Chromosomal Instability and Tumors Promoted by DNA Hypomethylation

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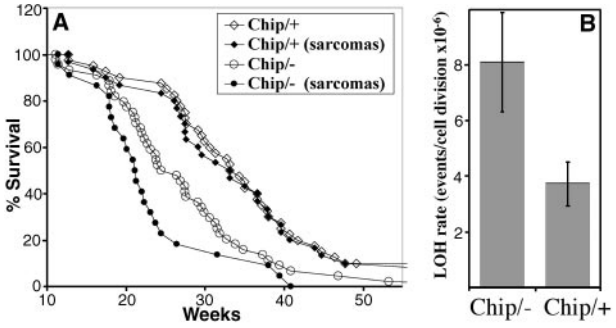
Human tumors often display changes in DNA methylation, including both genome-wide hypomethylation and site-specific hypermethylation (*1*, *2*). In mice, DNA hypomethylation is sufficient to induce T cell lymphomas with consistent gain of chromosome 15 (*3*), indicating that genome-wide hypomethylation plays a causal role in cancer.

To explore further the link between DNA hypomethylation and chromosomal instability, we studied the effect of DNA hypomethylation on tumor-prone mice carrying mutations in both the Neurofibromatosis 1 (*Nf1*) and *p53* tumor suppressor genes. The *Nf1* and *p53* genes are closely linked on mouse chromosome 11, and double heterozygotes carrying the mutations on the same chromosome (NPcis) develop soft tissue sarcomas, which show simultaneous loss of heterozygosity (LOH) of *Nf1* and *p53* (*4*). When we induced genomic hypomethylation in the $Nf1^{+/-}$ $p53^{+/-}$ (NPcis) mice by introducing a hypomorphic allele of DNA methyltransferase 1 ($Dnmt1^{Chip/-}$) (*3*), the mice developed sarcomas at an earlier age compared with NPcis littermates with normal levels of DNA methylation ($Dnmt1^{Chip/+}$) (Fig. 1A). To determine whether hypomethylation promotes the initial LOH required for tumor development in the NPcis mice, we compared the rates of LOH in methylated and hypomethylated primary embryonic fibroblasts. We developed an assay to score for $Nf1^{-/-}$ $p53^{-/-}$ cells within a population of heterozygous cells (i.e., cells that have undergone LOH) (fig. S1). We then used this assay in a fluctuation analysis (*5*) to calculate the rate of LOH in hypomethylated versus methylated cells. This analysis revealed a significant increase in LOH rate in hypomethylated cells (2.2-fold; $P = 0.01$) (Fig. 1B), consistent with the hypothesis that hypomethylation promotes tumor development in NPcis mice by increasing the rate of LOH.

To investigate the chromosomal mechanism leading to LOH in the hypomethylated cells and whether specific chromosomal regions are involved, we analyzed LOH along chromosome 11 in single colonies representing independent LOH events (Fig. 1C). In methylated ($Dnmt1^{Chip/+}$) cells, LOH affecting the whole chromosome (including at position 1.5 cM from the centromere) occurred in 45% of analyzed events. The remaining 55% showed heterozygosity at 1.5 cM but were homozygous for markers at 20 cM or 39 cM and were, therefore, the result of mitotic recombination or of loss of the distal portion of the chromosome. Interestingly, the frequency of LOH events affecting the whole chromosome (including at 1.5 cM) was significantly higher in hypomethylated cells (77% compared with 45% in methylated cells) (Fig. 1C), suggesting that the increase in LOH rate in hypomethylated cells is the result of a specific effect of hypomethylation on the stability of the centromeric or pericentric regions. A link between hypomethylation and the stability of whole chromosome arms is also found in the human Immunodeficiency–Centromeric Instability–Facial Anomalies (ICF) syndrome, in hypomethylation-induced T cell lymphomas in mice (*3*) and in human hepatocellular and prostate carcinomas (*6*, *7*).

These results suggest that DNA hypomethylation promotes cancer through effects on chromosomal stability. Further characterization of the relations between DNA methylation, chromatin composition, and chromatin structure may allow a better understanding how DNA hypomethylation affects chromosome structure and integrity.



C

	1.5cM	20cM	39cM	46cM	66cM
Chip/+	D11Mit226	D11Mit270	*p53*	*Nf1*	D11Mit224
Embryo 1 (n=9)	44%	78%	100%	100%	100%
Embryo 2 (n=13)	38%	83%	100%	100%	100%
Embryo 3 (n=14)	53%	83%	86%	86%	86%
Total (n=36)	**45%**	**82%**	**95%**	**95%**	**95%**
Chip/-					
Embryo 1 (n=17)	71%	88%	100%	100%	100%
Embryo 2 (n=17)	82%	86%	100%	100%	100%
Embryo 3 (n=13)	77%	84%	99%	99%	99%
Total (n=47)	**77%**	**86%**	**99.7%**	**99.7%**	**99.7%**

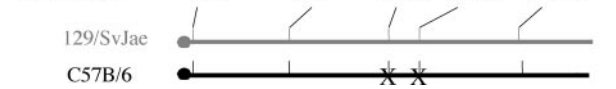


Fig. 1. (**A**) Survival curve of methylated [$Dnmt^{Chip/+}$ (Chip/+), $n = 42$] and hypomethylated [$Dnmt1^{Chip/-}$ (Chip/–), $n = 44$] $Nf1^{+/-}p53^{+/-}$cis (NPcis) mice. Survival curve for each genotype is plotted twice: One curve includes all mice (open symbols), and the second curve represents only mice that developed soft tissue sarcomas (solid symbols Chip/+), $n = 33$; Chip/–, $n = 22$). Mice of both genotypes typically developed one predominant, fast-growing sarcoma. Eighteen Chip/– mice developed T cell lymphomas [as described in (*3*)], including five mice that developed both a sarcoma and a lymphoma. (**B**) Increased rate of LOH events in hypomethylated mouse embryonic fibroblasts (MEFs), as determined by fluctuation analysis (*5*) (fig. S1). Graph shows the average rate in four independent experiments (±SD). The statistical significance was determined by Student's *t* test to be $P = 0.01$. (**C**) LOH analysis. Cells in this study were derived from C57/B6 × 129/svJae F_1 mice. The polymorphic markers indicated were used to examine heterozygosity along mouse chromosome 11 in cell colonies homozygous for *Nf1* and *p53* derived during fluctuation analysis. Only colonies from independent parallel cultures were considered independent events. We analyzed colonies obtained from three different fluctuation analyses (n = number of independent colonies). *Nf1* and *p53* mutant alleles (marked with "x") resided on the B6 chromosome, and LOH always resulted in the loss of the 129 allele. Results are shown as the percentage of colonies with only the B6 allele at the marked position.

References and Notes

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Fig. S1

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