Mechanisms of Disease: methyl-binding domain proteins as potential therapeutic targets in cancer

Owen J Sansom, Kathryn Maddison and Alan R Clarke*

SUMMARY

The methyl-CpG-binding domain (MBD) proteins 'read' and interpret the methylation moieties on DNA, and thus are critical mediators of many epigenetic processes. Currently, the MBD family comprises five members; MBD1, MBD2, MBD3, MBD4 and MeCP2. Although not a 'classical' MBD protein, Kaiso also mediates transcriptional repression by using zinc finger domains to bind its targets. Since DNA hypermethylation is a well-recognized mechanism underlying gene silencing events in both tumorigenesis and drug resistance, it is likely that the MBD proteins may be important modulators of tumorigenesis. We review the recent work addressing this possibility, and discuss several of the MBD proteins as potentially excellent novel therapeutic targets.

KEYWORDS Kaiso, MBD2, MBD4, MeCP2, methyl-binding domain

REVIEW CRITERIA

The information for this review was compiled using the PubMed and MEDLINE databases to search for articles published until 1 November 2005. Electronic early-release publications were also included. Only articles published in English were considered. The search terms used included "DNA methylation" in association the following terms: "MBD"; "epigenetics"; "Kaiso"; "Methyl binding domain"; "DNA binding"; "methylated CpG"; "DNMT"; "intestinal adenoma"; and "methylation status". When possible, primary sources have been quoted. Full articles were obtained and references were checked for additional material when appropriate. References were chosen on the basis of the best clinical or laboratory evidence, especially if the work had been corroborated by published work from other centers.

OJ Sansom is Group Leader at The Beatson Institute, Garscube Estate, Glasgow, K Maddison is a Postdoctoral Fellow, and AR Clarke is Professor of Genetics and Joint Head of Research, at the Cardiff School of Biosciences, Cardiff University, Cardiff, UK.

Correspondence
*Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US, UK
clarkear@cf.ac.uk

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INTRODUCTION

DNA can be methylated by the covalent addition of a methyl group at the fifth carbon position of cytosine to form 5-methyl cytosine. In mammals, the bulk of this methylation occurs in sequences of DNA where a cytosine is directly followed by a guanine residue to form a symmetrical CpG dinucleotide.[1] These methylated CpGs are formed by the actions of a family of DNA methyltransferase (DNMT) enzymes, which use S-adenosyl-L-methionine as the methyl donor.[2] DNA methylation is associated with diverse functions in the normal cell, including transcriptional repression, genomic imprinting, X-chromosome inactivation and genomic stability.[3] It is the role of DNA methylation in tumor cells, however, that has been most extensively studied over the past 20 years. Two contrasting changes in the pattern of methylation have been associated with neoplasia: global hypomethylation of the genome; and regional hypermethylation of CpG islands. The importance of hypomethylation for tumorigenesis is still somewhat unclear, although it has been associated with genomic instability.[4] The role for hypermethylation of CpG islands is clearer. These CpG islands are thought to account for around 1% of the genome,[5] and are frequently found in the promoter regions, first exons and 3' regions of a gene.[6] CpG islands are present on the promoters of an estimated 60% of RNA polymerase II transcribed genes,[2] and are generally unmethylated. During the neoplastic process, however, numerous important tumor suppressor genes, including *p16^{INK4a}* (also called *CDKN2A*), *RB1* and *BRCA1*, have been shown to be hypermethylated and transcriptionally repressed. Hypermethylation and gene silencing are important mechanisms in the process of drug resistance, the clearest example of which is the silencing of the mismatch-repair gene *MLH1* following cisplatin treatment in ovarian cell lines. Notably, sensitivity to cisplatin in these lines can subsequently be restored using inhibitors of DNA methylation.[7,8]

In essence, it is the potential reversibility of DNA hypermethylation (unlike DNA mutation) that has provoked significant excitement, as inhibition of DNA methylation and reactivation of genes may have therapeutic importance. Proof-of-principle data in mice have shown that inhibition of DNA methylation can slow tumor formation and growth, either through the use of drugs such as 5-azacytidine or through genetic modulation of the maintenance methylase gene *DNMT1*.[9] Thus, mice bearing an Apc^{Min} allele (a model for the human colorectal syndrome, familial adenomatous polyposis and multiple intestinal metaplasia) show strongly suppressed adenoma development on backgrounds hypomorphic for *DNMT1*.[9]

Recent studies have begun to unravel the role of DNA methyl transferases in the process of tumorigenesis. Using conditional inactivation of *DNMT3B* on an $Apc^{Min/+}$ background, Lin *et al.*[10] have shown that although the loss of *DNMT3B* does not affect the earliest stages of intestinal tumor formation (i.e. the initiation of microadenoma formation) it does seem to inhibit the formation of larger, macroscopic adenomas. The complexity of the role of DNMT3B is illustrated by the fact that, although the numbers of macroscopic adenomas were reduced in the absence of DNMT3B, many of the large tumors observed in this study did show regions of DNMT3B inactivation. Lin *et al.* suggest that this finding narrows the window of action of DNMT3B to the transitional stage between the initiation of the tumor and the outgrowth phase, suggesting that DNMT3B is not required for further growth of tumors.[10]

Given the robust preclinical data and the availability of DNA methylation inhibitors such as 5-azacytidine and 5-aza-2'-deoxycytidine (decitabine; Dacogen®, Supergen, Dublin, CA), it is perhaps surprising that it has taken so long for these drugs to reach the clinic. There are a number of reasons for this, including problems with drug specificity, toxic effects associated with these drugs, and the selection of patient cohorts. Perhaps most significantly, these agents have proven to be cytotoxic at high doses, and recent data indicate that lowering the levels of decitabine induces more DNA hypomethylation in the tumor and better preclinical results.[11] Despite these difficulties, reports are now emerging of improved patient outcome, with longer median times to progression or death following decitabine treatment.[12] One particular concern with targeting DNMT1 is that potent inhibition of this protein may be toxic to normal cells. Mice with complete inactivation of *DNMT1* die shortly after gastrulation,[13] and loss of *DNMT1* in fibroblasts leads to p53-dependent apoptosis.[14] Also of considerable concern is the potential for a protumorigenic effect in some tissues. Thus, mouse embryonic stem cells deficient in *DNMT1* showed enhanced levels of microsatellite instability,[15] and *DNMT1* hypomorphic mice expressing 10% of normal levels of this gene show runting at birth, and at 4–8 months of age develop aggressive T-cell lymphomas that display a high frequency of chromosome 15 trisomy.[16] Finally, despite the reduction in macroscopic intestinal tumors, mice hypomorphic for *DNMT1* on the Apc^{Min} background have been reported to show both increased microadenoma formation and multifocal liver tumors.[17]

Significant attention is focused upon the possibility that proteins that interpret DNA methylation patterns, rather than directly modifying them, may represent alternate or even improved therapeutic targets. The methyl-binding domain (MBD) proteins are examples of such proteins, and evidence is beginning to accumulate that inactivation of members of this family may effectively suppress tumorigenesis without the drawbacks of genomic instability and cytotoxicity.

THE METHYL-BINDING DOMAIN PROTEINS

Originally, two methyl-binding activities were identified within eukaryotic cells, and these identified complexes were termed methyl-CpG binding protein 1 (MeCP1) and MeCP2.[18,19] MeCP1 was subsequently revealed to be a complex of MBD2, MBD3 and the multisubunit protein corepressor NuRD complex,[20–23] whilst MeCP2 is a single protein. From these proteins, a common MBD was identified, which was subsequently used to identify a family of proteins that include MBD1, MBD2, MBD3, MBD4 and MeCP2.[24,25] A further protein, Kaiso, has also been shown to repress transcription in both a methylation-dependent and sequence-specific manner. Although structurally unrelated to the MBD proteins, Kaiso has also been shown to bind methylated 5'CGCG sequences through three Kruppel-like C2H2 zinc finger motifs.[26] The MBD proteins MBD1, MBD2, and MeCP2 repress transcription from methylated promoters *in vitro* and *in vivo* via the association of their transcription repression

domain (TRD) with a corepressor complex.[27–30] By contrast, MBD4 has been primarily characterized as a thymine DNA glycosylase,[31] with little role in transcriptional repression; however, other data suggest that this protein can also mediate transcriptional repression.[32] Of all the proteins with a MBD, only MBD3 cannot specifically bind methylated DNA,[24,33,34] and requires MBD2 to recruit it to methylated DNA. The precise interdependency of the MBD proteins is still unclear. Ballestar *et al.* have identified a number of MBD targets using a chromatin immunoprecipitation (ChIP) and a CpG island microarray (ChIP on chip) approach.[35] This research strategy revealed a gene-specific association for combinations of MBDs. For example, the ApoE promoter was found to be associated with all MBDs, whilst the RasF1A promoter was found to associate only with MeCP2. The ChIP on chip approach provides a high throughput method of identifying MBD-associated CpG islands, and can also be used to identify novel genes that may be epigenetically inactivated through hypermethylation in cancer cells. Recent studies by Lopez-Serra *et al.* reiterate the importance of MBD occupancy of CpG islands in normal rather than neoplastic cell lineages.[36] Simplified structures for each protein discussed are shown in Figure 1, with the relevant MBDs and TRDs indicated. The potential role for each of these proteins in modulating tumorigenesis is discussed below.

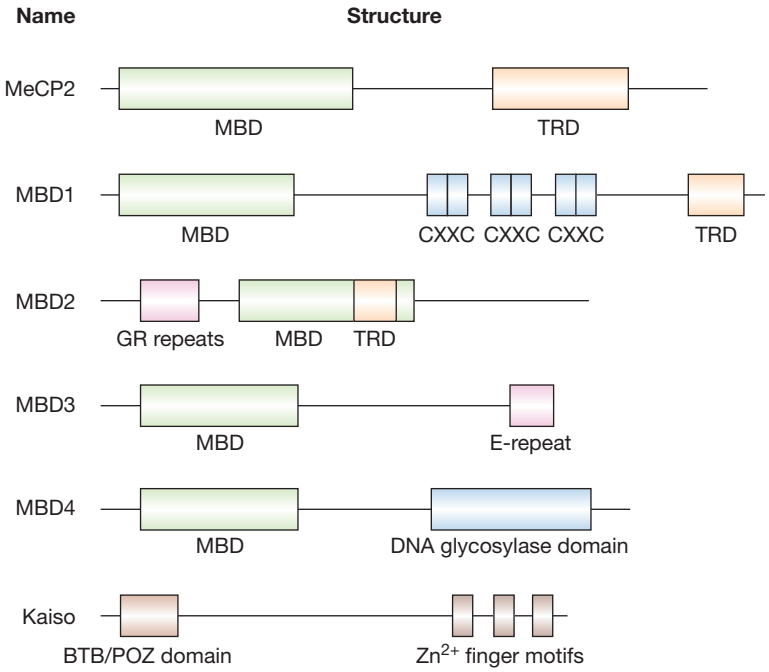

Figure 1 Schematic structure of the major MBD family members. Only the general structure of the protein is shown—some have alternative isoforms. N termini are on the left, C-termini are on the right. Abbreviations: BTB/POZ, broad complex, tramtrack, bric a brac/pox virus zinc finger; GR, refers to alternating glycine and arginine repeats; MBD, methyl-binding domain; MeCP2, methyl-CpG-binding protein 2; TRD, transcription repression domain. Permission obtained from the American Society of Microbiology © Wade PA (2001) *Bioessays* **23:** 1131–1137.[90]

METHYL-BINDING DOMAIN PROTEIN 1

MBD1 occurs in five isoforms that are differentially spliced in the cysteine-rich CXXC and C-terminal domains. Fujita *et al.* found that all five isoforms repress transcription from methylated promoters, and that MBD1v1 and MBD1v2, which contain three CXXC domains, also repress transcription from unmethylated promoters.[37] The relevance of the different splice isoforms *in vivo* remains unclear. To date, there is relatively little direct evidence linking mutation of *MBD1* to neoplasia, although MBD1 has recently been implicated in acute promyelocytic leukemia through its observed cooperation with PML–RAR-alpha in transcriptional repression.[38] Furthermore, MBD1 has been shown to cooperate with the DNA damage protein methylpurine-DNA glycosylase (also known as DNA-3-methyladenine glycosylase) in transcriptional repression and DNA repair, implicating a direct role for MBD1 in sensing base damage in chromatin.[39] Nevertheless, frequent MBD1 mutations have not yet been detected in human colon or lung tumors.[40] Furthermore, constitutive knockout of *MBD1* does not seem to generate any overt tumor phenotype in mice, although increased genomic instability has been reported in neural-stem cells.[41] Hence, on the basis of the current data in the literature, it is not clear whether MBD1 has a substantial role in modifying tumorigenesis.

METHYL-BINDING DOMAIN PROTEIN 2

MBD2 has been demonstrated to recognize a single methylated CpG pair sequence, although it is possible that MBD2 prefers more-densely methylated DNA, as early results suggested a requirement for at least 12 consecutive CpG sequences.[42] MBD2 is the methyl-binding component of the MeCP1 complex, which also contains the histone deacetylase (HDAC) proteins HDAC1, HDAC2 and the RbAp46 and RbAp48 proteins (also known as RBBP7 and RBBP4), allowing MBD2 to target HDAC/chromatin remodeling activity to methylated templates.[43] MBD2 can also

associate with MBD3, which is part of the Mi2/NuRD corepressor complex (Figures 2 and 3). MBD2 and MBD3 can form a complex with *DNMT1* on hemimethylated DNA at replication foci, which might help to retain the repressive transcription state after replication.[44] MBD2 can also act synergistically with other proteins; for example, the recently identified zinc finger protein MIZF interacts with MBD2 and acts as an HDAC-dependent transcriptional repressor.[45]

Unlike *DNMT1* deficient mice, *MBD2* null mice are viable and fertile, with normal imprinting patterns and methylation levels;[46] however, the *MBD2* knockout mice are not entirely benign, as they show impaired nurturing behavior. Deficiency of *MBD2* strongly suppresses intestinal tumorigenesis when crossed onto the Apc^{Min} background, with $Mbd2^{-/-}Apc^{Min}$ mice living longer than Apc^{Min} controls (median of 354 days versus 183 days) and showing a greatly reduced tumor burden.[47] Significantly, *MBD2* gene dosage also affects tumorigenesis, with *MBD2* heterozygotes showing a significant reduction in adenoma burden and increased survival compared with wild-type mice. The precise mechanism of this suppression remains unclear, but it is interesting to note that many important negative regulators of the WNT pathway are transcriptionally repressed in colorectal cancer, implicating these negative regulators as potential targets of *MBD2*-mediated transcriptional repression.[48] Other strong candidate mechanistic targets of *MBD2* include the *p16INK4A* and *p14ARF* tumor-suppressor genes, whose promoter regions have been identified as MBD2-binding targets.[49]

In addition to showing reduced intestinal tumor predisposition, mice bearing *DNMT1* hypomorphic mutations are also characterized by increased lymphomagenesis, which occurs spontaneously,[16] and by mutations in the mismatch-repair pathway.[50] Importantly, deficiency of *MBD2* does not recapitulate this phenotype, with no alteration in the predisposition to lymphomagenesis either alone or in the context of a null *p53* mutation.[51] These data indicate that strategies aimed at inhibiting MBD2 should not raise the same concerns as anti-DNMT1 strategies with respect to possible increases in genomic instability and tumorigenesis.

One critical issue relates to the therapeutic window within which anti-MBD2 strategies may be useful. Data from mouse knockout studies are derived from animals that bear constitutive mutations, and, therefore, these studies cannot differentiate between effects upon tumor initiation and tumor growth. Hence, although these studies highlight the potential of prophylactic use of anti-MBD2 strategies, they do not establish a rationale for therapeutic use. It has, therefore, been extremely encouraging to observe that sequence-specific antisense inhibitors of MBD2 can inhibit both anchorage-independent growth of human lung (A549) and colorectal (HCT116) cancer cell lines *in vitro* and tumorigenic growth of human cancer cell xenografts *in vivo*. Indeed some synergy has been observed between these antisense strategies and exposure to the clastogenic agent bleomycin.[52,53]

METHYL-BINDING DOMAIN PROTEIN 3

MBD3 shares significant DNA and protein sequence homology to MBD2, and is a component of the Mi2/NuRD chromatin-remodeling complex.[24,54] The binding properties of MBD3 vary between species: mammalian MBD3 does not bind methylated DNA,[24,34] whereas *Xenopus* MBD3 binds strongly to methylated DNA. *MBD2* and *MBD3* have a similar sequence, and it had been suggested that they might have a number of redundant functions *in vivo*. In contrast to MBD2, MBD3 is essential for normal embryogenesis, highlighted by the fact no normal $Mbd3^{-/-}$ embryos were identified beyond the implantation stage.[46] This observation shows that MBD2 and MBD3 are not functionally redundant *in vivo* and that MBD3 is required for more functions than simply the transcription repression conferred by the MeCP1 complex.

In terms of its potential as a therapeutic target, there is currently little direct evidence in the literature associating MBD3 with neoplasia, and indeed MBD3 has not been reported as a major target of genetic or epigenetic alteration in either lung or colon cancers.[55] It is clearly implicated in these processes, however, through its association with MBD2.[46,54] Furthermore, MBD3 has recently been associated with the mechanisms through which HDAC inhibitors can specifically inhibit the growth and survival of cancer cells. Thus, in lung tumor cells, treatment with HDAC inhibitors led to an MBD3-dependent overexpression of *p21* (also known as CDKN1A) and/or silencing of the *ERBB2* gene, with MBD3 released by the p21 promoter but recruited to the *ERBB2* gene.[56] The embryonic lethality of the *MBD3* knockout, which occurs early in development, makes it a somewhat less attractive

potential target for therapy than *MBD2*, as inhibition may be toxic to normal cells; however, as with any potential anti-DNMT1 agent, no inhibitor will be 100% effective and, therefore, reduction of MBD3 activity may represent a viable therapeutic approach.

In 2002, two novel genes were identified on human chromosome 19 that are 30–42% identical to human *MBD3* and *MBD2*, but which lack the MBD itself and were not capable of binding methylated DNA.[57] These genes, named *MBD3L1* and *MBD3L2* have been found to be overexpressed in germ-cell tumors, the human ovarian teratocarcinoma cell line PA1, and the mouse embryonal carcinoma cell line P19.[57] The MBD3L2 protein has been shown to interact with MBD3 *in vitro* and *in vivo*, and it has been suggested that it is a transcriptional modulator that can interchange with MBD2 as an MBD3-interacting component of the NuRD complex.[58] Thus, MBD3L2 has the potential to recruit the MeCP1 complex away from methylated DNA and reactivate transcription.[58] These data suggest that MBD3L2 might have a role in modulating tumorigenesis, although this remains to be established.

KAISO

Kaiso expression and localization is altered during tumorigenesis; its expression is elevated in intestinal adenomas arising in the $Apc^{Min/+}$ mouse and in human colon tumors.[59] The regulation of Kaiso is more complex than MBD2, where tumor suppression can be clearly linked with its ability to suppress transcription in a methylation-dependent manner. Kaiso belongs to the BTB/POZ (broad complex, tramtrack, bric a brac/pox virus zinc finger) family of proteins and unlike other methyl-CpG-binding domain proteins, it does not contain a classical MBD, but instead binds methylated CpG dinucleotides through a zinc finger motif located at the C-terminus. Kaiso recognizes and binds to sequences that contain at least two methyl-CpG dinucleotides, and is capable of repressing transcription from methylated templates in a methylation-dependent manner.[60] Critically, ectopic expression of Kaiso in $Mbd2^{-/-}$ fibroblasts has been shown to be capable of restoring repression of a methylated construct, indicating its potential role in modifying neoplasia.

Kaiso was first identified as a binding partner of the 120 kDa catenin protein $p120^{ctn}$.[61] The $p120^{ctn}$ protein contains several armadillo repeats with structural similarity to beta catenin 1, and is a component of the E-cadherin–catenin cell adhesion complex. Kaiso binds $p120^{ctn}$ at a distinct juxtamembrane site that has been implicated in the regulation of cell adhesion and motility.[60,61] This observation raises the possibility that Kaiso provides a link between cell-surface signaling and methylation-dependent gene regulation. Kaiso and $p120^{ctn}$ are associated in the nucleus, where it is thought that $p120^{ctn}$ may negatively regulate Kaiso-mediated transcriptional repression.[62,63] As $p120^{ctn}$ shows altered intracellular localization in some cancer cells compared with wild-type cells, manipulation of $p120^{ctn}$ localization may provide a ready mechanism for deregulation of Kaiso activity.[64]

In addition to mediating methylation-dependent transcriptional repression, Kaiso has been shown to be a sequence-specific transcriptional repressor. Kaiso recognizes the minimal core nucleotide sequence CTGCNA (where N is any nucleotide) in addition to the methyl-CpG dinucleotides, and indeed has a higher affinity for the consensus nucleotide binding site than for the methyl-CpG sites.[65] In mice, the *RAPSN* promoter has been shown to be regulated by Kaiso,[66] and in *Xenopus*, Kaiso has been shown to repress both canonical and noncanonical WNT signaling.[63,67] These data raise two distinct possibilities: first, that Kaiso could act as a methylation-dependent transcriptional repressor in a similar manner to MBD2 and promote tumorigenesis; second, that it may act as a negative regulator of WNT signaling and could suppress WNT-dependent tumorigenesis.

To determine the *in vivo* role of mammalian Kaiso, null mice have been generated.[59] In contrast to the phenotype of $Kaiso^{-/-}$ *Xenopus*, which resembles that of *DNMT1* null mice,[62] $Kaiso^{-/-}$ mice are viable, fertile and seem healthy, with no gross phenotypic abnormalities. This result establishes that the primary role of Kaiso is different in the two species, or that in mammals there is a degree of functional redundancy. To examine the role of Kaiso in tumorigenesis, Kaiso-deficient mice were crossed onto the $Apc^{Min/+}$ background. Deficiency of *Kaiso* expression strongly suppressed tumorigenesis in this model, indicating that the net effect of gene loss is to antagonize tumorigenesis in this tissue. The precise mechanism underlying this effect remains unclear; however, these data do identify Kaiso as another promising potential therapeutic target.

Two other proteins containing Kaiso-like zinc fingers, ZBTB4 and ZBTB38, have recently

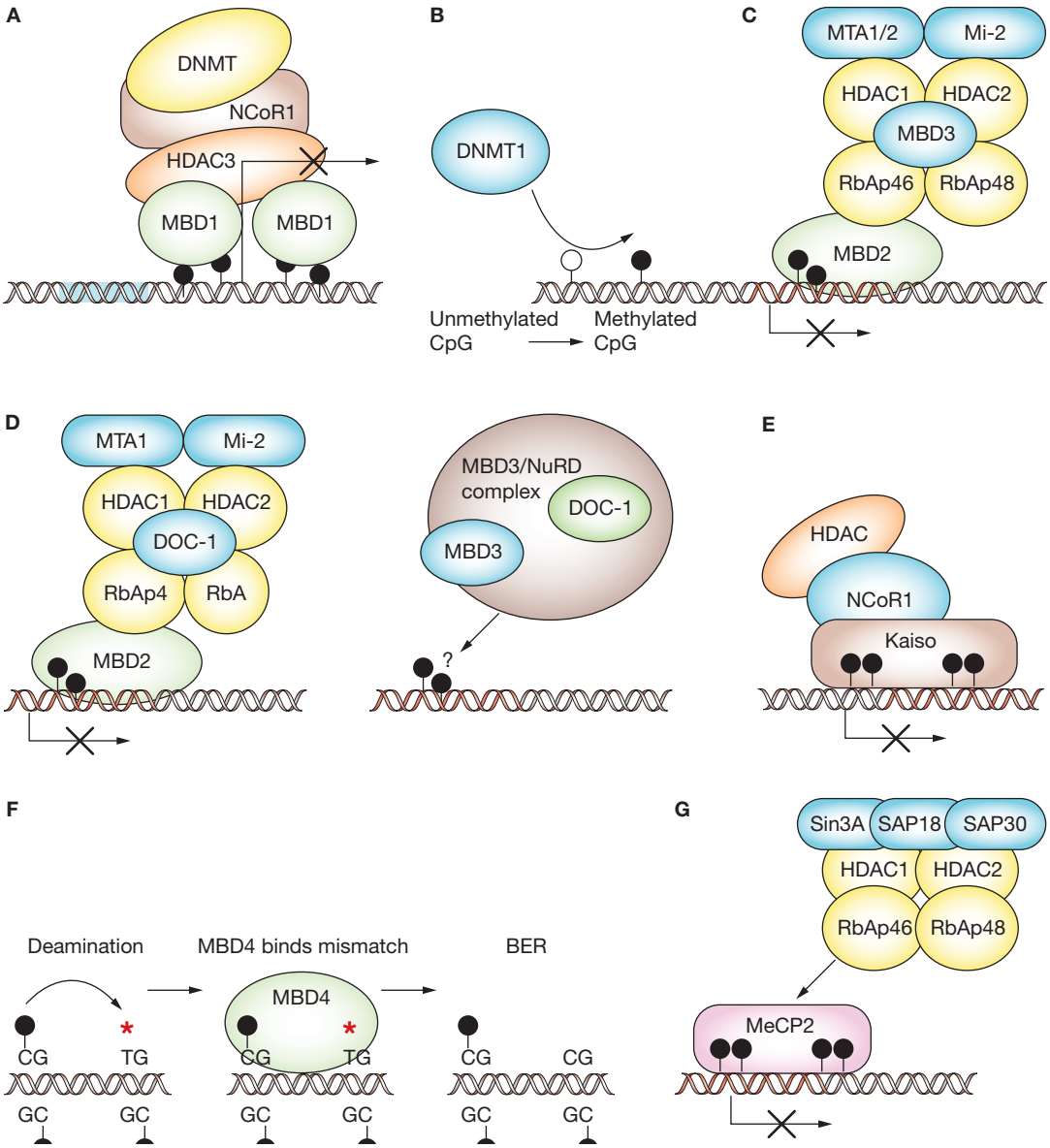

Figure 2 Simplified diagram showing the modes of action of DNMT1, MBD2, MBD3, Kaiso, MBD4 and MeCP2. (**A**) MBD1 binds to methylated CpGs and mediates repression through interactions with NCoR1, HDAC3 and the DNMT proteins. (**B**) DNMT1 is responsible for the methylation of cytosines that are 5' to guanine residues, creating the pattern of methylation in the mammalian genome. (**C**) MBD2 is a component of the Mi-2/NuRD complex, which binds to methylated CpGs and mediates transcriptional repression. MBD3 cannot directly bind methylated CpGs, but is a component of the recruited Mi-2/NuRD complex. (**D**) Recent work by Le Guezennec *et al*.[91] has shown that MBD2 and MBD3 do not coexist in the same complex, but rather inhabit mutually exclusive Mi-2/NuRD like complexes. DOC-1, a putative tumor suppressor, was also found to be a core subunit of both complexes. Since MBD3 does not bind methylated DNA, it is still unclear how the MBD3/NuRD complex is targeted to methylated DNA. (**E**) Kaiso does not contain a homologous MBD, but binds methylated CpGs through a zinc finger motif. Kaiso mediates both sequence-specific transcriptional repression, as well as methylation-dependent suppression. (**F**) The primary function of MBD4 is to repair mismatches that arise following spontaneous deamination events. Recent evidence suggests MBD4 also functions as a transcriptional repressor. (**G**) MeCP2 seems to bind the Sin3A repressor complex, which acts to remodel chromatin into a state refractory to transcription via the recruitment of HDACs. Abbreviations: BER, base-excision repair; DNMT, DNA methyltransferase; DOC-1, deleted in oral cancer 1; HDAC, histone deacetylase; MDB, methyl-binding domain; MTA, metastasis associated; NCoR1, nuclear receptor corepressor 1; NuRD, nucleosome remodeling and deacetylase; RbAp, retinoblastoma-binding protein.

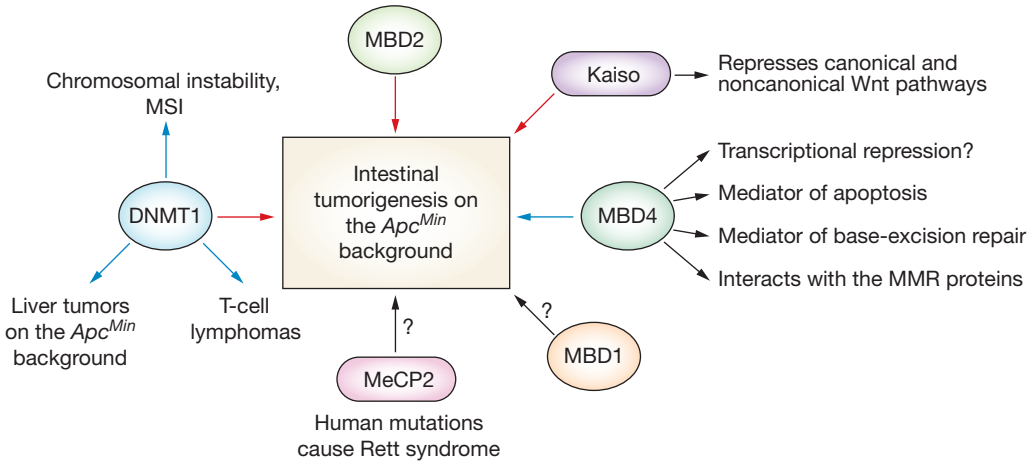

Figure 3 The MBD proteins and neoplasia. Red arrows indicate that a given protein has a tumor augmenting effect, and blue arrows indicate that a given protein has a protective effect against tumor formation. *DNMT1* null mice exhibit embryonic lethality at mid-gestation so hypomorphic alleles have been used to explore the consequences of lowered methylation levels. Reduced methylation leads to chromosomal instability and the induction of tumors and reduces intestinal tumorigenesis on an Apc^{Min} intestinal tumor model background, although it also increases liver tumor formation in this model. MBD2 has been shown to be involved in transcription repression of tumor suppressor genes such as $p16^{INK4A}$/p14ARF and deficiency of MBD2 has also been shown to greatly reduce the incidence of tumors in the Apc^{Min} model of intestinal tumorigenesis. Kaiso has also been shown to augment tumorigenesis, as deficiency of Kaiso represses tumor formation in the Apc^{Min} background. MBD4 is a multifunctional protein, with roles in genome surveillance, apoptosis and tumorigenesis, as well as links to the mismatch repair machinery. The primary consequence of loss of function seems to be tumor promotion due to loss of DNA repair activity. As yet, there are no clearly established tumorigenesis roles for either MeCP2 or MBD1; however, their roles in the interpretation of the methylation pattern of the genome indicate that a role in tumorigenesis seems likely. Abbreviations: DNMT, DNA methyltransferase enzyme; MBD, methyl-binding domain; MeCP2, methyl-CpG-binding protein 2; MMR, mismatch repair, MSI, microsatellite instability.

been characterized.[68] Both proteins have been found to bind methylated DNA *in vitro* and *in vivo* and to repress methylated template DNA. Unlike Kaiso, they are capable of binding single methylated CpGs. ZBTB4 and ZBTB38 are both highly expressed in mouse brain tissue, although their distinct expression patterns seem to suggest nonredundancy. Perhaps other methyl-binding motifs will be discovered, adding to the complexity of the methylation signal.

METHYL-CPG-BINDING PROTEIN 2

At present, very little evidence links MECP2 to cancer, although mutation of *MECP2* causes the human disorder Rett syndrome. This is a complex neurodevelopmental syndrome that affects an estimated 1 in 10,000–22,000 live births. Rett syndrome mainly affects females, and although rare male cases are reported, boys with Rett syndrome tend to be born with severe encephalopathy and die within 1–2 years of age.[69,70] As the syndrome causes premature death, no association between lower levels of MECP2 and tumorigenesis has been closely investigated.

MeCP2 contains an 85 amino acid MBD similar in structure to those found in other MBD proteins, a 104 amino acid TRD, a nuclear localization signal within the TRD, and a C-terminal portion that mediates binding to the nucleosome core. MeCP2 is localized to the CpG-rich heterochromatic pericentromic regions of mouse chromosomes. Studies by Nan *et al.* using rat tissues have shown that MeCP2 can repress transcription *in vitro* from methylated promoters, but not from nonmethylated promoters.[71] Nan *et al.* also found that MeCP2 seems to bind the Sin3A repressor complex, which acts to remodel chromatin into a state refractory to transcription via the recruitment of HDACs.[72] These data and the fact that MeCP2 binds to a single symmetrically methylated CpG pair (which are widespread in the genome) implies that MeCP2 is a global transcriptional repressor *in vivo*. Despite this, as

yet, very few transcriptional changes have been reported in the absence of MeCP2 *in vivo*, and those that have been identified are associated with relief of sequence-specific repression by MeCP2. Indeed, recent data have suggested rather pronounced sequence specificity for MeCP2.[73]

Recently, a number of studies have postulated a link between MeCP2 and cancer. Bernard and coauthors have shown that the androgen-free growth of human prostate cancer cells is MeCP2-dependent, a phenomenon that seems to rely upon retaining *MYC* expression.[74] Similarly, MeCP2 levels have also been found to correlate with estrogen-receptor status in breast cancer specimens.[75] In addition, it has been reported that MeCP2 mediates the transcriptional silencing of the DNA repair enzyme O-6-methylguanine-DNA methyltransferase.[76] These reports raise the possibility that modulation of MeCP2 levels might be of therapeutic relevance. At the present time there is insufficient experimental data to conclude that MeCP2 could be a viable therapeutic target.

METHYL-BINDING DOMAIN PROTEIN 4

Unlike the other MBD proteins, there is little evidence that the ability to repress transcription is important for MBD4 function. Instead MBD4 primarily acts as a methylation-specific DNA repair protein, interacting with the mismatch repair (MMR) family of DNA repair proteins. MBD4 has also been found to be mutated in nearly all MMR-deficient colorectal cancers and there has been much debate over the significance of MBD4 mutation and whether it can act as a bona fide tumor suppressor.

MBD4 has two functional domains: the MBD, which directs binding to hemi-methylated or fully methylated DNA; and a C-terminal domain that acts as a thymine DNA glycosylase to remove thymine or uracil from mismatched CpG sites *in vitro*.[31] The CpG dinucleotide is hypermutable, with G–T and G–U mismatches being formed by the hydrolytic deamination of 5-methyl-cytosine to produce thymine, and deamination of cytosine to produce uracil. These deamination events are frequent, occurring at a rate of 2–300/cell/day; if left unrepaired they would form G>C and A>T transition mutations during the next round of DNA replication.[33,77] By preventing these mutations, MBD4 may act as a caretaker of genomic fidelity at hypermutable CpG sites. Such mutations are known to contribute to tumorigenesis; nearly 50% of somatic *P53* mutations in colorectal cancers arise at 'hotspots' where cytosines in CpGs are deaminated to form transition mutations.[78]

MBD4-deficient mice have been generated and shown to be viable, with no overt phenotype.[79] As predicted, the frequency of mutation of 5-methyl-cytosine in a reporter transgene was significantly increased in *Mbd4*$^{-/-}$ mice.[79,80] Although *Mbd4*$^{-/-}$ mice did not show increased spontaneous tumorigenesis, deficiency did accelerate intestinal tumorigenesis in the *Apc*$^{Min/+}$ background.[79] Significantly, the tumors that formed in these mice showed an increase in the number of mutations at CpG sites in the *APC* allele. These data indicate that MBD4 functions as a tumor suppressor through its DNA repair activity. MBD4 has also been shown to directly interact with the MLH1 mismatch repair proteins. This originally led to the proposal that MBD4 may act as a homolog of *E. coli* mutH homolog functioning in MMR. The lack of an established endonuclease activity of MBD4 and the fact that *Mbd4*$^{-/-}$ mice showed increased mutation frequencies only at methylated CpGs, however, indicates that MBD4 does not have a significant role in canonical MMR.

It is possible that MBD4 may mediate other MMR-dependent functions, in particular signaling in response to DNA damage. Originally such a role for the MMR proteins was considered restricted to agents that mimic mismatches *in vivo*, such as O-6-methylguanine and 6-thioguanine. MMR has now been shown to be important in the response to numerous different DNA damage stimuli including cisplatin, 5-fluorouracil, ionizing radiation and UV irradiation.[81–83] Mice that are null for *MBD4* show a similar damage dependency, with significantly reduced apoptotic responses to a range of DNA-damaging agents such as 5-fluorouracil, temozolamide, γ-irradiation, and cisplatin. This observation indicates that the interaction between MBD4 and MMR may be important for the DNA damage signaling response.[84–87] Supporting this hypothesis, mice deficient for both *MBD4* and *MLH1* show no synergistic decrease in apoptosis when exposed to 5-fluorouracil or temozolamide, implying that *MBD4* and *MLH1* are involved in the same pathway and that MMR-dependent apoptosis might be mediated through MBD4. The MMR/MBD4 interaction is not the only route by which MBD4 modulates the apoptotic response, as MBD4 has also been shown to have a functional interaction with the death domain protein FADD.[88]

Even if MBD4 and the MMR proteins function in the same damage responsive pathway, this does not explain why MBD4 is frequently mutated in colorectal cancers lacking functional MMR. It seems most likely that the *MBD4* mutations found in cancer may simply reflect MMR status and the consequent increase in microsatellite instability. Indeed, Bellacosa *et al.* showed that the *MBD4* gene contains four simple repetitive sequences that would potentially generate instability.[77] Two separate studies have reported *MBD4* mutations in approximately 20–25% of human colorectal and gastric carcinomas.[77,89] Virtually all of these *MBD4* mutations were in a poly(A)$_{(10)}$ tract in the central portion of the gene, resulting in frame shifts that produced a truncated protein with no C-terminal domain and no catalytic ability. To test whether additional loss of *MBD4* accelerated tumorigenesis on a MMR background, we also intercrossed *MBD4* mice to mice mutant for either *MLH1* or *MSH2*.[87] On both backgrounds, additional *MBD4* deficiency did not alter tumorigenesis or mutation frequency at an endogenous locus, *DLB1* (also known as *DLEU1*). The most likely interpretation of these data is that the *MBD4* mutations seen in MMR-deficient tumors simply reflect the poly(A) tract repeat in human *MBD4*. It remains possible that mutations in *MBD4* confer a dominant, gain-of-function phenotype. It is clear that MBD4 has a role in suppressing deamination events *in vivo* and in mediating the DNA damage response *in vivo*; however, there is still some debate regarding the significance of *MBD4* mutations in cancer. Until MBD4 is found to be mutated in cancers other than those characterized by MMR deficiency, its true significance as a tumor suppressor will remain unclear.

CONCLUSION

Although only discovered relatively recently, the MBD family of proteins has a significant role in controlling gene expression *in vivo*. They have also been linked to multiple and diverse functions in tumorigenesis; several MBD proteins seem to augment tumorigenesis, whilst MBD4 seems to function primarily as a DNA repair protein. Of all the MBD proteins, MBD2 seems to be the most relevant as a potential therapeutic target. The fact that the *MBD2* knockout is viable and strongly resistant to tumorigenesis, and that the mechanism of this resistance is at least partially understood, makes *MBD2* an ideal target for therapeutic intervention.

KEY POINTS

- Modulation of DNA methylation patterns and the proteins that interpret those patterns modifies the neoplastic process
- Reduction in levels of DNMT1 reduces tumorigenesis in some tissues, but exacerbates it in other tissues
- MBD2 deficiency has been shown to suppress intestinal neoplasia in animal models, with apparently little protumorigenic role in other tissues, so identifying it as a promising new therapeutic target
- Kaiso deficiency has been shown to suppress intestinal neoplasia, identifying it as a promising therapeutic target
- MBD4 deficiency has multiple effects upon tumorigenesis, but its primary consequence is an increase in mutation rates through failed DNA repair of spontaneous deamination events
- Although implicated in the neoplastic process, the precise roles of MBD1, MBD3 and MeCP2 remain to be established

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Competing interests
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