sues and oppositely deregulated in hyperplastic colorectal polyps and early adenomas. *Am J Pathol* 2000, 156:595–605

predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998, 58:5248–5257). *Cancer Res* 1999, 59:249–256

Genetic and Epigenetic Modification of MLH1

To the Editor-in-Chief:

I read with great interest the article by Kuismanen et al¹ that appeared in a recent issue of *The American Journal of Pathology*. At the end of the abstract, Kuismanen et al report that the differences in frequency of methylation at the hMLH1 promoter between sporadic and hereditary MSI+ tumors "... might explain some differences that are known to occur in the clinicopathological characteristics and tumorigenic pathways between sporadic and hereditary MSI+ colorectal cancers."

However, description of these differences is nowhere in the paper to be found, other than the alleged slender differences in anatomical location between sporadic and hereditary large bowel tumors with MSI (83 to 94% vs. 70%, respectively). The reference given to support this difference (Kim et al²) is inappropriate.

Kim et al compared the proximal/distal distribution between MSI+ colorectal tumors and hereditary nonpolyposis colorectal cancer (HNPCC) tumors. Kuismanen's reference to Kim et al was doubly incorrect, since not all non-HNPCC colorectal tumors are sporadic (including those analyzed by Kim et al, where they found two MSI+ tumors with documented family history) and not all HNPCC tumors are MSI+.

The experimental evidence does not substantiate the artificial distinction in genotype and phenotype between hereditary and sporadic MSI+ colorectal tumors³ (other than the presence or absence of germline mutations in mismatch repair genes). Attempts such as those by Kuismanen et al to find differences in clinicopathological characteristics and tumorigenic pathways between sporadic and hereditary MSI+ tumors, depending on whether MLH1 inactivation is achieved by mutation, loss of heterozygosity, or methylation, are destined to failure because the functional consequences are the same: mismatch repair deficiency and a mutator phenotype.⁴

Manuel Perucho

The Burnham Institute
La Jolla, California

References

1. Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomäki P: Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. *Am J Pathol* 2000, 156:1773–1779
2. Kim H, Jen J, Vogelstein B, Hamilton SR: Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994, 145:148–156
3. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR, et al: Clues to the pathogenesis of familial colorectal cancer. *Science* 1993, 260:812–816
4. Perucho M: Correspondence (re: A National Cancer Institute workshop on microsatellite instability for cancer detection and familial

Authors' Reply:

Since the first descriptions of tumorigenesis driven by DNA mismatch repair deficiency, it has been observed that the clinicopathological characteristics of microsatellite-unstable (MSI+) colorectal tumors from hereditary nonpolyposis colorectal cancer (HNPCC) patients and those from sporadic cases are largely similar¹ but not identical. Sidedness is one of the documented differences: even though both share proximal predilection, sporadic MSI+ tumors are even more proximal (83 to 94% are proximal, as compared to 70% of HNPCC tumors). Kim et al² is cited in our paper for providing a summary of the most important reports leading to the figures mentioned above. Our own definition for sporadic *versus* hereditary MSI+ tumors³ is based on the demonstrated absence *versus* presence of germline mutations in the DNA mismatch repair genes. Using these criteria, we have detected a similar difference in the colonic location between sporadic and hereditary cases,^{3–5} as indicated by Kim et al.²

An essential observation of our study is that although promoter hypermethylation appears to be the single most important mechanism associated with mismatch repair gene inactivation and MSI in colorectal tumors from sporadic cases, it is significantly less frequent in HNPCC tumors. We have previously shown that MLH1 promoter hypermethylation occurs not only in tumor tissue but in normal colonic mucosa as well, and that the hypermethylator phenotype, whether occurring in normal mucosa or tumor tissue, is associated with proximal location in the bowel.⁶ Widespread DNA methylation in sporadic MSI+ tumors, its location, and its prevalence in MSI+ tumors from HNPCC patients may therefore underlie the distinct clinicopathological profiles (such as sidedness) of these tumors. However, the exact mechanism is not known.

It is well established that the methylation tendency has several other target genes besides MLH1, and recent studies indicate an association between epigenetic and genetic profiles (eg, in terms of *K-ras* and *p53* mutations) in colorectal tumors.⁷ Furthermore, Herman et al⁸ report a differential involvement of *p16* by promoter hypermethylation in HNPCC tumors *versus* sporadic MSI+ cases.

These and other examples suggest that DNA methylation differences, coupled with selective involvement of target genes, can be associated with diverging developmental pathways in sporadic and hereditary tumors despite a similar MSI+ phenotype. Further studies are necessary to unravel the specific biological mediators of the individual clinicopathological properties and their involvement in colorectal tumors.

Päivi Peltomäki
Albert de la Chapelle

Ohio State University
Columbus, Ohio

Shannon A. Kuusmanen
Mari T. Holmberg
Reijo Salovaara

*University of Helsinki
Helsinki, Finland*

References

1. Lynch HT, de la Chapelle A: Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet* 1999, 36:801–818
2. Kim H, Jen J, Vogelstein B, Hamilton SR: Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994, 145:148–156
3. Kuusmanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomäki P: Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. *Am J Pathol* 2000, 156:1773–1779
4. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomäki P, Chadwick RB, Percesepe A, Kaariainen H, Ahtola H, Eskelinen M, Harkonen N, Julkunen R, Kangas E, Ojala S, Tulikoura J, Valkamo E, Jarvinen H, Mecklin J-P, de la Chapelle A: Incidence of hereditary nonpolyposis colorectal cancer and molecular screening for the disease. *N Engl J Med* 1998, 338:1481–1487
5. Moisio A-L, Sistonen P, Mecklin J-P, Jarvinen H, Peltomäki P: Genetic polymorphisms in carcinogen metabolism and their association to hereditary non-polyposis colon cancer. *Gastroenterology* 1998, 115:1387–1394
6. Kuusmanen SA, Holmberg MT, Salovaara R, Schweizer P, Aaltonen LA, de la Chapelle A, Nystrom-Lahti M, Peltomäki P: Epigenetic phenotypes distinguish microsatellite-stable and unstable colorectal cancers. *Proc Natl Acad Sci USA* 1999, 96:12661–12666
7. Toyota M, Ohe-Toyota M, Ahuja N, Issa J-P: Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype. *Proc Natl Acad Sci USA* 2000, 97:710–715
8. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa J-PJ, Markowitz S, Willson JKV, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB: Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998, 95:6870–6875