Short Communication

Endometrial and Colorectal Tumors from Patients with Hereditary Nonpolyposis Colon Cancer Display Different Patterns of Microsatellite Instability

Shannon A. Kuusmanen,^{*†} Anu-Liisa Moisio,^{*} Pascal Schweizer,[‡] Kaspar Truninger,[‡] Reijo Salovaara,^{*§} Johanna Arola,[§] Ralf Butzow,[¶] Josef Jiricny,[‡] Minna Nyström-Lahti,^{||} and Päivi Peltomäki^{*†}

From the Department of Medical Genetics,^{*} University of Helsinki, Helsinki, Finland; the Division of Human Cancer Genetics,[†] Comprehensive Cancer Center, Ohio State University, Columbus, Ohio; the Institute of Medical Radiobiology,[‡] University of Zürich, Zürich, Switzerland; the Department of Pathology,[§] Haartman Institute, University of Helsinki, Helsinki, Finland; the Department of Obstetrics and Gynaecology,[¶] Helsinki University Central Hospital, Helsinki, Finland; and the Division of Genetics,^{||} Department of Biosciences, University of Helsinki, Helsinki, Finland

The colorectum and uterine endometrium are the two most commonly affected organs in hereditary non-polyposis colon cancer (HNPCC), but the genetic basis of organ selection is poorly understood. As tumorigenesis in HNPCC is driven by deficient DNA mismatch repair (MMR), we compared its typical consequence, instability at microsatellite sequences, in colorectal and endometrial cancers from patients with identical predisposing mutations in the MMR genes *MLH1* or *MSH2*. Analysis of non-coding (BAT25, BAT26, and BAT40) and coding mononucleotide repeats (*MSH6*, *MSH3*, *MLH3*, *BAX*, *IGF2R*, *TGFβRII*, and *PTEN*), as well as *MLH1*- and *MSH2*-linked dinucleotide repeats (D3S1611 and CA7) revealed significant differences, both quantitative and qualitative, between the two tumor types. Whereas colorectal cancers displayed a predominant pattern consisting of instability at the BAT loci (in 89% of tumors), *TGFβRII* (73%), dinucleotide repeats (70%), *MSH3* (43%), and *BAX* (30%), no such single pattern was discernible in endometrial cancers. Instead, the pattern was more heterogeneous and involved a lower proportion of unstable markers per tumor (mean 0.27 for endometrial cancers versus 0.45 for colorectal cancers, $P < 0.001$) and shorter allelic

shifts for BAT markers (average 5.1 bp for unstable endometrial cancers versus 9.3 bp for colorectal cancers, $P < 0.001$). Among the individual putative “target” loci, *PTEN* instability was associated with endometrial cancers and *TGFβRII* instability with colon cancers. The different instability profiles in endometrial and colorectal cancers despite identical genetic predisposition underlines organ-specific differences that may be important determinants of the HNPCC tumor spectrum. (Am J Pathol 2002, 160:1953–1958)

Hereditary nonpolyposis colon cancer (HNPCC) is a multi-organ cancer syndrome associated with DNA mismatch repair (MMR) deficiency. Besides colon cancer, cancer of the uterine endometrium is common and occurs even more frequently than colon cancer in female patients with HNPCC.¹ In genetically predisposed individuals, one copy of a DNA mismatch repair gene is defective in all cells and may occasionally result in measurable hypermutability even in a heterozygous state.² Given that all organs are similarly predisposed to this hypermutability, a question arises as to why only a few of them are preferentially affected by cancer, giving rise to the “HNPCC tumor spectrum.”

Observations that link a frequent occurrence of endometrial cancer to predominantly *MSH2* mutations (as compared to *MLH1* mutations),³ and *MSH6* mutations in particular,⁴ suggest a differential role for the different predisposing mutations. This is further substantiated by mouse models, in which patterns of tumor susceptibility vary depending on which MMR gene is defective.^{5,6} Dur-

Supported by grants from the Sigrid Juselius Foundation, the Maud Kuistila Foundation, the Finnish Medical Foundation, the Academy of Finland, the Finnish Cancer Foundation, the European Commission (QLG1-CT-2000-01230), and National Institutes of Health grants CA67941, CA82282, and P30 CA16058.

Accepted for publication March 7, 2002.

Address reprint requests to Päivi Peltomäki, M.D., Ph.D., Biomedicum Helsinki, Department of Medical Genetics, P. O. Box 63 (Haartmaninkatu 8), FIN-00014 University of Helsinki, Finland. E-mail: paivi.peltomaki@helsinki.fi.

ing tumor development, the wild-type copy of the gene that is mutated in the germline is typically lost or mutated or inactivated by an epigenetic mechanism in a target tissue, rendering the cells completely MMR-deficient and hence accelerating tumor progression. Tissue-specific inactivation of the wild-type allele therefore offers a possible explanation for the HNPCC tumor spectrum. However, a recent investigation of *Msh2* hemizygous mice reported a poor correlation between tumor incidence and the loss of the wild-type allele, suggesting that other factors, such as exposure to exogenous mutagens, may be more important determinants of organ specificity.⁷

Instability at microsatellite sequences (MSI), a hallmark of HNPCC, occurs in some 15 to 25% of sporadic colorectal and endometrial cancers as well, but the underlying mechanism seems different (epigenetic rather than genetic).⁸⁻¹⁰ Some microsatellites occur as part of coding regions of important growth regulatory genes making these genes particular targets for mutations. Studies on sporadic MSI-positive colorectal and endometrial cancers have shown that even identical mutations in the target genes may be associated with different growth advantages in different tissues. Thus, the coding polyadenosine tracts of the tumor suppressor gene *RIZ* are fairly evenly altered in both tumor types¹¹ whereas those of *TGF β RII*¹² and *TCF4*¹³ are mainly involved in gastrointestinal cancers. This implies that the genesis of gastrointestinal and endometrial tumors occurs by different routes even if driven by generalized MSI.

While the involvement of defined microsatellite repeats has been extensively explored in sporadic MSI-positive tumors, recent observations have emphasized the importance of studying the familial and sporadic groups of microsatellite-unstable tumors separately, because of their different evolutionary pathways.¹⁴ The aim of the present study was to investigate colorectal and endometrial tumorigenesis by comparing the MSI patterns and possible target genes for MSI in these tumors. A particular advantage of the present investigation was that the tumors studied were from carriers of identical predisposing mutations, and in some cases even from the same patients, making the two series fully comparable. We demonstrate important differences between endometrial and colorectal tumors that provide further insights into the role of MSI in the development of these tumors.

Materials and Methods

Patients and Samples

Colorectal ($n = 44$) and endometrial cancers ($n = 57$) were collected from a series of well-characterized HNPCC families segregating germline mutations in either *MLH1* or *MSH2*.^{15,16} The distribution of germline mutations was similar in both groups, and in eight cases, both the colorectal and the endometrial cancer originated from the same patient. The *MLH1* mutations (nos. 1 to 8 in Figure 1) were as follows: mutation 1, 3.5-kb genomic deletion affecting codons 578–632 of exon 16 and flanking intron sequences; mutation 2, g>a at 454–1 at splice ac-

ceptor of exon 6; mutation 3, G>C at 1975 (codon 659) of exon 17; mutation 4, g>c at 1409 + 1 at splice donor of exon 12; mutation 5, T>G at 320 (codon 107) of exon 4; mutation 6, g>a at 1039–1 at splice acceptor of exon 12; mutation 7, g>t at 1559–1 at splice acceptor of exon 14; mutation 8, C>T at 1975 (codon 659) in exon 17. The *MSH2* mutation (no. 9) consisted of a deletion of CA at 1550 (codon 518) of exon 10. All human investigations were performed after approval of the local Institutional Review Boards.

DNA was prepared from archival paraffin-embedded tumor and normal tissue samples according to the method of Isola et al.¹⁷ When both tumor and normal tissue were derived from the same block, cancerous and normal areas were identified and carefully dissected under microscopic visualization from non-coverslipped hematoxylin and eosin-stained slides. Twenty 5- μ m sections were used for DNA extraction. To ensure histological representativeness, tumor percentages were determined before and after sectioning. Additional sections were made from the same tissue blocks for immunohistochemical analysis of MMR proteins, as described elsewhere.¹⁸

Analysis of MSI

Three non-coding mononucleotide repeat markers, BAT25, BAT26, and BAT40 (containing A₂₅, A₂₆, and A₄₀ repeats, respectively) were studied as described.^{19,20} The following coding mononucleotide repeats were studied using published primers and conditions: *MSH6*-C₈,²¹ *MSH3*-A₈,²¹ *IGF2R*-G₈,²² *BAX*-G₈,²³ and *TGF β RII*-A₁₀.²⁴ Primers were designed to analyze two mononucleotide repeats located in exon 1 of the *MLH3* gene as follows: for A₈ repeat, forward 5'-CAAAGATTAGCCAGCACTT-3', reverse 5'-TTTGCTACCTTCCTGAAAAG-3'; for A₉ repeat, forward 5'-GCCTTTTGCAACAACATTAT-3', reverse 5'-TCGCCCATAACTAAAAACAT-3'. Two repeats in the *PTEN* gene were studied with the following primers: for A₆ repeat in exon 7, forward 5'-GACGGGAAGACAAGTTCAT-3', reverse 5'-TTTGATATTTCTCCCAATG-3'; for A₆ repeat in exon 8, forward 5'-CAGAGGAAACCTCAGAAAAA-3', reverse 5'-TTGGCTTTGCTTTATTTC-3'. Additionally, dinucleotide repeat markers D3S1611 (located within *MLH1*) and CA7 (located close to *MSH2*) were studied as described.²⁵

Statistical Analysis

Fisher's exact test (two-tailed) or *t*-test (two-tailed) was used to assess differences between frequencies and means, respectively.

Results and Discussion

Distinct MSI Profiles for Endometrial and Colorectal Cancers

This investigation was based on 44 colorectal cancers and 57 endometrial cancers from carriers of eight *MLH1*

mutations and one *MSH2* mutation (see Materials and Methods). The marker-specific MSI patterns for individual cases are shown in Figure 1. Despite identical genetic predisposition (most cases were derived from carriers of either one of two common founding mutations, mutation 1 or 2, affecting *MLH1*), the MSI profiles of endometrial and

colorectal cancers showed significant differences. Colorectal cancers displayed a predominant pattern that consisted of instability at the non-coding BAT loci (at least one being unstable in 89% of tumors), *TGF β RII* (73%), dinucleotide repeats (at least one being unstable in 70% of tumors), *MSH3* (43%), and *BAX* (30%). In endometrial cancers, the pattern was more heterogeneous, typically involving different coding repeats in different tumors (eg, *TGF β RII* and *PTEN* instability were often mutually exclusive, see Figure 1B).

When the instability frequencies of the individual marker loci were compared in colorectal and endometrial cancers, *TGF β RII* emerged as a "target" gene for the former tumors, being unstable in 32 of 44 colorectal cancers (73%) versus 10 of 57 (18%) in endometrial cancers ($P = 2.2 \times 10^{-8}$). On the other hand, *PTEN* instability was associated with endometrial cancers occurring in 11 of 56 (20%) of these tumors, as compared to 2 of 44 (5%) of colorectal cancers ($P = 0.04$). The importance of the *TGF β RII* and *PTEN* genes, respectively, in the suppression of colorectal and endometrial tumors, both microsatellite-stable and -unstable, is well established,^{26–28} and their reported mutation frequencies in sporadic MSI-positive tumors are comparable to those we observed in our HNPCC tumors, even though the mutational hot spot areas may vary.^{12,29–34}

Lower Proportion of Unstable Loci in Endometrial Cancers

The mean proportion of unstable markers per tumor was significantly lower for endometrial cancers (0.27) than colorectal cancers (0.45) ($P < 0.001$). As an accentuation of this trend, 13 of 57 endometrial cancers (23%) were stable with all microsatellite markers studied as compared to 5 of 44 colorectal cancers (11%) (the difference was not statistically significant). Although tumors arising in MMR gene mutation carriers (either humans²⁹ or mice⁷) occasionally lack MSI, microsatellite stability was somewhat unexpected, because immunohistochemical analysis regularly demonstrated inactivation of the MMR protein corresponding to the germline mutation (see reference 18 and Figure 1). Normal tissue "contamination" could provide one possible explanation; however, histological evaluation of hematoxylin and eosin-stained slides suggested that this was not necessarily the

Figure 1. Comparison of the MSI profiles in colorectal (A) and endometrial (B) cancers originating from *MLH1* or *MSH2* mutation carriers. Mutations 1–8 affect *MLH1* while mutation 9 affects *MSH2*. The expression patterns of the proteins corresponding to the genes mutated in the germline (*MLH1* for cases with mutations 1–8, *MSH2* for cases with mutation 9), as determined by immunohistochemistry (IHC), are shown on the right. Case F67:6 in A is a colorectal adenoma. Eight patients were diagnosed with both endometrial and colorectal cancer, and the identification numbers of these individuals are in italics. The tumors were grouped into four categories according to their BAT and coding region instability patterns (among colorectal cancers, there were no cases in the "coding region instability only" category). Among genes with coding repeats, *MSH6*, *MSH3*, and *MLH3* are MMR genes, *BAX* is a proapoptotic gene, and *IGF2R*, *TGFBR1*, and *PTEN* are tumor suppressor genes. The percentage of (informative) tumors showing instability for each marker is indicated below the marker columns. MSI: **black**, unstable; **white**, stable; **gray**, not determined. IHC: **diagonal stripes**, not expressed; **gray**, not determined.

Figure 2. Distribution of allelic size shifts in colorectal *versus* endometrial tumors. The values on the x-axis represent the means of size deviations at the BAT25, BAT26, and BAT40 loci from the length of the germline alleles, calculated for each tumor and plotted against the number of cases. All size shifts were deletions.

case (the mean percentage of tumor cells was 40% and 50%, respectively, for stable and unstable endometrial cancers, and 60% and 50%, respectively, for stable and unstable colorectal cancers). Even with sufficiently high overall tumor percentages, intratumoral heterogeneity could lead to microsatellite-stable subpopulations in addition to microsatellite-unstable ones^{7,35,36} and the MSI result could then be negative or positive depending on which clones prevail. We consider this as a likely explanation in our case, and more sensitive dilution techniques² may serve useful to further clarify the MSI status of apparently stable tumors from MMR gene mutation carriers.

Shorter Allelic Shifts for BAT Markers in Endometrial Cancers

Not only was the proportion of unstable markers lower in endometrial cancers as compared to colon cancers, but the former tumors also showed significantly shorter allelic shifts with BAT markers. In endometrial cancers, the mean deviation (bp) from the germline allele was 4.1 (range, 1–7) for BAT25, 8.5 (range, 4–13) for BAT26, and 6.1 (range, 3–9) for BAT40. The same values for colorectal cancers were 6.7 (range, 4–11), 13.5 (range, 9–17), and 9.6 (range, 3–13) for BAT25, BAT26, and BAT40, respectively ($P < 0.001$ for the difference between endometrial and colorectal cancers in all cases). The size shifts for the individual BAT markers within each tumor were closely correlated, and in Figure 2, the average of the size shifts for BAT25, BAT26, and BAT 40 is shown for each unstable tumor. Calculated this way, the mean value was 5.1 (range, 1–12) bp for endometrial cancers *versus* 9.3 (range, 3–16) bp for colorectal cancers ($P < 0.001$).

Patients with Both Colorectal and Endometrial Cancer

Eight patients (F19:48, F43:23, F66:47, F90:49, F67:11, F105:24, F39:50, and F40:9) representing five different

Figure 3. A: Autoradiographs of mononucleotide repeat instability analysis of the *PTEN* (exon 7), *TGFβRII*, and BAT26 loci in paired cases of colorectal (C) and endometrial (E) tumors from the same patients. These loci are essentially monomorphic, and the position of the normal-sized fragment can be determined from lanes N₁ and N₂ that represent normal DNA samples from control individuals. **Arrowheads** denote fragments of aberrant size resulting from gains (endometrial tumor from F19:48, *TGFβRII*) or losses of mononucleotides (all other unstable cases, *PTEN* and *TGFβRII*). Shortening of the A₂₆ repeat within BAT26 gives rise to a ladder of extra fragments in the lower portion of the autoradiograph (+) that are absent in stable cases (–). **B:** Autoradiograph of BAT40 results, coded (+) or (–) as BAT26 above. Since BAT40 is polymorphic, the results from normal DNA of each individual (N) are shown adjacent to tumor lanes.

MLH1 germline mutations, were diagnosed with both colorectal and endometrial cancer and provided an ideal comparative setting for the evaluation of tumorigenesis in these two types of cancers (Figures 1 and 3). These cases corroborated all of the main trends described above for the larger groups. First, the average allelic size shifts for the BAT markers were smaller for endometrial than colorectal cancers (6.1 *versus* 10.5; see Figure 3). Second, the proportion of unstable markers per tumor was lower for endometrial than colorectal cancers (0.42 *versus* 0.58; see Figure 1). Third, *TGFβRII* mutations were more frequent in colorectal than endometrial cancers (7 of 8, 88% *versus* 2 of 8, 25%) whereas *PTEN* mutations occurred more often in endometrial than colorectal cancers (2 of 8, 25% *versus* 1 of 8, 13%; see Figure 3).

Clinicopathological Correlations

We have previously reported a correlation between local stages and small allelic shifts at the BAT loci in (mainly)

sporadic colon cancers.³⁰ In the present study, the clinical stage (according to the Dukes and International Federation of Obstetrics and Gynecology, (FIGO) classification, respectively) did not distinguish colorectal and endometrial cancers since most tumors were diagnosed at local stages (typically of HNPCC³⁷). In contrast, these two sets of tumors differed relative to their histological grade: while a significant proportion (44%) of colon cancers were poorly differentiated (grade 3), tumors with grade 3 constituted only a small fraction (22%) of endometrial cancers and most endometrial cancers were moderately or well-differentiated (grades 1 to 2). The average size shift showed a direct correlation with increasing tumor grade, being 9 bp for colon cancers with grades 1 to 2 versus 11 bp for those with grade 3, and 5 versus 7 bp for endometrial cancers with grades 1 to 2 versus 3, respectively.

Concluding Remarks

Apart from the likely selection in the case of functionally important mutations (such as those affecting *TGFBRII* in colorectal cancers and *PTEN* in endometrial cancers), the basis for the more general differences in the MSI profiles between endometrial and colorectal cancers is unknown. MSI carcinogenesis has been proposed to occur in two phases; beginning with the counterselective loss of MMR function in phenotypically normal cells, followed by rapid progression to malignancy, provided that mutations blocking apoptosis and senescence are able to rescue a MSI cell, the progenitor of the malignant clone.^{38,39} Although further studies are necessary to clarify this issue, we propose that the observed differences in the MSI profiles between endometrial and colorectal cancers may, in part, reflect the duration of tumor development. Studies of microsatellite alterations in colorectal cancers from HNPCC patients suggest that these cancers may develop for up to 13 years before diagnosis.⁴⁰ Moreover, most divisions contributing to the estimated "age" of the tumors are believed to occur before terminal clonal expansion that results in the appearance of molecularly distinct subclones. These estimates are based on sophisticated calculations taking the magnitude of deviation from the size of the germline allele at a given locus as well as the variability in the modal lengths at different loci into account.⁴⁰ By a rough extrapolation, the smaller allelic shifts and the more heterogeneous clonal patterns we observed for endometrial as compared to colorectal cancers might indicate a lower "age" of evolution for the former tumors. This is further supported by epidemiological observations of a gradual increase of colon cancer risk in HNPCC as a function of age, starting from age 20 to 30 and extending up to 60 to 70 years (with an average age at diagnosis of 42 years).^{41,42} In contrast, endometrial cancer risk shows a rapid and age-restricted increase from age 40 until menopause (with an average age at diagnosis of approximately 50 years), suggesting that hormone-related proliferation of the endometrium may provide a more narrow time window optimal for the development of these tumors.

Analogous to our findings, tumor-specific patterns of MSI and target gene involvement are emerging from comparative studies of other tumors from MMR gene mutation carriers.^{43,44} Increased understanding of factors that influence the susceptibility of different organs to cancer development may eventually aid in the design of appropriate surveillance and prevention strategies in HNPCC.

Acknowledgments

We thank Salla Saarinen for expert technical assistance.

References

1. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, Peltomäki P, Mecklin J-P, Järvinen H: Cancer risk in mutation carriers of DNA mismatch repair genes. *Int J Cancer* 1999, 81:214–218
2. Parsons R, Li G-M, Longley M, Modrich P, Liu B, Berk T, Hamilton SR, Kinzler KW, Vogelstein B: Mismatch repair deficiency in phenotypically normal human cells. *Science* 1995, 268:738–740
3. Vasen HFA, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, Nagengast FM, Meijers-Heijboer EH, Bertario L, Varesco L, Bisgaard M-L, Mohr J, Fodde R, Meera Khan P: Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 1996, 110:1020–1027
4. Wijnen J, de Leeuw W, Vasen H, van der Klift H, Møller P, Stormorken A, Meijers-Heijboer H, Lindhout D, Menko F, Vossen S, Möslin G, Tops C, Bröcker-Vriends A, Wu Y, Hofstra R, Sijmons R, Cornelisse C, Morreau H, Fodde R: Familial endometrial cancer in female carriers of MSH6 germline mutations. *Nat Genet* 1999, 23:142–144
5. Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM: Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1, and Pms2 DNA mismatch repair. *Nat Genet* 1998, 18:276–279
6. de Wind N, Dekker M, Claij N, Jansen L, van Klink Y, Radman M, Riggins G, van der Valk M, van't Wout K, te Riele H: HNPCC-like cancer predisposition in mice through simultaneous loss of Msh3 and Msh6 mismatch repair protein functions. *Nat Genet* 1999, 23:359–362
7. de Wind N, Dekker M, van Rossum A, van der Valk M, te Riele H: Mouse models for hereditary nonpolyposis colorectal cancer. *Cancer Res* 1998, 58:248–255
8. Esteller M, Levine R, Baylin SB, Hedrick Ellenson L, Herman JG: MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* 1998, 16:2413–2417
9. 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
10. Wheeler JMD, Loukola A, Aaltonen LA, Mortensen NJ, Bodmer WF: The role of hypermethylation of the hMLH1 promoter region in HNPCC versus MSI+ sporadic colorectal cancers. *J Med Genet* 2000, 37:588–592
11. Piao Z, Fang W, Malkhosyan S, Kim H, Horii A, Perucho M, Huang S: Frequent frameshift mutations of RIZ in sporadic gastrointestinal and endometrial carcinomas with microsatellite instability. *Cancer Res* 2000, 60:4701–4704
12. Myeroff LL, Parsons R, Kim S-J, Hedrick L, Cho KR, Orth K, Mathis M, Kinzler KW, Lutterbaugh J, Park K, Bang Y-J, Lee HY, Park JG, Lynch HT, Roberts AB, Vogelstein B, Markowitz SD: A transforming growth factor- β receptor type II gene mutation common in colon and gastric but rare in endometrial cancers. *Cancer Res* 1995, 55:5545–5547
13. Duval A, Iacopetta B, Ranzani GN, Lothe RA, Thomas G, Hamelin R: Variable mutation frequencies in coding repeats of TCF-4 and other target genes in colon, gastric, and endometrial carcinoma showing microsatellite instability. *Oncogene* 1999, 18:6806–6809

14. Young J, Simms LA, Biden KG, Wynter C, Whitehall V, Karamatic R, George J, Goldblatt J, Walpole I, Robin S-A, Borten MM, Stitz R, Searle J, McKeone D, Fraser L, Purdie DR, Podger K, Price R, Buttenshaw R, Walsh MD, Barker M, Leggett BA, Jass J: Features of colorectal cancers with high level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. *Am J Pathol* 2001, 159:2107-2115
15. Nyström-Lahti M, Wu Y, Moisio A-L, Hofstra RMW, Osinga J, Mecklin J-P, Järvinen HJ, Leisti J, Buys CHCM, de la Chapelle A, Peltomäki P: DNA mismatch repair gene mutations in 55 verified or putative kindreds with hereditary nonpolyposis colorectal cancer. *Hum Mol Genet* 1996, 5:763-769
16. Holmberg M, Kristo P, Chadwick RB, Mecklin J-P, Järvinen H, de la Chapelle A, Nyström-Lahti M, Peltomäki P: Mutation sharing, predominant involvement of MLH1 gene, and description of four novel mutations in hereditary nonpolyposis colorectal cancer. *Hum Mutat* 1998, 11:482
17. Isola J, DeVries S, Chu L, Ghazvini S, Waldman F: Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. *Am J Pathol* 1994, 145:1301-1308
18. Schweizer P, Moisio A-L, Kuismäki SA, Truninger K, Vierumäki R, Salovaara R, Arola J, Bützow R, Jiricny J, Peltomäki P, Nyström-Lahti M: Lack of MSH2 and MSH6 characterizes endometrial but not colon carcinomas in hereditary nonpolyposis colorectal cancer. *Cancer Res* 2001, 61:2813-2815
19. Hoang J-M, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R: BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. *Cancer Res* 1997, 57:300-303
20. Zhou X-P, Hoang J-M, Cottu P, Thomas G, Hamelin R: Allelic profiles of mononucleotide repeat microsatellites in control individuals and in colorectal tumors with and without replication errors. *Oncogene* 1997, 15:1713-1718
21. Malkhosyan S, Rampino N, Yamamoto H, Perucho M: Frameshift mutator mutations. *Nature* 1996, 382:499-500
22. Souza RF, Appel R, Yin J, Wang S, Smolinski KN, Abraham JM, Zou TT, Shi Y-Q, Lei J, Cottrell J, Cymes K, Biden K, Simms L, Leggett B, Lynch PM, Frazier M, Powell SM, Harpaz N, Sugimura H, Young J, Meltzer SJ: The insulin growth factor II receptor gene in colon cancers of the microsatellite mutator phenotype. *Nat Genet* 1996, 14:255-257
23. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M: Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997, 275:967-969
24. Parsons R, Myeroff L, Liu B, Willson J, Markowitz S, Kinzler K, Vogelstein B: Microsatellite instability and mutations in the transforming growth factor- β type II receptor gene in colorectal cancer. *Cancer Res* 1995, 55:5548-5550
25. Hemminki A, Peltomäki P, Mecklin J-P, Järvinen H, Salovaara R, Nyström-Lahti M, de la Chapelle A, Aaltonen LA: Loss of the wild-type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat Genet* 1994, 8:405-410
26. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M, Willson JKW: Inactivation of the type II TGF- β receptor in colon cancer cells with microsatellite instability. *Science* 1995, 268:1336-1338
27. Grady WM, Myeroff LL, Swinler SE, Rajput A, Thiagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim S-J, Kinzler KW, Vogelstein B, Willson JKW, Markowitz S: Mutational inactivation of transforming growth factor- β receptor type II in microsatellite-stable colon cancers. *Cancer Res* 1999, 59:320-324
28. Mutter GL, Lin M-C, Fitzgerald JT, Kum JB, Baak JPA, Lees JA, Weng L-P, Eng C: Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst* 2000, 92:924-931
29. Fujiwara T, Stolker JM, Watanabe T, Rashid A, Longo P, Eshleman JR, Booker S, Lynch HT, Jass JR, Green JS, Kim H, Jen J, Vogelstein B, Hamilton SR: Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. *Am J Pathol* 1998, 153:1063-1078
30. Percecepe A, Pedroni M, Sala E, Menigatti M, Borghi F, Losi L, Viel A, Genuardi M, Benatti P, Roncucci L, Peltomäki P, Ponz de Leon M: Genomic instability and target gene mutations in colon cancers with different degrees of allelic shifts. *Genes Chromosomes Cancer* 2000, 27:424-429
31. Shin K-H, Park YJ, Park J-G: PTEN gene mutations in colorectal cancers displaying microsatellite instability. *Cancer Lett* 2001, 174:189-194
32. Kong D, Suzuki A, Zou T-T, Sakurada A, Kemp LW, Wakatsuki S, Yokoyama T, Yamakawa H, Furukawa T, Sato M, Ohuchi N, Sato S, Yin J, Wang S, Abraham JM, Souza RF, Smolinski KN, Meltzer SJ, Horii A: PTEN1 is frequently mutated in primary endometrial carcinomas. *Nat Genet* 1997, 17:143-144
33. Guanti G, Resta N, Simone C, Cariola F, Demma I, Fiorente P, Gentile M: Involvement of PTEN mutations in the genetic pathways of colorectal cancerogenesis. *Hum Mol Genet* 2000, 9:283-287
34. Cohn DE, Basil JB, Venegoni AR, Mutch DG, Rader JS, Herzog TJ, Gersell DJ, Goodfellow PJ: Absence of PTEN repeat tract mutation in endometrial cancers with microsatellite instability. *Gynecol Oncol* 2000, 79:101-106
35. Habano W, Sugai T, Nakamura S: Mismatch repair deficiency leads to a unique mode of colorectal tumorigenesis characterized by intratumoral heterogeneity. *Oncogene* 1998, 16:1259-1265
36. Barnetson R, Jass J, Tse R, Eckstein R, Robinson B, Schnitzler M: Mutations associated with microsatellite-unstable colorectal carcinomas exhibit widespread intratumoral heterogeneity. *Genes Chromosomes Cancer* 2000, 29:130-136
37. Lynch HT, de la Chapelle A: Genetic susceptibility to nonpolyposis colorectal cancer. *J Med Genet* 1999, 36:801-818
38. Janin N: A simple model for carcinogenesis of colorectal cancers with microsatellite instability. *Adv Cancer Res* 2000, 77:189-221
39. Loeb LA: A mutator phenotype in cancer. *Cancer Res* 2001, 61:3230-3239
40. Tsao J-L, Yatabe Y, Salovaara R, Järvinen HJ, Mecklin J-P, Aaltonen LA, Tavare S, Shibata D: Genetic reconstruction of individual colorectal tumor histories. *Proc Natl Acad Sci USA* 2000, 97:1236-1241
41. Aarnio M, Mecklin JP, Aaltonen LA, Nyström-Lahti M, Järvinen HJ: Life-time risk of different cancers in hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer* 1995, 64:430-433
42. Dunlop MG, Farrington SM, Carothers AD, Wyllie AH, Sharp L, Burn J, Kinzler KW, Vogelstein B: Cancer risk associated with germline DNA mismatch repair gene mutations. *Hum Mol Genet* 1997, 6:105-110
43. Chan TL, Yuen ST, Chung LP, Ho JWC, Kwan K, Fan YW, Chan ASY, Leung SY: Germline hMSH2 and differential somatic mutations in patients with Turcot's syndrome. *Genes Chromosomes Cancer* 1999, 25:75-81
44. Southey MC, Young M-A, Whitty J, Mifsud S, Keilar M, Mead L, Trute L, Aittomäki K, McLachlan S-A, Debinski H, Venter DJ, Armes JE: Molecular pathologic analysis enhances the diagnosis and management of Muir-Torre syndrome and gives insight into its underlying molecular pathogenesis. *Am J Surg Pathol* 2001, 25:936-941