtypes, due to the FDA approval of 5-aza-CR and 5-aza-CdR for clinical treatment of MDS and AML, and the reactivation of tumor suppressor genes in cell culture models of various human cancers [13]. Currently, 5-aza-CR or 5-aza-CdR, either alone or in combination with other chemotherapy agents, are being tested in the following cancer subtypes: lymphoma (NCT00109824 [121], NCT00089089 [122], NCT00079378 [123], NCT00543582 [124], NCT00589160 [125], NCT00336063 [126], NCT00005639 [127], NCT00349596 [128]), lung cancer (NCT00387465 [129], NCT00006019 [130]), esophageal cancer (NCT00041158 [131], NCT00019825 [132]), squamous cell cancer of the head/neck (NCT00443261 [133]), thyroid cancer (NCT00085293 [134], NCT00004062 [135]), melanoma (NCT00398450 [136], NCT00217542 [137], NCT00030615 [116], NCT00002980 [138]), kidney cancer (NCT00561912 [139], NCT00217542 [137]) and multiple myeloma (NCT00412919 [140], NCT00006019 [130]). Preclinical data from a variety of cancer cell lines have shown a

dramatic alteration of gene expression after cancer cells *in vitro* have been treated with either 5-aza-CR or 5-aza-CdR [100,101,102,103,104]. Through the genetic inhibition of DNMT1, cancer cells have responded by inducing growth arrest and cell death [12]. This preclinical data furthers the notion that DNA methylation abnormalities play a direct role in the genetic alterations leading to cancer.

Hemoglobin disorders: -thalassemia and sickle cell disorder

Sickle cell disorder is linked to a substitution mutation in the HBB (β-globin) gene, causing a deformation of red blood cells [141]. β-thalassemia is an hereditary disease resulting in decreased production of the adult HBB chain, resulting in the premature degradation of red blood cells [142]. In the past, researchers believed that inducing DNA hypomethylation by inhibiting DNA methyltransferases would lead to the reactivation of the fetal hemoglobin gene promoter and potentially compensate for the reduction of the adult HBB. Initial clinical studies showed that 5-aza-CR, a DNA methyltransferase inhibitor, led to a reactivation of fetal hemoglobin in β-thalassemia patients and those with sickle cell disorder [143,144]; however, studies were halted due to the mutagenic potential of cytidine analogs in animals [145]. Biochemical studies have shown that DNA methyltransferase-directed methylation of one CpG dinucleotide in the promoter of fetal γ-globin regulates the fetal-to-adult globin switch [146,147,148,149]. The FDA approval of 5-aza-CR and 5-aza-CdR as administered therapeutics to patients with MDS renewed interest in the use of these drugs in hemoglobin disorders (NCT00000623; [150]) [151,152]. Recently, however, there is a growing body of evidence suggesting that the regulation of globin genes is independent of DNA methylation and the previous reports of reactivation of fetal γ-globin after treatment with demethylating agents is occurring through an unknown mechanism [153,154].

Endometriosis

Gene regulation changes have been previously observed in endometrial cells from endometriosis patients, suggesting that alterations in gene expression underlie the disease [155,156]. Since DNA methylation mediates gene repression, researchers are currently investigating DNA methylation changes in endometrial tissue from patients diagnosed with endometriosis. An increase in DNMT1 in laser-capture micro-dissected endometrial cells of endometriosis patients has been reported; however, it is unclear if the increase in DNMT1 levels correlates with aberrant DNA methylation patterns in diseased cells [157].

Neurological disorders

While studying gene expression changes in post-mortem brains of schizophrenic patients, DNMT1 mRNA overexpression is observed in inhibitory cortical neurons [158]. Furthermore, tentative evidence suggests that DNMT1 overexpression leads to hypermethylation of RELN (reelin) and DCE1 (GAD67) genes in a subpopulation of inhibitory neurons of schizophrenic, but not bipolar, brains [159,160,161].

Imprinting disorders

Imprinted loci are no longer repressed in *Dnmt1*$^{-/-}$ embryos, thus implicating DNMT1 misregulation in imprinting disorders [95]. DNA methylation changes could be involved in Beckwith-Wiedemann syndrome, Silver-Russell syndrome, Prader-Willi syndrome, and Angelman syndrome [162,163,164,165].

Characteristic Structural Features

Domains and motifs

The DNMT1 (DNA [cytosine-5]-methyltransferase 1) enzyme has two domains: the regulatory N-terminal region and the C-terminal regarded as the catalytic domain (see Figure 1). The DNA TRD is located at the beginning of the N-terminal domain (amino acids 122–417) [166]. The N-terminal domain interacts with PCNA, RB, HDAC1/2 and DMAP1 [60,61,82]. A replication foci-targeting domain is also present in the N-terminal domain, which is required for import of DNMT1 into nuclei and association with the replication foci [167,168]. A cysteine-rich region containing zinc-binding sites (CXXC motif) is centered in the N-terminal. The CXXC motif is present in all mammalian cytosine methyltransferases, methyl-CpG-binding proteins and various proteins affecting cytosine methylation [168,169]. Two bromo-adjacent homology (BAH) domains are located at the end of the N-terminal domain. BAH motifs are found in origin recognition complex proteins and in chromatin regulatory proteins [170]. The C-terminal part of DNMT1 functions as the catalytic domain and is only active when the regulatory domain is also present [171,71].

The three-dimensional structure of the mammalian DNMT1 is currently unknown (as of 18 November 2008 and as reported in [172]), with crystal structure information available for only bacterial DNA methyltransferases [173,174]. Structural work on bacterial HhaI DNA methyltransferase from

Haemophilus haemolyticus demonstrates that the substrate cytosine is completely flipped out of the helix during the modification reaction [173,174]. Because the catalytic domain of bacterial HhaI DNA methyltransferase is highly homologous to mammalian DNMT1, the crystal structure of bacterial DNA methyltransferase has provided much insight into the possible enzymatic reaction of DNMT1. It is predicted that DNMT1 could also methylate cytosine through a base-flipping mechanism.

Targeted features

The mechanism of action of cytidine analogs as DNMT1 inhibitors is to incorporate into replicating strands of DNA in place of the endogenous cytosine bases [28,49]. Once cytidine analogs are incorporated into DNA, they covalently trap DNMT1 at the methyltransferase catalytic domain (amino acids 1139–1616), thus causing depletion of DNA methyltransferase enzyme through subsequent rounds of DNA replication [28,49]. RG108 is a small molecule that specifically targets and directly binds the DNA methyltransferase catalytic domain (amino acids 1139–1616) [57]. However, all of the above inhibitors target all members of the DNA methyltransferase family, including the *de novo* methyltransferases DNM3A (DNM3Ta) and DNM3B (DNMT3b) [28,49,57]. The antisense oligonucleotide MG98 targets DNMT1 mRNA for degradation, thus creating a transcriptional blockade of DNMT1 protein [11]. However, the largest obstacle in the search for novel DNA methyltransferase inhibitors is the lack of three-dimensional structure modeling of mammalian DNA methyltransferases. In order to develop small molecule inhibitors to different protein domains of DNMT1, the crystal structure of human DNMT1 is first necessary. It is theoretically possible that inhibition of the DNA TRD would also lead to hypomethylation. However, DNA sequence specificity and how DNMT1 targets specific DNA sequence for methylation, in either normal or cancer genomes, is currently unknown. These facts make it difficult to direct inhibition to the TRD of DNMT1.

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177 **Modulation of gene expression by combination therapy.** *Patent number US6953783, Thu Apr 27 00:00:00 BST 2000*

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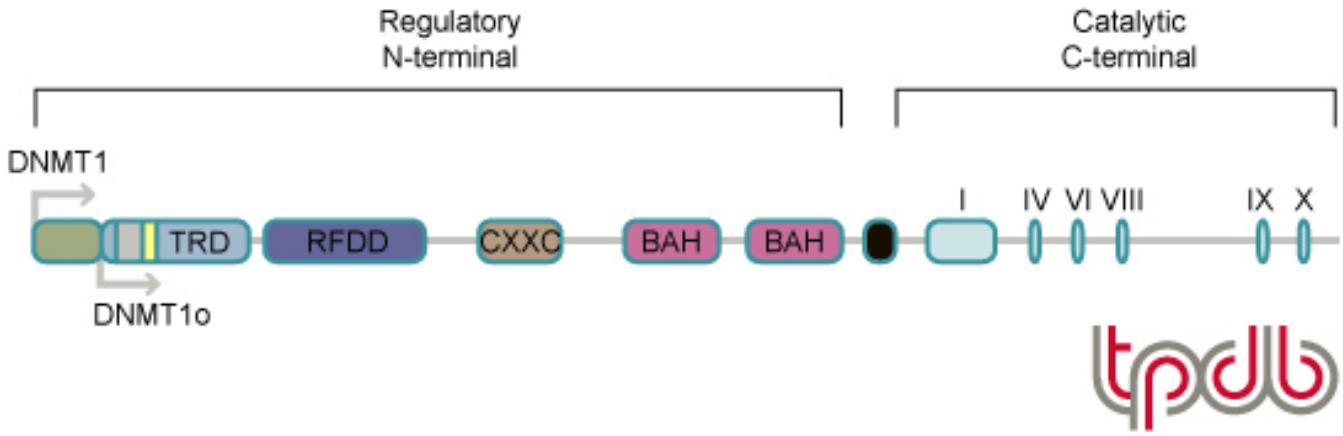

Figure 1 ***DNMT1 structure***

The DNMT1 protein (EC 2.1.1.37) comprises 1616 amino acid residues with multiple functional domains. Individual domains of DNMT1 are as follows: DMAP1-binding domain (green, amino acids 1–120), nuclear localization signal domain (putative) (grey, amino acids 117–205), TRD (blue, amino acids 122–417), PCNA-binding region (yellow, amino acids 163–174), RFDD (by similarity) (purple, amino acids 331–550), CXXC zinc finger domain (sepia, amino acids 646–692), BAH domains (pink, amino acids 775–880 and 972–1100), six glycine–lysine repeats (black, amino acids 1110–1122) and methyltransferase catalytic domain (amino acids 1139–1616) with conserved motifs (light blue bars). Arrows mark the beginning of the somatic isoform DNMT1 enzyme (DNMT1) or the oocyte-specific isoform DNMT1o.

Table 1

Patent/application	Date	Assignee(s)	Reference	Title	Comments
US7038038	23 September 2004	Pharmion	175	Synthesis of 5-azacytidine (VIDAZA)	The present invention provides a method for the preparation of 5-aza-CR, wherein 5-aza-CR is represented by the structure. The method involves the silylation of 5-aza-CR, followed by the coupling of silylated 5-aza-CR to a protected -D-ribofuranose derivative. The coupling reaction is catalyzed by trimethylsilyl trifluoromethanesulfonate. The present invention provides for the first time a method that synthesizes 5-aza-CR that is suitable for use in humans and is amenable to large-scale synthesis

Patent/application	Date	Assignee(s)	Reference	Title	Comments
US6982253	03 January 2006	SuperGen	176	Liquid formulation of decitabine and use of the same (Dacogen®)	Pharmaceutical formulations, kits and vessels are provided for delivering 5-aza-CdR to a patient suffering from a disease in need of treatment with 5-aza -CdR. The pharmaceutical formulation comprises 5-aza-CdR solvated in a non-aqueous solvent that comprises glycerin, propylene glycol, polyethylene glycol or combinations. Such formulations are more chemically stable than conventional liquid formulations of 5-aza-CdR containing more than 40% water in volume. The pharmaceutical formulations can be used for any disease that is sensitive to the treatment with 5-aza-CdR, such as hematological disorders and cancer

Patent/application	Date	Assignee(s)	Reference	Title	Comments
US6953783	11 October 2005	MethylGene	177	Modulation of gene expression by combination therapy (MG98 antisense oligonucleotide)	The invention relates to the modulation of gene expression. In particular, the invention relates to compositions comprising antisense oligonucleotides which inhibit expression of a gene in operable association with protein effectors of a product of that gene and methods of using the same. In addition, the invention relates to the modulation of mammalian gene expression regulated by methylation

Patent/application	Date	Assignee(s)	Reference	Title	Comments
US7250416	31 July 2007	SuperGen	178	Azacytosine analogs and derivatives	Compounds and compositions of 5-aza-CR analogs and derivatives are provided. In one aspect of the invention, analogs or derivatives of 5-aza-CdR and 5-aza-CR are provided with modification at the 4- and 6-position of the triazine ring, at the 1'–6' position of the ribose ring or combinations thereof. Methods of synthesizing and manufacturing these analogs and derivatives are also provided. These compounds can be formulated into pharmaceutical compositions that can be used for treating any disease that is sensitive to the treatment with 5-aza-CdR or 5-aza-CR, such as hematological disorders and cancer

Patent/application	Date	Assignee(s)	Reference	Title	Comments
WO03012051	30 July 2002	Selker EU, Matsen CB, Jones PA, Cheng J, Greer SB and Marquez VE	179	Inhibitor of DNA methylation (zebularine)	*Zebularine has hypomethylating activity, and can be used to inhibit, reverse and/or reduce DNA methylation in vivo and in vitro*. Methods are provided for treating methylation-linked conditions through the application of 2-pyrimidinone derivatives, such as zebularine. Compositions, including pharmaceutical compositions, comprising such derivatives are also provided. Also provided are kits for use in inhibiting DNA methylation, which kits include an amount of a 2-pyrimidinone derivative
EP2005002437	02 August 2005	Deutsches Krebsforschungszentrum (Germany) and Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Poland)	180	Inhibitors of DNA methylation in tumor cells (RG108 and derivatives)	The compounds covered are capable of binding to DNA methyltransferases, particularly human DNMT1, and inhibiting their catalytic activity. Preferably, such inhibitors should have a different mode of action than structural analogs of cytidine, and they should be more specific and less toxic than other inhibitors of DNA methylation

Patent/application	Date	Assignee(s)	Reference	Title	Comments
US2006252723	30 March 2006	MethylGene	181	Combined therapy utilizing reduction of DNA methyltransferase expression and/or activity in interferon	The invention provides methods for the treatment of cancer comprising a reduction of DNA methyltransferase expression and/or activity and treatment and/or induction of interferon. The invention overcomes resistance of cancer cells to interferon

Patent/application	Date	Assignee(s)	Reference	Title	Comments
WO2007041071	29 September 2005	Phiasivongsa P and Redkar S	182	Oligonucleotide analogues incorporating 5-aza-cytosine therein	Oligonucleotide analogs are provided that incorporate 5-aza-CR in the oligonucleotide sequence, e.g. in the form of 5-aza-CdR or 5-aza-CR. In particular, oligonucleotide analogs rich in 5-aza -CdR-deoxyguanosine islets (DpG and GpD) are provided to target the CpG islets in the human genome, especially in the promoter regions of genes susceptible to aberrant hypermethylation. Such analogs can be used for modulation of DNA methylation, such as effective inhibition of methylation of cytosine at the C5 position. Methods for synthesizing these oligonucleotide analogs and for modulating nucleic acid methylation are provided. Also provided are phosphoramidite building blocks for synthesizing the oligonucleotide analogs, methods for synthesizing, formulating and administering these compounds or compositions to treat conditions, such as cancer and hematological disorders

Table 2

Manufacturer	Details
Abcam	Mouse anti-human DNMT1 monoclonal antibody, unconjugated, ab54759 Rabbit anti-DNMT1 polyclonal antibody, unconjugated, ab16632 Chicken anti-DNMT1 polyclonal antibody, unconjugated, ab14290 Mouse anti-DNMT1 monoclonal antibody, unconjugated, clone 60B1220, ab13537
Abgent	Rabbit anti-DNMT1 C-terminal S1602 RB1848 polyclonal antibody, unconjugated, ap1032a
Abnova	Mouse anti-human DNMT1 monoclonal antibody, unconjugated, clone 2b5, h00001786-m01 Anti-DNMT1 monoclonal antibody, unconjugated, clone 60B1220.1, MAB0079
Imgenex	Mouse anti-DNMT1 monoclonal antibody, unconjugated, clone 60B1220.1, IMG-261 (putative chromatin immunoprecipitation grade)
Millipore	Chicken anti-DNMT1 antibody, AB3429 Rabbit anti-DNMT1 polyclonal antibody, 07-688
New England Biolabs	Human DNMT1 N-terminal, M0231S

Table of experimental models for DNMT1

Target	Model/assay	Disease/phenotype/assay	Reference	Source (if applicable)
DNMT1	*Dnmt1* homozygous knockout murine ES cells	Reduced genomic methylation and targeting	1	Jaenisch R laboratory, Whitehead Institute for Biomedical Research, USA
DNMT1	*Dnmt1* homozygous knockout mouse	Reduced genomic methylation, apoptosis, mid-gestational embryonic lethality	1	Jaenisch R laboratory, Whitehead Institute for Biomedical Research, USA
DNMT1	*Dnmt1* conditional knockout mouse: nervous system	*NEST (nestin)-Cre (whole CNS) conditional deletion of Dnmt1*, genomic hypomethylation in neurons and glia, perinatal lethality	2	Fan G, University of California Los Angeles, USA
DNMT1	*Dnmt1* conditional knockout mouse: T-cells	Impaired survival of T-cell receptor / double-positive T-cells and generation of atypical CD8+; T-cell receptor /+ cells (LCK-Cre line); differential cytokine expression in peripheral T-cells (CD4+-Cre line)	3	Wilson CB, Department of Immunology, University of Washington, USA

Target	Model/assay	Disease/phenotype/assay	Reference	Source (if applicable)
DNMT1	*Dnmt1* hypomorphic allele mouse	*Reduces Dnmt1* expression to 10% of wild-type levels, genome-wide hypomethylation in all tissues, runted at birth, aggressive T-cell lymphomas (4–8 months of age)	5	Jaenisch R laboratory, Whitehead Institute for Biomedical Research, USA
*Dnmt1; Apc*Min/+	*Dnmt1 hypomorphic allele mouse crossed with Apc*Min/+ mouse	*Intestinal cancer model (ApcMin/+ mice): crossing onto Dnmt1* hypomorphic allele mouse relieved intestinal cancer formation; liver microtumor formation	6,7	Laird PW, Norris Comprehensive Cancer Center, USA
DNMT1	DNMT1 deletion of exons 2–5 in human HCT116 cells	Colon cancer cells, limited reduction of genomic methylation 8; incorrect targeting leading to hypomorphic allele and a truncated, catalytically active DNMT1 protein 9,10	8,9,10	Vogelstein B, Johns Hopkins Oncology Center, USA
DNMT1	siRNA knockdown of DNMT1 in human HCT116 cells	Colon cancer cells, reduced genomic methylation, tumor suppressor gene reactivation, maintains 10–20% of normal DNMT1 protein level	11	Szyf M, McGill University, Canada
DNMT1	DNMT1 conditional deletion in human HCT116 cells	Colon cancer cells, reduced genomic methylation, checkpoint arrest, mitotic catastrophe, cell death	12	Li E, Novartis Institutes for BioMedical Research, China