Commentary

Cancer Prevention Research

All Things in Moderation: Prevention of Intestinal Adenomas by DNA Hypomethylation

Kwang-Ho Lee and Peter W. Laird

Abstract

DNA hypomethylation can prevent intestinal tumorigenesis, presumably by reducing epigenetic silencing of tumor-suppressor genes. A study in this issue by Sheaffer and colleagues challenges this notion by showing that severe DNA hypomethylation by tissue-specific *Dnmt1* knockout can actually *promote* intestinal adenoma formation. *Cancer Prev Res; 9(7); 509–11. ©2016 AACR.*

See related article by Sheaffer, et al., p. 534

A study in this issue of *Cancer Prevention Research* by Sheaffer and colleagues reports that extreme DNA hypomethylation can promote intestinal tumorigenesis in a mouse model system, in apparent contradiction to prior reports of reduced adenoma formation by DNA hypomethylation. Using an elegant inducible intestine-specific knockout system, the authors found that a complete knockout of *Dnmt1* in the mouse intestinal epithelium led to a more than 6-fold increase in macroscopic adenoma formation in the $Apc^{Min/+}$ mice (1). This observation challenges the current notion that DNA methylation inhibition represses intestinal adenoma formation by suppressing promoter CpG island (CGI) hypermethylation and may have important implications for the prevention and treatment of human cancer (2–7).

Widespread hypomethylation and CGI hypermethylation are commonly observed in human cancers (8). Besides extensive, but descriptive evidence accumulated by human cancer epigenomic profiling studies, animal studies have provided more direct evidence for the causal contribution of DNA methylation to cancer. *In vivo* experimental evidence for a causal role of DNA methylation in tumorigenesis was provided in the $Apc^{Min/+}$ mouse model, genetically predisposed to develop intestinal polyps, mimicking human familial adenomatous polyposis (9). Pharmacologic and/or genetic reduction in the expression of the *Dnmt1* maintenance DNA methyltransferase in this system resulted in a substantial suppression of intestinal polyp formation (5). A follow-up study relying solely on *Dnmt1* hypomorphic alleles, $Dnmt1^{N/R}$ (~20% *Dnmt1* expression in comparison with wild-type mice) without the use of demethylating drugs showed a complete suppression of intestinal polyp formation in the $Apc^{Min/+}$ model, reinforcing the notion that DNA methylation plays an essential role in intestinal tumorigenesis (2). Various models have been put forward to explain this dependency, but most evidence suggests that tumor- and growth-suppressing gene silencing by promoter CGI hypermethylation is the predominant mechanism affected by reduced DNA methyltransferase. Most interpretations have

focused on DNA methylation itself; however, the DNA methyltransferase protein has transcriptional repressive capability independent from its methylating capacity (10). Nevertheless, the case for a role mediated by DNA methylation *per se* was strengthened by the observation that knockout of a DNA methylation reader protein, Mbd2, also caused a reduction in adenoma size and multiplicity in $Apc^{Min/+}$ mice (11).

Intestinal mouse tumor model systems relying on loss of mismatch repair function showed a similar reliance on sufficient DNA methylating capacity. $Mlh1^{-/-}$ mice with reduced *Dnmt1* expression displayed a substantial reduction of intestinal tumor formation, in line with the results from $Apc^{Min/+}$ mice. However, these same mice developed invasive T- and B-cell lymphomas earlier and at a much higher frequency than their *Dnmt1* wild-type littermates, suggesting that DNA hypomethylation could also promote oncogenesis (6). The T- and B-cell lymphoma promotion by DNA hypomethylation was also demonstrated using a more severe *Dnmt1* hypomorphic allele, $Dnmt1^{Chip/-}$ (10% *Dnmt1* expression; ref. 4). In this mouse model, the lymphomas were induced without any genetic predisposition, demonstrating oncogenic sufficiency of *Dnmt1* inhibition for lymphoma formation. A high frequency of chromosome 15 trisomy with accompanying *c-Myc* oncogene amplification was evident in the tumors, suggesting that increased chromosomal instability in these *Dnmt1* hypomorphic mice might underlie the tumor formation (4). The tumorigenicity of DNA hypomethylation was further demonstrated in the context of *NF1* and *p53* double heterozygosity (3). Eden and colleagues showed that DNA hypomethylation results in increased soft-tissue sarcoma by increasing LOH of the two tumor-suppressor genes (3). These studies not only demonstrated that DNA hypomethylation can contribute to tumorigenesis by increasing genomic instability but also provided an important insight that DNA methylation inhibition can have opposite outcomes depending on tumor cell type and genetic background.

A more detailed view of the role of DNA methylation in intestinal tumorigenesis emerged from a study utilizing the $Apc^{Min/+}$ model combined with the severe *Dnmt1* hypomorphic allele, $Dnmt1^{Chip/-}$ (7). As observed in previous studies, intestinal polyp formation was strongly suppressed in the model; however, colonic microadenoma formation associated with LOH of *Apc* was actually increased (7). These results suggested a more refined model for intestinal tumorigenesis in which DNA hypomethylation contributes to tumor initiation by increasing

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genomic instability but suppresses progression from microadenoma to macroadenoma by inhibiting tumor-suppressor gene silencing (7).

The study by Sheaffer and colleagues reports that a complete knockout of *Dnmt1* in the mouse intestinal epithelium promotes intestinal adenoma formation in *Apc^{Min/+}* mice (1). This result is in sharp contrast to the aforementioned studies in which reduced *Dnmt1* expression results in either complete or substantial suppression of intestinal adenoma formation (2, 5–7). To gain mechanistic insight into the causes of elevated tumor formation, the authors examined effects of *Dnmt1* knockout on DNA methylation, genomic stability, and intestinal cell growth. *Dnmt1* knockout induced substantial hypomethylation of Long Interspersed Nucleotide Element 1 (LINE1) at 1 week post-*Dnmt1* deletion in the intestinal epithelium, indicating widespread DNA hypomethylation as expected. In addition to this broad hypomethylation, gene-specific hypomethylation was found at a few genes in adenomas from the *Dnmt1*-mutant mice in comparison with tumors from control mice, including potential oncogenes, *Dusp6* and *c-Fos*. Hypomethylation at these potential oncogenes is intriguing, but the small number of genes analyzed and the lack of functional validation data do not allow us to conclude decisively that these alterations explain the observed elevated adenoma formation.

Interestingly, the LINE1 DNA methylation appeared to be fully restored to normal levels at 2 months post-deletion of *Dnmt1*, whereas the gene-specific hypomethylation persisted. In contrast, restoration of repetitive element DNA methylation was not observed in systemic *Dnmt1* hypomorphic mice (2, 4–7). The restoration of LINE1 DNA methylation observed in this new report raises the question whether there had been overgrowth of nonrecombined crypt cells with retained *Dnmt1* expression. However, IHC showed a continued absence of *Dnmt1* expression. Nevertheless, a DNA-based assay for the ratio of recombined alleles after 2 months would have laid this issue to rest. It has been reported by the Kaestner group that *Dnmt1* knockout induces upregulation of a *de novo* DNA methyltransferase, *Dnmt3b*, in the mouse epithelium, which has been found to be essential for the viability of *Dnmt1* knockout mice (12). Whether this phenomenon of upregulation of *Dnmt3b* by *Dnmt1* knockout is responsible for the restoration of LINE1 methylation remains to be tested. The full restoration level suggests that the DNA methylation recovery is a systemic cellular response to the *Dnmt1* knockout occurring in most, if not all, cells and that global hypomethylation is temporal in the knockout mice. This raises an interesting possibility that the observed elevated adenoma formation could be a combinatorial effect of acute hypomethylation and subsequent hypermethylation possibly by the upregulated *Dnmt3b*. In support of this, *Dnmt3b* overexpression has been shown to promote intestinal adenoma formation by inducing widespread gene-specific promoter hypermethylation, including Wnt antagonists *Sfrp2*, *4*, and *5* (13, 14). It is also worth noting that a knockout of human *DNMT1* employing a partial deletion strategy similar to the one used in the study by Sheaffer and colleagues (exons 3–5 deletion vs. exons 4–5) resulted in a truncated DNMT1 protein with a substantial residual DNA methylation capability through an alternative splicing (15). The exact same alternative splicing event from exon 1 to exon 6 in the mouse *Dnmt1* would also result in a truncated Dnmt1 protein with the same reading frame as wild-type Dnmt1. Regardless of the completeness of the *Dnmt1* knockout, the discovery that DNA hypomethylation can promote intestinal adenoma formation remains valid. However, whether the knockout is complete may affect other interpretations and conclusions, such as whether *Dnmt1* is dispensable for intestinal cell viability, differentiation, and transformation.

Sheaffer and colleagues also reported that acute hypomethylation induces genomic instability and increased cell proliferation, both of which would promote LOH of *Apc*. However, increased LOH of *Apc* alone would not account for the elevated adenoma formation in the *Dnmt1* null *Apc^{Min/+}* mice as most microadenomas with LOH of *Apc* in the *Dnmt1* hypomorphic mice did not progress to a macroscopic adenoma even at 6-month age (7). These disparate trajectories of microadenomas between *Dnmt1* null and hypomorphic *Apc^{Min/+}* mice imply that the more severe hypomethylation in the *Dnmt1* null mice resulted in additional genetic or epigenetic lesions that promoted further progression to adenoma (Fig. 1). Thus, the answer to the perplexing opposite outcomes between the *Dnmt1* null and hypomorphic mice may simply lie in the fact that each mouse model induces different degrees of DNA hypomethylation.

The findings by Sheaffer and colleagues extend our understanding of the tumorigenic potential of DNA hypomethylation beyond the intestinal microadenoma stage and suggest a continuous spectrum of oncogenic contribution of DNA hypomethylation and hypermethylation encompassing initiation to progression, rather than each playing a discrete, stage-specific role in the course of intestinal tumorigenesis (Fig. 1). In this revised view, the cellular DNA methylation capacity determines the degrees of DNA hypomethylation and hypermethylation, which together influence the extent and types of both genetic and epigenetic alterations that ultimately mold tumorigenic potential (Fig. 1). It would be of great interest to compare
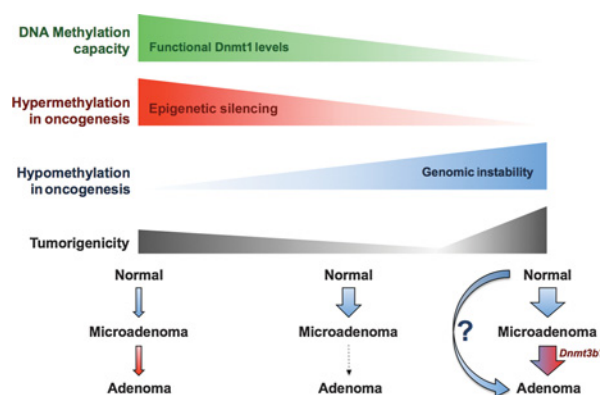
Figure 1.
Intestinal tumorigenesis affected by the degree of DNA hypomethylation. Cellular DNA methylating capacity affects the potential for tumor-associated DNA hypermethylation and hypomethylation, which contribute to oncogenesis by inducing epigenetic gene silencing or genomic instability, respectively. The degrees of tumorigenicity determined collectively by hypermethyation and hypomethylation potentials influence the course of intestinal tumorigenesis. At reduced levels of DNA methylating capacity, microadenoma formation is promoted by increased genomic instability, whereas progression from microadenoma to adenoma is suppressed by reduced epigenetic gene silencing. Severely reduced levels of DNA methylating capacity may promote both microadenoma and adenoma formation, possibly involving compensatory *Dnmt3b* expression or may cause a rapid direct progression from normal cells to adenoma.

epigenetic and genetic alterations among the *Dnmt1*-deficient, *Dnmt1*-hypomorphic, and *Dnmt1*-proficient tumors as the tumors from the three groups might have evolved through distinct molecular paths under the different degrees of epigenetic influence.

Mouse studies with null and hypomorphic *Dnmt1* have been integral in understanding the role of DNA methylation in tumorigenesis and have shed light on epigenetic mechanisms contributing to human cancer. However, there is little evidence for cancer-associated *DNMT1* mutations or downregulation of *DNMT1* in human cancers. This may indicate that the mechanistic bases of hypomethylation in human cancers might be fundamentally different from those of *Dnmt1* hypomorphic mice. This might account for the considerable differences in the patterns, profiles, and degrees of hypomethylation between human cancers and *Dnmt1* hypomorphic mouse models (16, 17). For example, the degrees of hypomethylation induced in the mouse models are more severe than those found in human cancers (17). In addition, hypomethylation in human colorectal cancer is primarily found at late-replicating lamina-associated domains (16, 17). Thus, we should view with caution the insights obtained from *Dnmt1* hypomorphic mouse models.

The tumor promotion by severe DNA hypomethylation reported by Sheaffer and colleagues raises a concern that the use of demethylating agents, such as 5-azacitidine (AZA) and 5-Aza-2′-deoxycitidine (DAC), in human cancer may cause a secondary malignancy or exacerbate primary tumors in patients. However, it has been shown that leukemia patients treated with DAC displayed relatively mild levels of demethylation of Alu (an average of 7.7%), which is much lower than hypomethylation found in the *Dnmt1* null and hypomorphic mice (2, 4, 5, 18). Nevertheless, development and deployment of new demethylating drugs and regimes with improved demethylation efficacy should be undertaken with caution.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P.W. Laird
Writing, review, and/or revision of the manuscript: K.-H. Lee, P.W. Laird
Study supervision: P.W. Laird

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