An Unstable Liaison

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Changes in the pattern of DNA methylation are common in human tumors (*1*). Both genome-wide hypomethylation and region-specific hypermethylation seem to be important in the formation and progression of cancers (carcinogenesis). The global level of DNA methylation is generally lower in tumor cells than in normal cells (*2*), but this hypomethylation is curious in light of the increased expression of DNA methyltransferase (an enzyme that adds methyl groups to specific cytosines in DNA) in many tumor cells (*3*). In two papers on pages 455 and 489 of this issue (*4*, *5*), Jaenisch's group presents evidence for a potential link between DNA hypomethylation, genomic instability, and cancer.

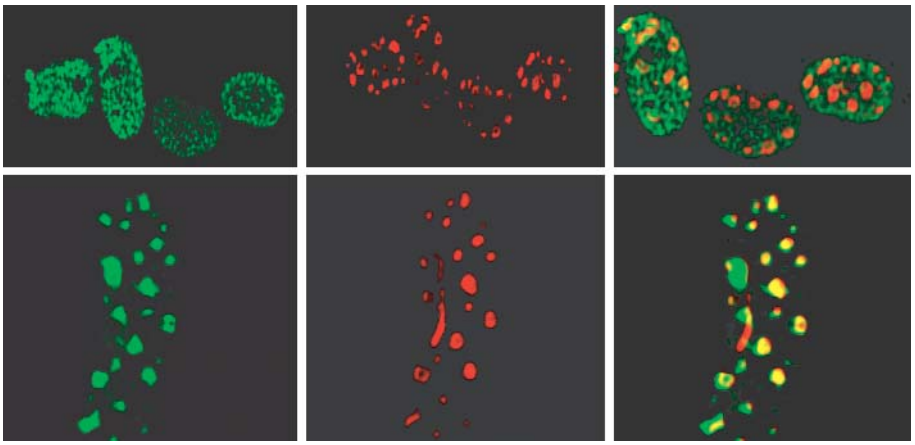
DNA hypermethylation is associated with the inappropriate transcriptional silencing of tumor suppressor genes, explaining its pervasive role in oncogenesis. The biological significance of DNA hypomethylation in cancer is less clear. Early experiments using DNA methylation inhibitors in vivo and in vitro seemed to support the involvement of DNA hypomethylation in carcinogenesis. Such experiments resulted in conversion of low-metastatic tumor cell lines to high-metastatic versions and formation of transformed foci. Feeding methyl-deficient diets to rats and mice resulted in global DNA undermethylation, the formation of liver tumors, and demethylation of proto-oncogenes (*6*).

Two analyses of DNA methyltransferase-deficient mice have complicated the interpretation of established hypotheses about methylation and cancer. Jaenisch's group reduced DNA methyltransferase 1 (Dnmt1) activity in the Min mouse model, which mimics familial adenomatous polyposis, a human disease characterized by formation of numerous precancerous polyps in the colon. They achieved this by giving a low dose of 5-azadeoxycytidine to mice heterozygous for the DNA methyltransferase gene. The decrease in DNA methylation significantly reduced the number of intestinal adenomas formed in the mice (*7*). These results conflicted with conventional wisdom at the time because they implied that DNA hypomethylation had no oncogenic effect. Further confusing the issue, Trinh *et al.* found that a reduction in DNMT1 activity had significant but opposing effects on the development of two different types of tumor (*8*). To study the interaction between DNA mismatch repair deficiency and DNA methylation, they introduced Dnmt1 mutations into a mouse strain deficient in the MLH1 protein, which is required for repair of mistakes made in DNA replication (*8*). Mice harboring hypomorphic Dnmt1 mutations showed diminished DNA methylation but developed normally and were tumor-free. When crossed with $Mlh1^{-/-}$ homozygous mice lacking the MLH1 protein, the resulting offspring were less likely to develop the intestinal cancers characteristic of their mismatch repair–deficient parent. However, these same mice developed invasive T and B cell lymphomas earlier and at a much higher frequency than did their Dnmt1 wild-type littermates.

How does hypomethylation influence neoplasia? Because both genomic instability and hypomethylation are observed early during carcinogenesis (*1, 9*), it is tempting to speculate that genomic hypomethylation, by destabilizing the genome, provides the incipient cancer cells with a way of acquiring more mutations. Earlier studies have suggested that defects in DNA methylation might contribute to the chromosomal instability observed in aneuploid human colorectal cancer cell lines (*10*). Also, DNA hypomethylation of very discrete locations within the genome has been associated with abnormal chromosomal structures, such as those observed in cells from patients with ICF syndrome (immunodeficiency–centromeric instability–facial abnormalities). This rare recessive disease is caused by mutations in the catalytic domain of the *Dnmt3b* gene, which encodes another DNA methyltransferase (*11*). Murine embryonic stem (ES) cells lacking Dnmt1 exhibit an increased frequency of chromosomal deletions (*12*). In their two new studies, Jaenisch's group now extends its ES cell work to somatic cells (*4*, *5*).

Mice carrying two *Dnmt1* null alleles die during gestation (*13*). To overcome this problem, Jaenisch and colleagues (*5*) combined a hypomorphic allele ($Dnmt1^{chip}$) with a null allele to generate $Dnmt1^{chip/-}$ compound heterozygote animals with substantially reduced DNA methylation (10% of wild type). Such a genetic approach is superior to previous pharmacological studies by the same group (*7*) because the *Dnmt1* hypomorphic allele causes genome-wide hypomethylation in all tissues while avoiding the detrimental effects of mutations. This severe DNA hypomethylation is sufficient to induce formation of T cell lymphomas. Using array-based comparative genomic hybridization, the authors compared genomic DNA from $Dnmt1^{chip/-}$ tumors with that from Moloney virus–induced tumors. They observed a subtle but statistically significant increase in gains and losses of chromosomes in the hypomethylated tumors (*5*).

In their companion study, the investigators provide further support for the potential destabilizing effect of hypomethylation on genomic DNA (*4*). Purposely avoiding models in which hypermethylation and gene silencing might be involved, they selected a different mouse model in which a chromosomal event was the rate-limiting step and essential for tumor formation (*4*). They introduced a hypomorphic $Dnmt1^{chip/-}$ allele



DNMT1 and DNA methylation. (**Top**) In cultured mouse cells in early S phase of the cell cycle, DNMT1 (green) is distributed into small foci where it maintains the sparse methylation pattern of early-replicating euchromatic regions of the DNA (red). (**Bottom**) During late S-phase replication, DNMT1 (green) becomes localized to densely methylated blocks of heterochromatin (red) where it maintains the hypermethylation of these silenced regions. (**Top** and **Bottom**) The images on the far right represent the superimposition of the first two images; regions of overlap appear in yellow.

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into animals with mutations in *p53* and *Nf1* alleles on the same copy of chromosome 11. Loss of Nf1 activates the proto-oncogene *ras* and cooperates with inactivating mutations in the *p53* tumor suppressor gene during malignant transformation. All of the mice harboring null *Nf1* and *p53* alleles developed soft tissue sarcomas between 3 and 7 months of age (*14*). These sarcomas exhibited loss of heterozygosity (LOH) at both gene loci. Consistent with the fact that DNA hypomethylation increased the rate of LOH in cultured fibroblasts, sarcomas formed earlier in the hypomethylated animals (*4*). This phenotype is strikingly consistent with that of histone methyltransferase (Suv39h) mutant mice, which provides a compelling link between DNA and histone methylation, pericentric chromatin structure, and the maintenance of chromosomal stability (*15*).

The new data provide the most direct evidence so far for an effect of hypomethylation on chromosomal stability. However, the mechanisms by which hypomethylation causes genomic instability remain unclear. The authors show that in hypomethylated cells, LOH occurs preferentially in the centromeric region, but they have been unable to determine whether loss of the region carrying the wild-type copies of *Nf1* and *p53* is the result of mitotic recombination or of whole chromosome loss. Identifying cells with only one copy of chromosome 11 (per diploid genome) would be an indication of whole chromosome loss, but such an analysis is complicated by the fact that both methylated and hypomethylated tumor cells are mostly aneuploid (*16*).

Are these mouse data relevant to human cancers? Maybe. Although DNMT1 accounts for most of the methylation in normal mouse cells, human colorectal cancer cells lacking Dnmt1 retain significant genomic methylation and associated gene silencing (*17*). Rhee *et al*. have also disrupted the *Dnmt3b* gene in human cell lines that codes for another DNA methyltransferase (*18*). This deletion reduced global DNA methylation by less than 3%. Surprisingly, genetic disruption of both *Dnmt1* and *Dnmt3b* in human cell lines nearly eliminated methyltransferase activity and reduced genomic DNA methylation by more than 95%. These marked changes resulted in demethylation of repeated sequences, loss of insulin-like growth factor II imprinting, abrogation of silencing of the tumor suppressor gene *p16INK4a*, and growth suppression (*18*). These results provide compelling evidence that the two enzymes cooperatively maintain DNA methylation and gene silencing in human colorectal cancer, and that such methylation is essential for optimal neoplastic proliferation. However, disruption of *Dnmt1* and/or *Dnmt3b* did not lead to a dramatically increased rate of gain or loss of chromosomes in these cells (*18*).

Again, such discrepancies might reflect differences in model systems, varying mechanisms in different species, or tissue specificity. Therefore, any implications for the treatment of human cancers need to be drawn with extreme caution. DNA methyltransferase inhibitors such as 5-aza-CdR have shown some efficacy in treating leukemias (*19*). DNA hypomethylation seems to promote LOH and genomic instability, and so DNA methyltransferase inhibitors might fatally accelerate tumor progression by increasing chromosomal instability just enough to promote tumorigenesis. Alternatively, DNA methyltransferase inhibitors could drive the cancer to self-destruct by increasing chromosomal instability enough to push tumor cells with already unstable genomes into death (*20*). This model explains why genomic demethylation may protect against some cancers such as intestinal tumors in the APC^{Min} mouse model (*7*), but may increase the risk of cancers in other tissues, exemplified by the tumors arising in the hypomethylated mutant mice.

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MATERIALS SCIENCE

The More Elements, the Merrier

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On 17 December 1903, the Wright brothers piloted the first powered airplane at Kitty Hawk, North Carolina. The upcoming centennial of their achievement also marks the anniversary of using a lightweight aviation alloy that made the flight possible. The airplane was powered by a revolutionary engine: the first aluminum-alloy, gas-powered engine block. Its low weight (<100 kg) kept the plane light, while its strength was sufficient to withstand the stresses associated with delivering the power (~12 hp) to "drive the machine through the air" (*1*).

Craftsmen, not scientists, had designed the alloy, and the origin of its strength was not fully understood until 1919: The aluminum was supersaturated with copper, leading to a precipitation-hardened alloy. This method has been used to improve alloy properties ever since. However, metal alloys have been advanced mostly by trial and error—an approach that goes back to the bronze age. After a hundred years of human combinatorial effort—expensive and slow, with incremental improvements and occasional breakthroughs—metal alloy development is now considered by many to be "mature": Continued improvements in properties such as strength, modulus, and toughness are expected to be incremental (<20% improvement).

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However, on page 464 of this issue, Saito *et al.* (*2*) show that it is the trial-and-error approach that is mature, not metal-alloy development itself. By adding several "solute" elements to a metallic element "solvent" (in this case titanium), they achieve major advances in specific material properties that would be exceedingly difficult to achieve by trial and error. The difficulty is that alloys can be made with a nearly infinite number of elemental compositions, very few of which improve any useful property. To overcome this problem, Saito *et al.* use computational methods in concert with experimental studies. An alloy of this complexity probably cannot be designed without computational tools. An exciting new landscape is opening up for advanced materials based on metals as the primary component.

Almost all metallic-based structural materials are alloys, composed most often with Fe, Al, Ti, or Ni as the major component (or solvent); rarely is a pure element used by itself. For instance, the Al alloy used by the Wright brothers contained Cu as a solute that formed nanocrystalline precipitates (*3*). Traditionally, alloy designers relied on experimentally determined phase diagrams (see the figure). But a new multicomponent alloy would require millions of phase diagrams (binary, ternary, quaternary, and so forth), just for mixtures of common metal elements like Fe, Cr, Mn, Ni, etc. Most of this