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Cancer epigenetics

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The field of cancer epigenetics is evolving rapidly on several fronts. Advances in our understanding of chromatin structure, histone modification, transcriptional activity and DNA methylation have resulted in an increasingly integrated view of epigenetics. In response to these insights, epigenetic therapy is expanding to include combinations of histone deacetylase inhibitors and DNA methyltransferase inhibitors. Zebularine, an orally administerable DNA methyltransferase inhibitor, has been a very promising recent addition to our arsenal of potentially useful drugs for epigenetic therapy. Aberrant DNA methylation patterns provide three powerful diagnostic applications as classification markers, sensitive detection markers, and risk assessment markers. Classification studies continue to increase in marker complexity, now incorporating microarrays, high-throughput bisulfite genomic sequencing and mass spectrometry, as the field moves to human epigenome projects. Sensitive detection technology has expanded from primarily blood-based cancer detection to include applications on a wide diversity of sample sources and is now also making inroads as a molecular risk assessment tool.

INTEGRATED EPIGENETICS

Epigenetics refers to alternate phenotypic states that are not based in differences in genotype, and are potentially reversible, but are generally stably maintained during cell division. The narrow interpretation of this concept is that of stable differential states of gene expression. A much more expanded view of epigenetics has recently emerged in which multiple mechanisms interact to collectively establish alternate states of chromatin structure, histone modification, associated protein composition, transcriptional activity, and in mammals, cytosine-5 DNA methylation at CpG dinucleotides (1–4). It has long been known that cancer cells undergo changes in 5-methylcytosine distribution including global DNA hypomethylation (5) and the hypermethylation of promoter CpG islands associated with tumor-suppressor genes (6–9). It is now realized that CpG island hypermethylation is just one facet of an integrated change in chromatin structure and in histone modifications (10,11), including histone H3 and H4 deacetylation (12), histone H3 lysine 9 methylation (13), histone H4 sumoylation (14) and reduced histone H3 lysine 4 methylation (15,16), among others, collectively resulting in a transcriptionally silenced state (Fig. 1).

DNA methylation is a useful marker for assessing the epigenetic state of a locus, because it is preserved in purified isolated DNA, and can be measured by PCR-based techniques, but it should be realized that this is a one-dimensional measurement of a multidimensional state. Indeed, investigators have begun to incorporate an integrated analysis of epigenetic states in cancer cells (17,18) such as combinations of DNA methylation and transcription factor binding (19), gene expression and DNA methylation (20), DNA methylation and histone modifications (19,21), combinations of gene expression and DNA methylation with the use of epigenetic inhibitors (22–24) and also triple combinations of gene expression, DNA methylation and histone acetylation (25). In genetic studies, it has been found that an integrated analysis of genome-wide linkage analysis and gene expression can help to identify genetic determinants of quantitative traits (26). It is anticipated that the inclusion of genome-wide epigenetic analysis in similar studies will help to identify novel genetic and quantitative determinants of epigenetic states.

CAUSE AND EFFECT

Given the multidimensional character of epigenetic phenomena, the question arises, as to which mechanism is the driving force in systems undergoing epigenetic change. The cyclical nature of mutually reinforcing interactions makes

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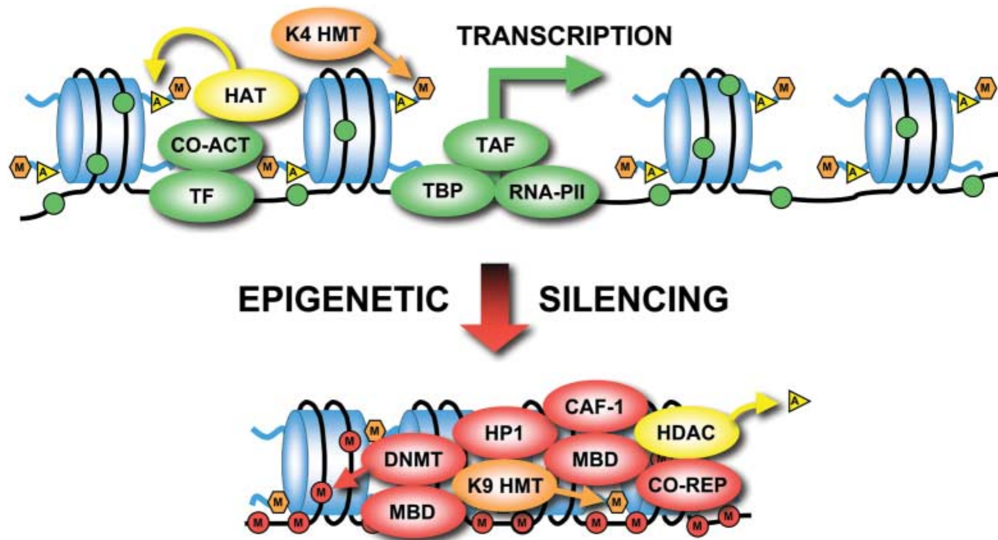

Figure 1. Epigenetic Silencing. Schematic of some of the molecular events that occur at CpG-rich promoters undergoing epigenetic silencing in cancer cells. The open chromatin structure of a transcriptionally active gene with loosely spaced nucleosomes (blue cylinders) is shown at the top and the transcriptionally silenced state with more tightly packed nucleosomes is shown at the bottom. DNA is indicated by a thin black line wrapped around nucleosomes. Proteins are indicated by shaded ovals, histone H3 acetylation is indicated by yellow triangles, hstone H3 methylation is indicated by orange hexagons and CpG dinucleotides are indicated by circles strung along the DNA, with green circles denoting an unmethylated state and red circles indicating a methylated state. Proteins involved in transcriptional activation are indicated in green (TF, tanscription factor; CO-ACT, co-activator; TBP, tata-binding factor; TAF, TBP-associated factor; RNA-PII, RNA polymerase II). Histone acetyl transferases (HAT) and histone deacetylases (HDAC) are indicated in yellow. Histone H3 lysine-4 (K4 HMT) and lysine-9 (K9-HMT) are indicated in orange. Proteins involved in transcriptional silencing are indicated in red (DNMT, DNA methyltransferase; MBD, methyl-binding domain protein; CO-REP, co-repressor; HP1, heterochromatin protein 1; CAF-1, chromatin assembly factor-1).

this question challenging to address experimentally and renders its significance debatable. The concepts of cause and effect have clear meaning in linearly causal relationships, where necessary and sufficient components can be defined by adding and subtracting these elements from the system and where the temporal order of events may be clear. In a cyclically reinforcing system, the removal of any one component may affect all other elements, not just downstream components, and temporal relationships become less clear as all elements change in concert. Indeed, numerous reports documenting the antecedence or predominance of each of the main epigenetic mechanisms have been published. For example, histone deacetylation and histone H3 lysine 4 methylation precede DNA methylation and histone H3 lysine 9 methylation in some model systems (27), whereas Sp1 transcription factor binding appears to be an important block to epigenetic silencing in other systems (28). Nevertheless, DNA methylation can itself interfere with Sp1 binding (29) and can even attenuate transcription elongation (30). Consistent with this more pronounced role for DNA methylation, removal of transcription by promoter deletion appeared to have little effect on local DNA methylation patterns in some systems (31), whereas in others, removal of Sp1 binding sites was shown to be necessary, but not sufficient to achieve transcriptional silencing and CpG island hypermethylation (32). In this latter system, ‘seeding’ by sporadic CpG methylation was found to be an additional requirement for subsequent histone deacetylation, further spread of DNA methylation, MBD2 binding and histone H4 lysine 9 methylation (33). Other systems have also been reported to require a critical density of seeded DNA methylation for subsequent spreading and chromatin closure (34). In further support of the central role for DNA methylation in mammalian epigenetic control, maintenance of the developmentally regulated expression pattern of the human *SERPINB5* gene has been shown to require DNA methylation (35), and aberrantly silenced genes in cancer cells can be transcriptionally activated by DNA methyltransferase inhibitors but not by histone deacetylase inhibitors alone (36–39). Moreover, in such inhibitor experiments, DNA demethylation occurs first, followed by transcriptional reactivation and subsequently histone code reversal (39). However, prolonged culture in the absence of inhibitor can lead to resilencing of gene expression and histone H3 lysine 9 methylation prior to the reacquisition of promoter DNA methylation (40). This distinction between the initial perturbation of an epigenetic state, and the reversal of that perturbation is nicely exemplified by the disruption of estrogen receptor signaling in human breast cancer cells, which results in stable repression of the progesterone receptor gene (41), accompanied by recruitment of polycomb repressors and histone deacetylases, and by promoter DNA methylation. However, merely reestablishing estrogen signaling was found to be insufficient to reactivate expression of the *PR* gene (41), indicating that, although loss of transcriptional activity triggered the epigenetic change, reversal of that event was no longer able to switch back the epigenetic state, once it had been established.

There are a few take-home lessons from these and other similar studies. First, perturbation of an existing epigenetic state may serve to show that a particular mechanism is required for maintenance of the epigenetic state, but does not necessarily imply that, under normal circumstances,

Table 1. Epigenetic inhibitors

Inhibitor	Alternate name	Comments	References
DNA methyltransferase inhibitors			
5-Azacytidine	Vidaza	FDA approved for MDS	(189,190)
5-Aza-2′-deoxycytidine	Decitabine, dacogen	Phase I/II	(55,191–193)
Arabinosyl-5-azacytidine	Fazarabine	Phase I/II	(194,195)
5-6-Dihydro-5-azacytidine	DHAC	Phase I/II	(195,196)
5-Fluoro-2′-deoxycytidine	Gemcitabine	Phase I/II	(197)
EGX30P	Oligonucleotide	Allosteric inhibitor	(198)
Epigallocatechin-3-gallate	EGCG	Green tea polyphenol	(199)
Hydralazine		Cardiovascular drug	(200)
MG98	DNMT1 antisense	Phase II	(201)
Procainamide		Cardiovascular drug	(202)
Procaine		Anesthetic	(203)
RNAi			(204–206)
Zebularine			(56,57)
Histone deacetylase inhibitors			
Apicidin			(207)
Butyrates		Phenylbutyrate, phase II	(208–210)
m-Carboxycinnamic acid bishydroxamide (CBHA)	CBHA		(211,212)
Cyclic hydroxamic-acid-containing peptide 1	CHAP1	TSA-Trapoxin Hybrid	(213)
Depudecin	Epoxide		(214)
FK228	Depsipeptide, FR901228	Phase I/II	(215)
MS-275	Benzamidine	Phase I	(216)
LAQ824		Phase I	(217)
Oxamflatin			(218)
MGCD0103		Phase I	(219)
PXD101		Phase I	(220)
Pyroxamide		Phase I	(221)
RNAi			(222)
Suberic Bishydroxamic Acid	SBHA		(223)
Suberoylanilide Hydroxamic Acid	SAHA	Phase I/II	(224)
Trichostatin A	TSA	High toxicity	(225)
Trapoxin A		Irreversible inhibitor	(226)
Valproic acid			(227)

this mechanism is the initiating event. Secondly, one has to be very careful about making sweeping generalizations based on observations with one particular system. However, there does appear to be a tendency towards change being initiated by transcriptional regulation or sequence-specific protein binding, associated with alterations in the histone code. In the case of epigenetic silencing, DNA methylation often appears late. However, once the silenced state has been achieved, DNA methylation appears to be a very powerful signal blocking reactivation of gene expression, regardless of perturbations to other parts of the system.

EPIGENETIC THERAPY

The potential reversibility of epigenetic states offers exciting opportunities for novel cancer drugs that can reactivate epigenetically silenced tumor-suppressor genes (12,42–44). Blocking either DNA methyltransferase or histone deacetylase activity could potentially inhibit or reverse the process of epigenetic silencing (Fig. 1). DNA methyltransferases and histone deacetylases are the two major drug targets for epigenetic inhibition to date, although others are expected to be added in the near future (Table 1). Most of the drugs with promising clinical prospects or those used widely as research tools are included in Table 1. Among those not listed are analogs of the methyl group donor *S*-adenosylmethionine, which inhibit a broad spectrum of cellular methyltransferases and have been shown to be potentially mutagenic (45). Although most reports on the inhibitors listed in Table 1 assume a high drug target specificity, many of these drugs in fact have pleiotropic effects. The most broadly used DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (5-aza-CdR), clinically referred to as decitabine, has been shown to be have toxic effects aside from its demethylating properties (46) and has been found to be mutagenic *in vivo* (47). Moreover, 5-aza-CdR has been shown to be capable of transcriptionally activating genes with unmethylated promoters (48), also leading to increased acetylation and H3 lysine 4 methylation (37), suggesting this drug can induce chromatin remodeling independently of its effects on cytosine methylation. Gene expression microarray experiments confirmed that many genes activated by 5-aza-CdR lack promoter methylation, and for reasons that are not entirely clear, appear to be enriched for genes involved in interferon signaling (22–24,49–51). Similar studies have now also been performed using zebularine (52).

The network of multiple reinforcing interactions involved in epigenetic silencing suggests that combination therapy would be a particularly appropriate strategy to achieve clinical

efficacy (42–44). Indeed, combinations of DNA methyltransferase and histone deacetylase inhibitors appear to synergize effectively in the reactivation of epigenetically silenced genes (25,36,38,53,54). Combination trials are underway to test this concept in the clinic.

Several other exciting developments in epigenetic therapy have emerged recently. First, insight into the mode of action, the mechanism of toxicity and the pharmacokinetics of decitabine inspired an improved protocol with prolonged administration at lower doses, with equal, if not better efficacy than previous regimens (55). Toxicity has been one of the major problems with this drug and the efficacy of such low-dose protocols is an important advance. Even more exciting is the recent addition of zebularine to the arsenal of DNA methyltransferase inhibitors (56,57). In contrast to decitabine, zebularine is stable in aqueous solution and can be administered orally, greatly simplifying continuous low-dose therapy (56,58). Zebularine appears to be more effectively incorporated by cancer cells than by fibroblasts (57). Such a preferential response of cancer cells to epigenetic therapy is shared by decitabine (51) and histone deacetylase inhibitors (59), also in non-proliferating cancer cells, suggesting that epigenetic therapy could be an effective strategy for treating tumors with a low mitotic index (59).

EPIGENETIC DIAGNOSTICS

Epigenetic changes in cancer cells not only provide novel targets for drug therapy but also offer unique prospects for cancer diagnostics (60). The three main approaches to assess the epigenetic state of individual gene loci are to (1) measure gene expression, (2) determine histone modifications and chromatin protein composition and (3) analyze promoter DNA methylation status. Chromatin immunoprecipitation has been an extremely useful research tool to analyze chromatin protein composition and modifications. However, it has not advanced sufficiently yet to become a clinically useful diagnostic method, in contrast to serum proteomics by mass spectrometry, which is progressing rapidly in clinical feasibility studies (61). Gene expression microarray analysis has proved to be a powerful method for identifying novel subclasses of cancer and predicting clinical outcome or response to therapy (62,63). However, gene expression analysis is generally not viewed as epigenetic analysis, in part because mechanistic understanding of gene regulation evolved from studies of transcriptional control by transcription factors, which does not necessarily involve mitotically stable epigenetic change, although the fields of gene regulation and epigenetics are moving closer. The major interest in cancer epigenetics as a diagnostic tool is in localized epigenetic silencing. The use of gene expression microarray studies to identify non-transcribed genes as candidates for promoter CpG island hypermethylation has had limited success, because lack of gene expression can stem from other causes aside from epigenetic silencing (22–24,49–51). For the most part, cancer epigenetics has relied on measurements of CpG island DNA hypermethylation (7,60).

DNA methylation markers are used in cancer diagnostics for both disease classification and disease detection. As a classification tool, CpG island hypermethylation is generally analyzed on sufficient quantities of primary tissue such as a surgically resected tumor sample. The DNA methylation status of individual gene promoters can be used for general prognosis or to predict response to a particular therapy. There have been numerous reports describing an association between hypermethylation of individual genes and overall clinical outcome (prognosis) for various types of cancer (64–70). Individual methylation markers have also been linked to breast cancer metastasis (71). In particular, methylation of the E-cadherin (*CDH1*) promoter appears to be required for invasion and metastasis (72–75). It is more difficult to make a convincing case that a DNA methylation marker is a predictor of response to a specific therapy, and not just a general prognostic marker of clinical outcome, independent of therapy. One of the best cases has been made for hypermethylation of the O^6-methylguanine methyltransferase (*MGMT*) promoter, which is associated with increased survival in glioma patients treated with alkylating agents (76–78). Melanoma cells with acquired resistance to the antineoplastic alkylating compound fotemustine, by repeated *in vitro* drug exposure, were shown to have reactivated the *MGMT* gene (79).

Increasingly, the profiling of a broader set of DNA methylation markers is used for prognosis and prediction, often facilitated by hierarchical cluster analysis (80). A screen of 10 genes in 145 neuroblastoma samples was able to delineate three main clinical risk groups (81), whereas a panel of 35 methylation markers revealed an association of DNA methylation profiles with hormone receptor status and response to tamoxifen treatment in 148 breast cancer patients (82). Unsupervised clustering of 956 unselected CpG islands in 19 late-stage ovarian tumors allowed discrimination between two major subgroups differing in progression-free survival rates (83). The increasing use of DNA methylation microarrays, high-throughput bisulfite genomic sequencing, mass spectrometry and other genome-wide techniques such as restriction landmark genome scanning is rapidly genomicizing epigenetics (25,84–87). A human epigenome project is now underway in Europe (88) (Murrell, this issue), and plans are being hatched for a similar effort in the USA. These molecular efforts are complemented by advances in epigenomic bioinformatics (80,89,90).

DNA methylation markers hold perhaps even greater promise as detectors of disease, as opposed to classifiers of existing disease (60). Disease detection is useful not only for the early detection of undiagnosed malignancies but also for the monitoring of recurrent disease as a measure of therapeutic efficacy (60). The demands placed on sensitive detection are quite different than those needed for the classification technology. Sensitive detection requires a high signal-to-noise ratio for the detection of aberrant DNA methylation patterns against a background of normal DNA methylation patterns. In principle, this can consist of the measurement of a single locus, although multiple loci may be needed to achieve sufficient sensitivity and specificity. Sensitive detection technologies tend to rely on sodium bisulfite-based methylation-specific PCR (MSP) to achieve sufficiently high signal-to-noise ratios (91). Molecular classification, on the other hand, requires that the methylation information is sufficiently

complex that subclasses of DNA methylation patterns can be defined. Hence, the interest in microarray methods and other genome-wide techniques. However, these high-throughput methods are usually based on either methylation-sensitive restriction enzyme digestion or methylation-independent bisulfite PCR, as opposed to MSP, and have modest signal-to-noise ratios. Therefore, classification technologies usually require fairly homogeneous samples, such as primary tumor tissue, and are generally unsuited for sensitive detection purposes. Automated variants of MSP, such as the real-time PCR-based MethyLight technique (92), have both a sufficient signal-to-noise ratio and the throughput capacity to sensitively analyze a broad set of markers (82,93,94).

The use of DNA methylation markers to detect cancer sensitively is based on the premise that tumor-derived DNA is released into bodily fluids or other remote samples and can be detected by the abnormal DNA methylation patterns specific for malignant cells. Most studies have used serum or plasma as the source of cell-free DNA (60). However, in recent years, a number of other novel sources of DNA have been used including nipple aspirate fluid (95), breast fine needle washings (96), bronchial brush samples (97), needle biopsies (98), prostatic fluid or ejaculate (99,100), lymph nodes (101,102), bronchioalveolar lavage (103), pancreatic juice (104), sputum (105), mouth and throat rinsing fluid (106), exfoliated bladder cells (107), urine or urine sediments (108–111), peritoneal fluid (112,113), stool (93) and vaginal tampons (114,115).

As more types of samples are analyzed for a variety of different loci, it is becoming clear that epigenetic changes, including silencing of tumor-suppressor genes, may occur early in malignant progression and can sometimes be detected even in non-malignant or precancerous tissues. For example, promoter methylation of the *p16^{INK4a}* (*CDKN2A*) gene is detectable in preinvasive bronchial lesions (116), in histologically normal human mammary epithelia (117) and in non-adenomatous pituitaries from patients with Cushing's disease (118). Promoter methylation of multiple genes has been reported in non-malignant gastric tissues (119–123), non-neoplastic prostate tissue (124,125), chronic cholecystitis (126) and ulcerative colitis (127). Some of these changes have been suggested to be age-related (121,127), whereas others have been proposed to be premalignant (123,128), in some cases associated with environmental risk exposures (129) and/or diet (130). The detection of epigenetic abnormalities in histologically normal or premalignant tissues at risk for progression to malignancy paves the way for the use of DNA methylation markers in risk assessment. Epigenetic markers should be particularly well suited as risk assessment tools, compared to germline genetic markers, because somatic epigenetic alterations presumably capture lifetime environmental and dietary exposures. Thus, a single class of markers can be used for assessing risk stemming from genotype and environmental exposure, for early detection of malignancy, and for classifying existing disease. In the next few years, the task will be to identify the markers and technologies best suited for each application. Moreover, if epigenetic changes occur in premalignant tissues, then this opens new avenues for cancer chemoprevention based on the inhibition or reversal of epigenetic alterations before the onset of malignancy (131).

EPIGENETIC CONTROL DEFECTS

The large estimated number of epigenetic alterations found in cancer cells (84,132) raises the question whether these reflect stochastic occurrences that accumulate with age, and are selected for during malignant outgrowth, or whether the large number of changes is caused by a defect in one of the components of the epigenetic machinery. By analogy, both stochastic mutations and mutator phenotypes are involved in producing the genetic alterations found in cancer. Likewise, it is anticipated that both stochastic errors and defects in epigenetic control participate in cancer epigenetics. The identification of these defects will likely emerge in the next few years as we acquire a fuller understanding of the normal mechanisms of maintenance and alteration of epigenetic states. Chromatin protein composition and histone modifications vary throughout the genome, with segregation maintained in part by chromatin insulators and boundaries (133–138) (West and Fraser, this issue). Therefore, it is likely that many epigenetic control defects will target a subset of genetic loci, rather than all loci equally. This has been modeled experimentally by overexpression of the *DNMT1* DNA methyltransferase (139,140). Even this generic component of epigenetic control appeared to display locus specificity, leading to the identification of a set of putative DNA hypermethylation target consensus sequences with a striking purine/pyrimidine strand bias (141). Likewise, *DNMT*-deficient cells display distinct subsets of affected loci (24,142–145). Another nice example of the target specificity of an epigenetic control defect is the increased histone acetylation and transcriptional reactivation of repeat sequences and of the paternally imprinted allele of the *Cdkn1c* gene in cells deficient for the chromatin remodeling protein Lsh (146–148). Recent reports of transcriptional silencing and promoter DNA methylation induced by small interfering RNAs in human cells raise the interesting prospect of an involvement of microRNAs in epigenetic silencing in cancer cells (149,150) (Mattick, this issue).

One controversial putative epigenetic control defect is the CpG island methylator phenotype (CIMP) first reported in colorectal cancer (151), but since described for several other types of cancer as well (152). The essence of CIMP is the concerted hypermethylation of multiple CpG islands in a subset of cancer cases. The source of controversy stems from the fact that only a subset of CpG islands appears to be affected, primarily those that are cancer-specifically methylated. This is exactly the phenotype that one would predict for a cancer-specific epigenetic control defect, but an extensive study that included a broader analysis of CpG island hypermethylation failed to confirm the existence of CIMP (153), giving rise to heated debate concerning the existence of CIMP. Other groups have found associations between CIMP and various clinicopathological criteria in colorectal cancer including survival benefit from 5-FU treatment (154), genetic mutation profiles (155), location in the right-sided colon and microsatellite instability (156) and association with family history (157), although this could not be confirmed by another group (158).

Genetic predisposition and/or environmental exposures could lead to systemic epigenetic control defects that either

increase the risk of developing cancer or at the very least represent surrogate markers for an increased risk. One such systemic epigenetic control defect appears to be loss of imprinting of the *IGF2* locus, which occurs in the colorectal tumors, and in the normal colonic mucosa and white blood cells of individuals with colorectal cancer, or with a family history of colorectal cancer (159,160). If epigenetic control defects can occur systemically, then perhaps they can also contribute to cancer risk through transgenerational inheritance. Promoter methylation of the mismatch repair gene *MLH1* is responsible for the majority of colorectal adenocarcinomas with microsatellite instability (161–164). This promoter methylation has been found to occur in normal colonic epithelium (165). Recently, strong evidence has accrued that *MLH1* methylation can be transmitted through the germline, although true transgenerational inheritance in humans has not yet been formally demonstrated (166–168). Such transgenerational epigenetic inheritance has been well documented to occur in mice (169,170) (Ruden, this issue). Recently, paramutation, in which one allele can affect the epigenetic state of the other allele, has also been reported to occur in mice (171). It will be interesting to see if the mechanism responsible for this epigenetic cross-talk between alleles contributes to the biallelic CpG island hypermethylation frequently seen in cancer.

Mouse models of epigenetic control defects have been particularly useful in demonstrating the important contribution of epigenetics to tumorigenesis. A combination of *Dnmt1* hypomorphic alleles and drug treatment was shown to severely inhibit tumor formation in $Apc^{Min/+}$ mice which are predisposed to the development of intestinal adenomas (172). Subsequent work showed that both the size and growth rates of polyps were affected (173) and that a complete suppression of the tumor phenotype could be achieved without drug treatment by using more severe hypomorphic *Dnmt1* alleles (174). In other words, intestinal polyp formation in this system is as much dependent on sufficient levels of functional Dnmt1 expression, as it is on the *Apc* mutation. These observations are consistent with a model in which polyp formation requires DNA methylation-dependent epigenetic silencing of unidentified tumor-suppressor genes, in addition to loss of heterozygosity of the wild-type *Apc* allele. Methyl-binding domain containing proteins (MBDs) bind to areas of dense DNA methylation and recruit histone deacetylases and transcriptional repressor complexes (Fig. 1). As such, MBDs are considered important mediators of epigenetic gene silencing, at the interface between DNA methylation and histone code modification. If the requirement of sufficient Dnmt1 expression for intestinal polyp formation is mediated through epigenetic silencing, then one would anticipate that polyp formation would also depend on other mediators of epigenetic silencing such as MBDs. In an elegant demonstration of the logical consistency of this proposed mechanism, $Apc^{Min/+}$ mice deficient in *Mbd2* were found to have substantially reduced numbers of intestinal polyps (175).

One of the criticisms of these mice model experiments has been that $Apc^{Min/+}$ mice develop benign polyps, as opposed to malignant tumors (176). It has now been shown that malignant colorectal adenocarcinomas that arise in $Mlh1^{-/-}$ mice are also severely attenuated in *Dnmt1* hypomorphic mice (177). However, interestingly, lymphomagenesis is increased in this same model (177). Increased lymphomagenesis in *Dnmt1* hypomorphic mice was subsequently confirmed in mice without an *Mlh1* mutation (178). Enhanced tumorigenesis in *Dnmt1* hypomorphic mice was shown to be related to increased chromosomal instability (179). These findings are consistent with the previously reported increased mutation rates seen in Dnmt1-deficient mouse embryonic stem cells (180) and with the large body of literature describing associations between DNA hypomethylation, particularly of pericentromeric repeats and chromosomal instability in various human experimental and disease models (5,181–183). However, genomic instability can be attributed to multiple different mechanisms, not all of which may respond to DNA hypomethylation or DNMT1 deficiency in equal ways. Indeed, others have reported that Dnmt1 deficiency can result in a reduced mutation and deletion rate in embryonic stem cells (184), consistent with the important contribution of 5-methylcytosine to deamination-mediated mutagenesis in mammalian cells. The diverse roles that Dnmt1 and DNA methylation play in the maintenance of genomic integrity has been further emphasized by the recent discovery that Dnmt1 is required for efficient DNA mismatch repair and that rates of microsatellite instability increase under Dnmt1-deficient conditions (185–188).

It is clear from this bird's-eye overview that the field of cancer epigenetics is in flux. We can expect to see clinical implementation of both epigenetic cancer therapy and epigenetic cancer diagnostics in the next decade. Epigenetic control defects in cancer cells represent an emerging new area of investigation, where significant breakthroughs in the identification of the underlying molecular defects are anticipated in the next few years.

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