Commentary

Cancer epigenetics takes center stage

Andrew P. Feinberg

Institute of Genetic Medicine, Departments of Medicine, Molecular Biology and Genetics, and Oncology, Johns Hopkins University School of Medicine, 1064 Ross, 720 Rutland Avenue, Baltimore, MD 21205

Next year will mark 20 years since I developed a Southern blot showing altered DNA methylation in cancer. This discovery (1) was met with some skepticism, primarily because it was thought that aberrant methylation in cancer was an epiphenomenon, somehow linked to a generalized disruption of gene regulation in cancer cells and arising after the cancer, rather than playing a causal role itself. This essay will address how cancer epigenetics has overcome these objections, and a report in this issue by Nakagawa *et al.* (2) adds significantly to this argument.

Epigenetics is defined as modifications of the genome, heritable during cell division, that do not involve a change in the DNA sequence. Examples include methylation induced premeiotically in *Ascobolus*, repeat-induced gene silencing and paramutation in plants, mating type silencing and telomere silencing in yeast, position effect variegation in *Drosophila*, and genomic imprinting in mammals and flowering plants. There are several features that distinguish epigenetics from conventional genetic mechanisms: reversibility; position effects, i.e., the ability to act over unexpected distances larger than a single gene; apparent mutations at unexpectedly high frequency; and the involvement of gene domains. One common thread to most epigenetic phenomena is DNA methylation, a covalent modification of the C5 position of cytosine. This methylation pattern is stably maintained at CpG dinucleotides by a family of DNA methyltransferases that recognize hemimethylated CpG dinucleotides after DNA replication. DNA methyltransferases belong to multiprotein complexes, and they contain sequence motifs for multiple such interactions, including interactions with chromatin components, some of which have been directly identified (3). Another common thread to epigenetics is a link to transcriptional regulation, generally involving gene silencing. DNA methylation in particular is generally but not exclusively linked to transcriptional silencing, including methylation induced premeiotically, paramutation, and mammalian gene silencing.

Epigenetic alterations in cancer include global hypomethylation (4), hypomethylation of individual genes (1), and hypermethylation of CpG islands (5), CpG-rich sequences in the promoters of housekeeping genes that are generally protected from methylation. This hypermethylation may lead to aberrant silencing of tumor suppressor genes (6). In addition, we and others have discovered loss of imprinting (LOI) in cancer (7, 8). Genomic imprinting, the subject of the report by Nakagawa *et al.* (2), is an epigenetic modification of a specific parental allele of a gene, or the chromosome on which it resides, in the gamete or zygote, leading to differential expression of the two alleles of the gene in somatic cells of the offspring. LOI involves loss of the normal pattern of expression of a specific parental allele, and in cancer it can lead to activation of growth-promoting imprinted genes such as insulin-like growth factor II (*IGF2*) (7, 8), as well as silencing of potential tumor suppressor genes such as $p57^{KIP2}$ (9) and *ARH1* (10).

Furthermore, we found that LOI can occur in the *normal* colonic mucosa of colorectal cancer patients with LOI in their tumors (11), overcoming the objection that epigenetic alterations are simply late consequences of neoplasia. This LOI was linked to cases showing microsatellite instability (MSI) in the tumors (11). MSI is a form of genetic instability found in patients with hereditary nonpolyposis colorectal cancer and caused by defects in DNA mismatch repair (12, 13). MSI occurs much more commonly in sporadic nonfamilial colon cancer, affecting about 25% of such patients. However, these patients do not have mutations in mismatch repair genes (14). One potential cause of MSI in these sporadic cancers is hypermethylation and epigenetic silencing of the hMLH1 mismatch repair gene (15), a target of conventional mutations in hereditary nonpolyposis colorectal cancer (16, 17). LOI of *IGF2* has also been previously linked to increased methylation in embryonal and other tumors, specifically at a CpG island that represents a differentially methylated region (DMR) upstream of the maternal H19 gene; methylation of the DMR in turn regulates the silencing of the IGF2 gene on the same chromosome (18, 19).

Nakagawa *et al.* (2) now confirm the original study of Cui *et al.* that LOI occurs in both tumor and normal tissue of patients with MSI-positive colorectal cancer (11), a result also confirmed earlier by Nishihara *et al.* (20). Furthermore, Nakagawa *et al.* find that LOI was not a feature of tumors in patients with a germline mismatch repair gene mutation (2), indicating that LOI is not merely a consequence of a mismatch repair defect. The study of Nakagawa *et al.* (2) also helps to close the circle among cancer, LOI, and DNA methylation, by demonstrating directly that sequences within the *H19* DMR are specifically methylated in these tumors. Not surprisingly, these same patients show hypermethylation of other CpG islands throughout the genome, as generalized hypermethylation and MSI have been previously linked (21, 41). Methylation of the H19 DMR was also observed in the matched normal tissue of patients with LOI (2), although it should be noted that this occurred at a lower frequency than LOI in these tissues, and only partial methylation was observed.

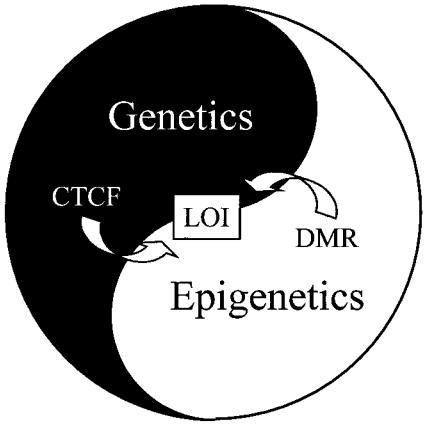

Fig. 1. The interrelationship of cancer genetics and epigenetics. Rather than a traditional Venn diagram, cancer genetics and epigenetics are drawn as a yin-yang. For example, loss of imprinting might be caused by genetic disruption of CTCF or by altered methylation of the DMR (illustrated). Other examples of overlap or in which the distinction between genetics and epigenetics are blurred are provided in the text.

See companion article on page 591.

Furthermore, generalized hypermethylation of CpG islands is *not* present in the normal tissue of these patients (2), consistent with the idea that LOI precedes a generalized disruption of CpG island methylation (11).

The present study (2) also offers an intriguing mechanistic hypothesis to explain the relationship between *H19* DMR methylation and LOI in these patients, as the methylated nucleotides include those to which the chromatin insulator CTCF has been shown to bind specifically in regulating genomic imprinting. Whether the partial methylation seen in their study is sufficient to disrupt CTCF binding remains to be proven. Nevertheless, the study calls attention to this remarkable highly conserved multifunctional protein, first discovered by Lobanenkov and colleagues as a multivalent transcription factor (22–24) that also serves as a chromatin insulator. In this capacity, CTCF binds specifically to the *H19* DMR *in vivo* (25) and *in vitro* (26–28) when it is unmethylated, separating *IGF2* from its enhancer and allowing monoallelic DMR methylation-dependent expression of *IGF2* (25–28). Lobanenkov and colleagues (including us) have found mutations in *CTCF* in diverse tumors, that selectively impair binding to target sequences, altering the functional spectrum of the protein and, as well as methylation of CTCF binding sites in tumors (G. N. Filippova, D. I. Loukinov, E. M. Pugacheva, J. E. Ulmer, J. M. Moore, Y. J. Hu, H. Moon, J. Breen, C.-F. Qi, P. E. Grundy, *et al.*, unpublished work), suggesting a general role for CTCF in cancer.

The potential link to CTCF suggested by this study also calls our attention to the link among DNA methylation, epigenetics, and chromatin. Boveri, the father of cancer genetics, described a generalized disturbance of chromatin that distinguished cancer cells from normal cells (30). Although his writing preceded our understanding of DNA and thus he was not truly distinguishing genetics and epigenetics, his thoughts were based on his observation of widespread disruption of chromosomal organization and nuclear structure, akin to what we might call chromatin today. In this regard, a gem in the recent studies of CTCF was the observation of Ohlsson and colleagues that CTCF binding may depend on nucleosome phasing (31), suggesting that imprinting and methylation may both ultimately be bound to the assembly of DNA into organized structures.

A clue to the link between MSI and epigenetics may be provided by another sometimes overlooked common thread in epigenetics, namely DNA replication. For example, repeat-induced gene silencing is thought to be propagated through hemimethylated intermediates during DNA replication; silencing in yeast depends on an origin of replication complex; and mating type silencing, position effect variegation, and genomic imprinting are all linked to delayed replication timing. Given that MSI is attributable to defective replication-linked mismatch repair, necessitating that the cell distinguishes between parent and daughter strands, all of these phenomena may involve disrupted chromatin.

The studies of Cui *et al.* (11), Nishihara *et al.* (20), and Nakagawa *et al.* (2) suggest a new and provocative view of the timing of epigenetic changes in cancer. It should be noted that LOI was observed in these studies generally throughout the colon, not just at the site of the tumor. Although colorectal cancer in particular involves a series of genetic alterations in the evolution of an advanced metastatic tumor, elegantly described by Vogelstein (32) and others, perhaps the likelihood of developing clinical cancer when a mutation arises depends in part on a preexisting epigenetic defect affecting much or all of the normal colonic mucosa. Why would this be? Studies of transgenic mice with constitutive biallelic expression of *IGF2*, comparable to LOI, show reduced apoptosis and increased tumor formation on introduction of an oncogenic transgene (33, 34). Perhaps preexisting LOI alters the balance between growth and apoptosis when conventional oncogenic mutations arise in the colon. Although this idea is admittedly speculative, these studies (2, 11, 20) nevertheless demonstrate such a field defect in nontumor tissue, and from an epidemiological perspective, what distinguishes a cancer patient from a noncancer patient may be such epigenetic alterations involving DNA methylation and imprinting. This hypothesis will require direct confirmation that the epigenetic alterations temporally precede the genetic changes and the tumors themselves, a subject of intense current clinical study. Such studies may have profound clinical significance, because they might offer the opportunity for detection of large numbers of patients in the general population at increased risk of colorectal cancer, and the eventual possibility of enhanced surveillance or even chemoprevention in such patients.

I conclude by noting that the distinction between cancer genetics and epigenetics has blurred considerably in recent years (Fig. 1). Many conventional ''genetic'' mechanisms directly affect proteins that regulate chromatin, such as the rearrangement of the trithorax family member ALL in common childhood leukemia (29, 35), mutation of the chromatin remodeling complex core member Snf5 in rhabdoid tumors (36), and rearrangement of a candidate histone acetyltransferase in acute myeloid leukemia (37). In addition, physical interactions have been found between critical tumor genes and chromatin proteins, such as Rb and histone deactylase (38, 39), and the promyelocytic leukemia nuclear body protein P100 with heterochromatin protein HP-1 (40), whose *Drosophila* homologue is also a suppressor of position effect variegation. While less is known of the mechanism of epigenetic alterations in cancer, CTCF itself offers a striking example of convergence of traditional genetics and epigenetics, as the same protein regulates genomic imprinting as well as the ''traditional'' oncogene c-*myc* (23). We may well find that the geneticists and the epigeneticists converge on Boveri's definition of cancer as a disease of ''the chromatin.''

I thank R. Ohlsson, V. Lobanenkov, H. Cui, J. Ravenel, and E. Niemitz for thoughtful comments. This work was supported by National Institutes of Health Grant CA65145.

1. Feinberg, A. P. & Vogelstein, B. (1983) *Nature (London)* **301,** 89–92.
2. Nakagawa, H., Chadwick, R. B., Peltomäki, P., Plass, C., Nakamura, Y. & de la Chapelle, A. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 591–596. (First Published December 19, 2000; 10.1073/pnas.011528698)
3. Robertson, K. D., Ait-Si-Ali, S., Yokochi, T., Wade, P. A., Jones, P. L. & Wolffe, A. P. (2000) *Nat. Genet.* **25,** 338–342.
4. Feinberg, A. P., Gehrke, C. W., Kuo, K. C. & Ehrlich, M. (1988) *Cancer Res.* **48,** 1159–1161.
5. Baylin, S. B., Hoppener, J. W., de Bustros, A., Steenbergh, P. H., Lips, C. J. & Nelkin, B. D. (1986) *Cancer Res.* **46,** 2917–2922.
6. Jones, P. A. & Laird, P. W. (1999) *Nat. Genet.* **21,** 163–167.
7. Rainier, S., Johnson, L. A., Dobry, C. J., Ping, A. J., Grundy, P. E. & Feinberg, A. P. (1993) *Nature (London)* **362,** 747–749.
8. Ogawa, O., Eccles, M. R., Szeto, J., McNoe, L. A., Yun, K., Maw, M. A., Smith, P. J. & Reeve, A. E. (1993) *Nature (London)* **362,** 749–751.
9. Thompson, J. S., Reese, K. J., DeBaun, M. R., Perlman, E. J. & Feinberg, A. P. (1996) *Cancer Res.* **56,** 5723–5727.
10. Yu, Y., Xu, F., Peng, H., Fang, X., Zhao, S., Li, Y., Cuevas, B., Kuo, W. L., Gray, J. W., Siciliano, M., *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96,** 214–219.
11. Cui, H., Horon, I. L., Ohlsson, R., Hamilton, S. R. & Feinberg, A. P. (1998) *Nat. Med.* **4,** 1276–1280.
12. Thibodeau, S. N., Bren, G. & Schaid, D. (1993) *Science* **260,** 816–819.
13. Parsons, R., Li, G. M., Longley, M. J., Fang, W. H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B. & Modrich, P. (1995) *Science* **268,** 738–740.

14. Boland, C. R., Thibodeau, S. N., Hamilton, S. R., Sidransky, D., Eshleman, J. R., Burt, R. W., Meltzer, S. J., Rodriguez-Bigas, M. A., Fodde, R., Ranzani, G. N., *et al.* (1998) *Cancer Res.* **58,** 5248–5257.
15. Herman, J. G., Umar, A., Polyak, K., Graff, J. R., Ahuja, N., Issa, J. P., Markowitz, S., Willson, J. K., Hamilton, S. R., Kinzler, K. W., *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6870–6875.
16. Papadopoulos, N., Nicolaides, N. C., Wei, Y. F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., *et al.* (1994) *Science* **263,** 1625–1629.
17. Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Earabino, C., Lipford, J., Lindblom, A., *et al.* (1994) *Nature (London)* **368,** 258–261.
18. Steenman, M. J. C., Rainier, S., Dobry, C. J., Grundy, P., Horon, I. L. & Feinberg, A. P. (1994) *Nat. Genet.* **7,** 433–439.
19. Moulton, T., Crenshaw, T., Hao, Y., Moosikasuwan, J., Lin, N., Dembitzer, F., Hensle, T., Weiss, L., McMorrow, L., Loew, T., *et al.* (1994) *Nat. Genet.* **7,** 440–447.
20. Nishihara, S., Hayashida, T., Mitsuya, K., Schulz, T. C., Ikeguchi, M., Kaibara, N. & Oshimura, M. (2000) *Int. J. Oncol.* **17,** 317–322.
21. Toyota, M. & Issa, J. P. (1999) *Semin. Cancer Biol.* **9,** 349–357.
22. Lobanenkov, V. V., Nicolas, R. H., Plumb, M. A., Wright, C. A. & Goodwin, G. H. (1986) *Eur. J. Biochem.* **159,** 181–188.
23. Klenova, E. M., Nicolas, R. H., Paterson, H. F., Carne, A. F., Heath, C. M., Goodwin, G. H., Neiman, P. E. & Lobanenkov, V. V. (1993) *Mol. Cell. Biol.* **13,** 7612–7624.
24. Filippova, G. N., Fagerlie, S., Klenova, E. M., Myers, C., Dehner, Y., Goodwin, G., Neiman, P. E., Collins, S. J. & Lobanenkov, V. V. (1996) *Mol. Cell. Biol.* **16,** 2802–2813.
25. Kanduri, C., Pant, V., Loukinov, D., Pugacheva, E., Qi, C. F., Wolffe, A., Ohlsson, R. & Lobanenkov, V. V. (2000) *Curr. Biol.* **10,** 853–856.
26. Hark, A. T., Schoenherr, C. J., Katz, D. J., Ingram, R. S., Levorse, J. M. & Tilghman, S. M. (2000) *Nature (London)* **405,** 486–489.
27. Bell, A. C. & Felsenfeld, G. (2000) *Nature (London)* **405,** 482–485.
28. Szabo, P., Tang, S. H., Rentsendorj, A., Pfeifer, G. P. & Mann, J. R. (2000) *Curr. Biol.* **10,** 607–610.
29. Tkachuk, D. C., Kohler, S. & Cleary, M. L. (1992) *Cell* **71,** 691–700.
30. Boveri, T. (1929) *The Origin of Malignant Tumors* (Williams & Wilkins, Baltimore).
31. Kanduri, C., Holmgren, C., Pilartz, M., Franklin, G., Kanduri, M., Liu, L., Ginjala, V., Ulleras, E., Mattsson, R. & Ohlsson, R. (2000) *Curr. Biol.* **10,** 449–457.
32. Fearon, E. R. & Vogelstein, B. (1990) *Cell* **61,** 759–767.
33. Christofori, G., Naik, P. & Hanahan, D. (1994) *Nature (London)* **369,** 414–418.
34. Christofori, G., Naik, P. & Hanahan, D. (1995) *Nat. Genet.* **10,** 196–201.
35. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M. & Canaani, E. (1992) *Cell* **71,** 701–708.
36. Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A. & Delattre, O. (1998) *Nature (London)* **394,** 203–206.
37. Borrow, J., Stanton, V. P., Jr., Andresen, J. M., Becher, R., Behm, F. G., Chaganti, R. S., Civin, C. I., Disteche, C., Dube, I., Frischauf, A. M., *et al.* (1996) *Nat. Genet.* **14,** 33–41.
38. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J. P., Troalen, F., Trouche, D. & Harel-Bellan, A. (1998) *Nature (London)* **391,** 601–605.
39. Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J. & Kouzarides, T. (1998) *Nature (London)* **391,** 597–601.
40. Seeler, J. S., Marchio, A., Sitterlin, D., Transy, C. & Dejean, A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7316–7321.
41. Lengauer, C., Kinzler, K. W. & Vogelstein, B. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 2545–2550.