Frequent loss of heterozygosity at the DNA mismatch-repair loci \textit{hMLH1} and \textit{hMSH3} in sporadic breast cancer

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Summary To study the involvement of DNA mismatch-repair genes in sporadic breast cancer, matched normal and tumoral DNA samples of 22 patients were analysed for genetic instability and loss of heterozygosity (LOH) with 42 microsatellites at or linked to \textit{hMLH1} (3p21), \textit{hMSH2} (2p16), \textit{hMSH3} (5q11–q13), \textit{hMSH6} (2p16), \textit{hPMS1} (2q32) and \textit{hPMS2} (7p22) loci. Chromosomal regions 3p21 and 5q11–q13 were found hemizygously deleted in 46\% and 23\% of patients respectively. Half of the patients deleted at \textit{hMLH1} were also deleted at \textit{hMSH3}. The shortest regions of overlapping (SRO) deletions were delimited by markers \textit{D3S1298} and \textit{D3S1266} at 3p21 and by \textit{D5S647} and \textit{D5S418} at 5q11–q13. Currently, the genes \textit{hMLH1} (3p21) and \textit{hMSH3} (5q11–q13) are the only known candidates located within these regions. The consequence of these allelic losses is still unclear because none of the breast cancers examined displayed microsatellite instability, a hallmark of mismatch-repair defect during replication error correction. We suggest that \textit{hMLH1} and \textit{hMSH3} could be involved in breast tumorigenesis through cellular functions other than replication error correction.

Keywords: mismatch-repair; \textit{hMLH1}; \textit{hMSH3}; loss of heterozygosity; breast cancer

Breast cancer is the most frequent neoplasm that affects women in the Western world. It is a heterogeneous disease, which displays a broad spectrum of clinical and pathological characteristics, and like most solid tumours is thought to develop through the accumulation of genetic alterations leading to uncontrolled cellular growth. Loss of heterozygosity (LOH) studies in non-hereditary breast tumours have shown deletions at a frequency ranging from 20\% to 50\% in several chromosomal arms (reviewed in Sato et al, 1990; Cornelisse et al, 1992; Bièche and Lidereau, 1995), suggesting the involvement of several tumour-suppressor genes in breast carcinogenesis.

Recently, another type of gene, encoding components of the DNA mismatch-repair system, has been linked to hereditary non-polyposis colorectal cancer (HNPPCC) (Fishel et al, 1993; Leach et al, 1993; Bronner et al, 1994; Nicolaides et al, 1994; Papadopoulos et al, 1994, 1995). These genes have been found mutated in HNPPCC and are presumably involved in certain sporadic forms of cancer (Leach et al, 1993; Nicolaides et al, 1994; Papadopoulos et al, 1994, 1995; Liu et al, 1995; Risinger et al, 1996). Their defects generally lead to a genome-wide instability of microsatellites in tumoral cells referred to as the replication error (RER) phenotype. In addition to HNPPCC, the RER phenotype was observed in a number of sporadic cancers (Yee et al, 1994; Karnik et al, 1995; Paulson et al, 1996), thus suggesting that deficiency in DNA repair could be involved in breast carcinogenesis. Like suppressor genes (Knudson, 1971), mismatch-repair mutants are inherited as recessive traits that eventually become dominant because of somatic mutations inactivating the second allele. This second mutational step may be revealed as LOH which was reported at the \textit{hMLH1} locus in HNPPCC patients (Hemminki et al, 1994) as well as in sporadic colorectal cancers (Tomlinson et al, 1996).

Based on these observations, we examined the involvement of mismatch-repair genes in sporadic breast cancer by microsatellite instability and LOH analyses. We have screened 22 primary breast carcinomas using 42 polymorphic microsatellites within or closely linked to \textit{hMLH1}, \textit{hMSH2}, \textit{hMSH3}, \textit{hMSH6}, \textit{hPMS1} and \textit{hPMS2} loci. We found that \textit{hMLH1} and \textit{hMSH3} were frequently deleted in tumoral cells, suggesting their possible involvement in sporadic breast cancer.

MATERIALS AND METHODS

DNA samples

Matched tumoral and normal sample pairs were obtained from 22 breast carcinoma patients (ages 40–90; mean, 58.09; median, 60), including ten metastatic cases, who underwent surgery at the Montreal Hôtel-Dieu Hospital. This is an unselected group of apparent sporadic cases with limited clinical information. Because family histories were unavailable, it was expected that, if any, only 5–10\% of the samples would be from patients with a familial form of the disease (Newman et al, 1988). DNA was isolated from fresh material by a standard procedure using digestion with proteinase K and phenol/chloroform extractions.
Microsatellite analysis

Matched DNA sample pairs were genotyped by polymerase chain reaction (PCR) at the 42 following highly polymorphic (62–90% heterozygosity) microsatellite loci: on chromosome 3p14–p26 (hMLH1), D3S1286, D3S1266, D3S1745, D3S1561, D3S1611, D3S1612, D3S1298, D3S1260, D3S3559, D3S3582, D3S3647, D3S1611, D3S1298, D3S1260, D3S3559, D3S3582, D3S1613, D3S1234, D3S1300 and D3S1312; on 5p14–q21 (hMSH3), D5S416, D5S477, D5S651, D5S674, D5S426, D5S395, D5S418, D5S430, D5S491, D5S398, D5S426, D5S424, D5S668, D5S647, D5S629, D5S428 and D5S433; on 2q32 (hPMS1), D2S318 and D2S118; on 2p16 (hMSH2/hMSH6), D2S391 and D2S288; on 7p22 (hPMS2), D7S531 and D7S517. The corresponding PCR primers were provided by Research Genetics. The chromosomal assignment of these microsatellites and genes was performed by integrating genetic, radiation hybrid and STS/YAC data from several sources (Gyapay et al, 1994; Hudson et al, 1995; Gemmill et al, 1995). Thirty amplification cycles of 1 min at 94°C, 1 min at 50–60°C and 1 min at 72°C were carried out in 20 μl of 10 mM tris-HCl (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride containing 0.2 mM of each primer, 50 μM dNTPs, 1 μCi of [32P]dCTP (ICN; specific activity 3000 Ci mmol−1), 5 ng of genomic DNA, and 0.4 U Taq DNA polymerase (BRL). The products were fractionated by denaturing electrophoresis in a 6% polyacrylamide gel, subsequently dried and autoradiographed. LOH was defined visually as the disappearance or significant reduction in the intensity of one allele in tumoral DNA compared with the normal DNA sample as described in Baccichet et al (1997). Only informative (heterozygous) loci were considered for LOH frequency calculations.

Single-strand conformational polymorphism (SSCP) analysis

The typing of hMLH1 exon 8 polymorphism by SSCP analysis using previously published oligonucleotides (Han et al, 1995) was performed as described in Zietkiewicz et al (1992).

RESULTS

Our microsatellite analysis (Figure 1) revealed LOH in 10 out of the 22 patients in at least one of the mismatch-repair loci tested (Table 1).

Detection of LOH on chromosome 3p21

Out of the 22 patients, ten (46%) exhibited LOH in at least one of the microsatellite markers located on chromosome 3p21. Patient 3 lost an allele at D3S1745 and D3S1561, but maintained heterozygosity at the distal neighbouring locus D3S1266; patient 43 showed LOH at every marker distal to D3S1611 and D3S1612, but retained both alleles at D3S1298 (Figure 2). These results suggested that the shortest region of overlapping (SRO) deletions delimited by D3S1298 and D3S1266 included hMLH1 at 3p21–p22 (Figure 2). In addition to the intragenic D3S1611 marker (Papadopoulos et al, 1994), we analysed by SSCP a biallelic polymorphism in exon 8 of hMLH1 to show hemizygous deletion in the three informative cases (not shown).

Several genes have been shown to be included in LOH regions on chromosome 3p (Figure 2). We extended the alleotyping to investigate the possible involvement of the SCLC region, which was shown to be homozygously deleted in small-cell lung cancer cell lines (Daly et al, 1993), as well as the FHIT and PTPRG genes (Figure 2). Among the ten patients with LOH at hMLH1, four

| Cases | hMLH1 (3p21) | hMSH3 (5q11–q13) | hPMS1 (2q32) | hMSH2 hMSH6 (2p16) | hPMS2 (7p22) |
|-------|-------------|----------------|--------------|------------------|--------------|
| 1     | LOH         | H             | H            | H                | H            |
| 3     | LOH         | H             | H            | H                | H            |
| 5     | H           | H             | H            | H                | H            |
| 7     | H           | H             | H            | H                | H            |
| 9     | LOH         | LOH           | LOH          | LOH              | LOH          |
| 11    | LOH         | H             | H            | H                | H            |
| 13    | LOH         | H             | H            | H                | H            |
| 15    | H           | H             | H            | H                | H            |
| 17    | LOH         | H             | H            | H                | H            |
| 19    | H           | H             | H            | H                | H            |
| 21    | H           | H             | H            | H                | H            |
| 23    | H           | H             | H            | H                | H            |
| 25    | H           | H             | H            | H                | H            |
| 27    | H           | H             | H            | H                | H            |
| 29    | LOH         | LOH           | H            | H                | H            |
| 31    | H           | H             | H            | H                | H            |
| 33    | LOH         | H             | H            | H                | H            |
| 35    | H           | H             | H            | H                | H            |
| 37    | LOH         | H             | H            | H                | H            |
| 39    | H           | H             | H            | H                | H            |
| 41    | H           | H             | H            | H                | H            |
| 43    | LOH         | H             | H            | H                | H            |

Table 1: Summary of the LOH data for the analysed mismatch repair-related chromosomal regions

LOH, loss of heterozygosity, H, heterozygote, NI, non-informative.

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The deleted alleles in tumours; (N) normal and (T) tumoral DNA

Patients 3, 5, 11 and 13 analysed with marker D5S427; arrowheads indicate regions. Patients 9, 11, 25 and 35 analysed with marker D3S1745 and Figure 1

Examples of LOH and heterozygosity of the 3p21 and 5q11–q13 regions. Patients 9, 11, 25 and 35 analysed with marker D5S427; arrowheads indicate the deleted alleles in tumours; (N) normal and (T) tumoral DNA

Detection of LOH on chromosome 5q11–q13

As shown in Figure 3, 5 out of the 22 informative cases (23%) were hemizygously deleted at one or more marker loci tightly linked to the hMSH3 locus. All of these patients with LOH at 5q11–q13 were also deleted in the 3p21 region (Figure 2 and 3). This non-random distribution of concomitant deletions was statistically significant (P ~ 0.02, chi-squared test). Large deletions were seen in patients 9 and 37, whereas others exhibited restricted LOH: patient 13 with LOH at DSS430 retained heterozygosity at every marker proximal to DSS418, whereas patient 29 was heterozygous at DSS647 thus delimiting the SRO between DSS418 and DSS647 at 5q11–q13 (Figure 3). The tumour-suppressor genes APC and MCC both located on chromosome 5q21 were excluded from deletions involving hMSH3 in two of the patients (13 and 29) heterozygous for markers linked to APC and MCC regions (Figure 3). Thus, hMSH3 is a good candidate as the target of 5q11–q13 deletions.

In contrast to hMLH1 and hMSH3, we found only two patients that were affected by allelic deletions at other tested loci: 1 out of 21 informative cases at hPMS2, 1 out of 20 at hMSH2/hMSH6 and 2 out of 20 at hPMS1 (Table 1), including patient 9 who displayed LOH at all investigated loci. The low rate of allelic losses (5–10%) affecting the chromosomes 2p16, 2q31–q33 and 7p15–pter may reflect the baseline frequency of LOH in breast cancer (Chen et al, 1992).

Microsatellite instability

None of the 22 tumours displayed the RER phenotype as judged by the absence of instability at the 42 microsatellites tested (a total of 572 independent comparisons). Although our markers were already shown as sensitive to detecting RER in sporadic colorectal cancers (Benachenhou et al, 1998a), we additionally examined two markers GGAA4D07 and GGAA2E02 recently reported as unstable in 30% (11 out of 37) and 41% (15 out of 37) of breast cancer patients respectively (Paulson et al, 1996). These two markers did not reveal any instability in the 22 tumours examined here (data not shown). Our findings were thus consistent with studies of two large cohorts of breast cancer patients that failed to detect significant levels of microsatellite instability (Lothe et al, 1993; Wooster et al, 1994).
DISCUSSION

The activation of oncogenes, the loss or inactivation of repressor genes and impaired mismatch-repair function are known to be involved in the development of solid tumours. Defects in DNA mismatch-repair genes lead to replication errors revealed as instability in microsatellite markers (Leach et al, 1993; Brunner et al, 1994; Papadopoulos et al, 1994). A proposal that deficient DNA repair was a predisposing factor in sporadic breast cancer (Helzlzouer et al, 1996; Parshad et al, 1996) was promoted by reports of microsatellite instability in breast tumours (Yee et al, 1994; Karnik et al, 1995; Paulson et al, 1996). By allelotyping the mismatch-repair genes hMLH1, hMSH2, hMSH3, hMSH6, hPMS1 and hPMS2, we have shown that 46% and 23% of the breast tumours tested were affected by allelic losses at hMLH1 and hMSH3 respectively. Because none of the tumour tissues were microdissected, these figures should be considered conservative as some allelic losses could have been masked by contaminating genetic material of normal cells. Other alterations such as small deletions, point mutations, gene rearrangements, or DNA methylation, if they also contribute to inactivation of these loci, could escape detection by our approach. Further studies are required to explore these possibilities.

Interstitial deletion of chromosome 3p is one of the most common genetic rearrangements observed in tumour cells (Pandis et al, 1993). The region 3p14–p23 has been shown to be deleted in small-cell lung carcinomas (Petersen et al, 1997), non-small-cell lung carcinomas (Benachenhou et al, 1998b) renal cell carcinomas (Foster et al, 1994) and uterine cervical carcinomas (Kohno et al, 1993). In breast cancer, LOH ranging from 30% to 47% were observed at two separate regions, 3p13–p14 and 3p21–p25 (Chen et al, 1993). The region 3p14–p23 has been shown to be deleted in common genetic rearrangements observed in tumour cells (Pandis et al, 1993; Karnik et al, 1995; Paulson et al, 1996). By allelotyping the mismatch-repair genes hMLH1, hMSH2, hMSH3, hMSH6, hPMS1 and hPMS2, we have shown that 46% and 23% of the breast tumours tested were affected by allelic losses at hMLH1 and hMSH3 respectively. Because none of the tumour tissues were microdissected, these figures should be considered conservative as some allelic losses could have been masked by contaminating genetic material of normal cells. Other alterations such as small deletions, point mutations, gene rearrangements, or DNA methylation, if they also contribute to inactivation of these loci, could escape detection by our approach. Further studies are required to explore these possibilities.

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development of cancer through defects in genes sensitive to exoge-
nous factors (Mellon et al, 1996; DeWeese et al, 1997). In addition,
a heterozygous mutation in hMLH1 in a human-derived cancer cell
line was shown to significantly reduce transcription-coupled repair
involved in selective removal of DNA damage from the tran-
scribed strands of active genes (Mellon et al, 1996). The possibility that allelic deletion of hMLH1 and/or hMSH3 could have the same effect. We propose that a subtle defect in the repair of DNA damage, which is less likely to be lethal to the carrying cells, could have an even more profound impact on tumorigenesis, thus placing individuals at increased cancer risk.

In conclusion, our allelotyping analysis of sporadic breast carci-
nomas demonstrated that two DNA mismatch-repair loci, hMLH1
and hMSH3, are frequently affected by LOH at chromosomal regions 3p21 and 5q11–q13 respectively. We suggest that hMLH1
and hMSH3 deletion could promote cancer progression through a
dosage effect affecting cellular functions other than replication
errors correction. Whether or not hMLH1 and hMSH3 are real
targets of the deletions is still under investigation, but the identifi-
cation of genes with suppressor activity for malignancy at 3p21
and 5q11–q13 is extremely important considering the frequency of
LOH at these regions in several major forms of cancer.

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REFERENCES

Arzimanoglou II, Gilbert F and Barber HRK (1998) Microsatellite instability
in human solid tumors. Cancer 82: 1808–1820
Shion-Rickart PG, Wyllie AH, Bird CC, Dunlop MG, Steel CM, Morris RG, Piris
J, Romanooski P, Wood R, White R, Nakamura Y (1991) MCC, a candidate
familial polyposis gene in 5p15.3 shows frequent allele loss in colorectal
and lung cancer. Oncogene 6: 1881–1886
Baccchiet A, QuaIman SA and Sinnett D (1997) Allelic loss in childhood acute
lymphoblastic leukemia. Leukemia Res 21: 817–823
Barnes LD, Garrison PN, Supravilul Z, Guranowski A, Robinson AK, Ingram SW,
Croce CM, Oltla M and Huebner K (1996) Phl, a putative tumor suppressor in
humans, is a dinucleoside 5′,5′′-P1,P3 triphosphate hydrolase. Biochemistry
35: 11529–11535
Benachenhou N, Guiral S, Gorska-Flipot I, Michalski R, Labuda D and Sinnett D
(1998a) Allelic losses and DNA methylation at DNA mismatch-repair loci in
sporadic colorectal cancer. Carcinogenesis (in press)
Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D and Sinnett D (1998b) High
resolution deletion mapping reveals frequent allelic losses at the DNA
mismatch-repair loci in hMLH1 and hMSH3 in non-small cell lung cancer. Int J
Cancer 77: 173–180
Blecher I and Liderau R (1995) Genetic alterations in breast cancer. Genes
Chromosomes Cancer 14: 227–251
Boynton RF, Blouant PL, Yin J, Brown VL, Huang Y, Tong Y, McDaniel T, Newkirk
C and Resau JH (1992) Loss of heterozygosity involving the APC and MCC
loci occurs in the majority of human esophageal cancers. Proc Natl
Acad Sci USA 89: 3385–3388
Bromer CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescce MK, Kane M,
Earabin C, Lipfard J, Lindblom A, Tannergard P, Bollag R, Godwin AR,
Ward DC, Nordenskjold M, Fisel R, Kolodner R and Liskay RM (1994)
Mutation in the DNA mismatch repair gene homologue hMLH1 is associated
with hereditary non-polyposis colon cancer. Nature 368: 258–261
Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore Jr D and Smith HS (1992)
Heterogeneity for allelic loss in human breast cancer. J Natl Cancer Inst 84:
506–510
Chen LC, Matsumura K, Deng G, Kurisu W, Ljung BM, Lerman ML, Waldman FM
and Smith HS (1994) Deletion of two separate regions on chromosome 3p in
breast cancers. Cancer Res 54: 3021–3024
Cornellie CJ, Kuipers-Dijkstra N, van Vliet M, Hermans J and Devilee P (1992)
Fractional allelic imbalance in human breast cancer increases with
tetraploidyization and chromosome loss. Int J Cancer 50: 544–548
Daly MC, Xiang RR, Buchhagen D, Hensel CH, Garcia DK, Killary AM, Minna J
and Naylor SL (1995) A homozygous deletion on chromosome 3 in a small cell
lung cancer cell line correlates with a region of tumor suppressor activity.
Oncogene 8: 1721–1729
D’Amico D, Carbone DP, Johnson BE, Meltzer J and Minna JD (1992) Polymorphic
sites within the MCC and APC loci reveal very frequent loss of heterozygosity
in human small cell lung cancer. Cancer Res 52: 1996–1999
DeWeese TL, Bucci JM, Larrier NA, Cutler RG, te Riele H and Nelson WG (1997)
Tolerance of oxidative DNA damage results from disruption of Msh2 alleles:
implications for carcinogenesis. In Proceedings of the 88th Annual Meeting
of the American Association for Cancer Research, Vol. 38, AACR (eds).
CADMUS Journal Services: Lithicum MD, USA
Futcher B, Derks NC, Ruddle FH, Ferrans VJ, Schachter CE, Reddel RR, Booser
D, Wallinger T, Gocker M, Fishel R and Rüschoff J (1997) Diagnostic microsatellite instability: definition and correlation with mismatch
repair protein expression. Cancer Res 57: 4749–4756
Drummond JT, Li GM, Longley MD and Medrich P (1995) Isolation of an
hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells.
Science 268: 1099–1112
Fishel R, Lescce MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M
and Kolodner R (1993) The human mutator gene homolog MSH2 and its
association with hereditary nonpolyposis colon cancer. Cell 75: 1027–1038
Foster K, Crossey PA, Cairns P, Hetherington JW, Richards FM, Jones MH, Bentley
E, Affara NA, Ferguson-Smith MA and Maher ER (1994) Molecular genetic
investigation of sporadic renal cell carcinoma: analysis of allele loss on
chromosomes 3p, 5q, 11p, 17 and 22. Br J Cancer 69: 230–234
Gemmill RM, Chumakov I, Scott P, Waggoner B, Rigault P, Cypser J, Chen Q,
Weisbenach J, Gardiner K, Wang H, Pekarsky Y, Le Gall I, Le Paslier D,
Guilou S, Li E, Robinson L, Hahner L, Toid S, Cohen D and Drabkin HA
(1995) A second-generation YAC contig map of human chromosome 3. Nature
377 (suppl.): 299–319
Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S,
Bernardi G, Lathrop M and Weissenbach J (1994) The 1993–94 Genethon
human genetic linkage map. Nature Genet 7: 246–339
Hammack P, Gabbani B, Osterholm A-M, Hellgren D and Lambert B (1995)
Spontaneous long segment microsatellite DNA from human T-cell clones.
Genes Chromosomes Cancer 14: 215–219
Han HJ, Yangasawa A, Kato Y, Park JG and Nakamura Y (1993) Genetic instability
in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer
Res 53: 5087–5089
Han HJ, Murayama M, Baba S, Park JG and Nakamura Y (1995) Genomic structure
of human mismatch repair gene, hMLH1, and its mutation analysis in patients
with hereditary non-polyposis colorectal cancer (HNPCC). Hum Mol Genet 4:
237–242
Hawnt MT, Umar A, Carethers JM, Marra G, Kunkel TA, Boland CR and Koi M
(1995) Evidence for a connection between the mismatch repair system and the
G2 cell cycle checkpoint. Cancer Res 55: 3721–3725
Helzouker RJ, Harris EL, Parasbud R, Perry HR, Price FM and Sanford KK (1996)
DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl
Cancer Inst 88: 754–755
Hermminki A, Peltonakki P, Mecklin JP, Haag J, Salovaara R, Nystrom-Lahti M,
de la Chapelle A and Aaltonen LA (1994) Loss of the wild-type MLH1 gene
is a feature of hereditary nonpolyposis colorectal cancer. Nature Genet 8:
405–410
Hislop TJ, Stein LD, Gerety SM, Ma J, Castle AB, Silva J, Slamik DM, Baptista R,
Kruglyk L, Xu S-H, Hu X, Colbert AME, Rosenberg C, Reeves-Daly MP,
Rozen S, Hui L, Wu X, Vestergaard C, Wilson KM, Bae JS, Maira S,
Gianatas S, Evans CA, DeAngelis MM, Ingals K, Nahil RW, Horton JR, LT,
Anderson MO, Collimore AJ, Ye W, Kouyoumjian V, Zemsteva IS, Yam T,
Devine R, Courtney DF, Renaud MA, Nguyen H, O’Connor TJ, Fizames C,
Fauré S, Gyapay G, Dib C, Morissette J, Orlando RJ, Birren BW, Goodman N,
Weisbenach J, Hawkins TL, Foiite S, Page DC and Lander ES (1995) An
STS-based map of the human genome. Science 270: 1945–1954
Jones M, Wagner R and Radman M (1987) Mismatch repair and recombination in
E. coli. Cell 50: 621–626
Kohno T, Takayama H, Hamaguchi M, Takano H, Yamaguchi N, Tsuda H, Hirohashi Knudson Jr AG (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820–823

Kohno T, Takaayama H, Hamaguchi M, Takano H, Yamaguchi N, Tsuda H, Hirohashi (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820–823

Kat A, Thilly WG, Fang WH, Longley MJ, Li GM and Modrich P (1993) An L Liu B, Nicolaides NC, Markowitz S, Willson JK, Parsons RE, Jen J, Papadopolous Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM and Huebner K (1993) Receptor protein–tyrosine phosphatase is a candidate tumor suppressor gene at human chromosome region 3p21. Proc Natl Acad Sci USA 88: 5036–5040

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Pelтомаки P, Sistonen P, Ailtonon LA, Nystrom-Lahti M, Guan XY, Zhang J, Meltzer PS, Yu JW, Kao FT, Chen DJ, Cerossaleti KM, Fourner REK, Todd S, Lewis T, Leach RJ, Naylòr SL, Weissenbach J, Mecklin JP, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch TT, Trent JM, de la Chapelle A, Kinzler KW and Vogelstein B (1993) Mutation of a mismatch repair gene in hereditary nonpolyposis colorectal cancer. Cell 75: 1215–1225

Liu B, Nicolaides NC, Markowitz S, Willson JK, Parsons RE, Jen J, Papadopoulos N, Pelтомаки P, de la Chapelle A, Hamilton SR, Kinzler KW and Vogelstein B (1993) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nature Genet 4: 48–55

Lothe RA, Pelтомаки P, Meling GI, Ailtonon LA, Nystrom-Lahti M, Pyhlikan L, Heimdal K, Andersen TI, Moller P, Rognum TO, Fossa SD, Halvorsen T, Langmark F, Brogger A, de la Chapelle A and Borresen AL (1993) Genomic instability in colorectal cancer: relationship to clinico-pathological variables and family history. Cancer Res 53: 5849–5852

Man S, Ellis IO, Sibberring M, Blamey RW and Brooker JK (1996) High levels of allele loss at the FHIT and ATM genes in non-comedo ductal carcinoma in situ and grade I tubular invasive breast cancers. Cancer Res 56: 5484–5489

Matsumoto S, Kasumi F, Sakamoto G, Onda M, Nakamura Y and Emi M (1997) Detailed deletion mapping of chromosome arm 3p in breast cancers: a 2-cM region on 3p14.3–21.1 and a 5-cM region on 3p24.3–25.1 commonly deleted in carcinomas. Genes Chromosomes Cancer 20: 268–274

Medeiros AC, Nagai MA, Neto MM and Brentani RR (1994) Loss of heterozygosity in colorectal cancer: evidence for autosomal dominant transmission in high risk families. Cancer Res 54: 2673–2675

Mellon I, Rajpal DK, Koi M, Boland CR and Champe N (1996) Transcription-coupled deficiency and mutations in mismatch repair genes. Science 272: 557–560

Mu D, Turson M, Duckett DR, Drummond JT, Modrich P and Sanxcar A (1997) Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. Mol Cell Biol 17: 760–769

Negri M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K and Croce CM (1996) The FHIT gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res 56: 3173–3179

Newman B, Austin MA, Lee M and King MC (1998) Inheritance of human breast cancer: evidence for autosomal dominant transmission in high risk families. Proc Natl Acad Sci USA 85: 3044–3048

Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haselwina TA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Petersen GM, de la Chapelle A, Vogelstein B and Kinzler KW (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371: 75–80

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM and Huebner K (1996) The human FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma associated translocation breakpoint, is abnormal in digestive tract cancers. Cell 84: 587–597

Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T and Jircy J (1996) The hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. Curr Biol 6: 1181–1184

Pandis N, Jin Y, Limon J, Bardi G, Idvall I, Mandahl N, Mitalien F and Heim S (1993) Interstitial deletion of the short arm of chromosome 3 as a primary chromosome abnormality in carcinomas of the breast. Genes Chromosomes Cancer 6: 151–155

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haselwina TA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Pelтомаки P, Mecklin JP, de la Chapelle A, Kinzler KW and Vogelstein B (1994) Mutation of a mismatch repair gene in hereditary colon cancer. Science 263: 1625–1629

Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D’Arrigo A, Markowitz S, Willson JK, Kinzler KW, Jircy J and Vogelstein B (1995) Mutations of GTTPB in genetically unstable cells. Science 286: 1915–1917

Parshad R, Price FM, Bohl VA, Cowans KH, Zajewski JA and Sanford KK (1996) Deficient DNA repair capacity, a predisposing factor in breast cancer. Br J Cancer 74: 1–5

Paulson TJ, Wright FA, Parker BA, Russack V and Walh GM (1996) Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res 56: 4021–4026

Peter sensor I, Langreck H, Wolf G, Schwendel A, Psille R, Vogt P, Reichel MB, Ried T and Dietel M (1997) Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. Br J Cancer 75: 79–86

Risinger JL, Umar A, Boyd J, Berchuck A, Kunkel A and Barrett JC (1996) Mutation in MSH2 in endometrial cancer and evidence for its functional role in repair. Nature Genet 4: 102–105

Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G and Nakamura Y (1990) Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res 50: 7184–7189

Solomon E, Voss R, Hall V, Bodmer WF, Jass JR, Jeffreys AJ, Lucibello FC, Patel I and Rider SH (1978) Chromosome 5 allele loss in human colorectal carcinomas. Nature 328: 616–619

Speicher MR (1995) Microsatellite instability in human cancer. Oncol Res 7: 267–275

Tavassoli M, Steingrimsdottir H, Pierce E, Jiang X, Alagoz F, Farzanefar H and Campbell IG (1996) Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by TP53 mutation and identifies a tumor suppressor gene locus at 5q13.1–21. Br J Cancer 74: 115–119

Thompson AM, Morris RG, Wallace M, Wyatt M, Steel CM and Carter DC (1993) Allele loss from 5q21 (APC/MCC) and 18q21 (DCC) and DCC mRNA expression in breast cancer. Br J Cancer 68: 64–68

Tolminson IP, Ilyas M and Bodmer WF (1996) Allele loss occurs frequently at hMLH1, but rarely at hMSH2, in sporadic colorectal cancers with microsatellite instability. Br J Cancer 74: 1514–1517

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525–532

Wooster R, Clanton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A, Wiestler OD, Corneilles CJ, Devilee P and Stratton MR (1994) Instability of short tandem repeats (microsatellites) in human cancers. Nature Genet 6: 152–156

Yee CJ, Roodi N, Verrier CS and Parl FF (1994) Microsatellite instability and loss of heterozygosity in breast cancer. Cancer Res 54: 1641–1644

Zietkiewicz E, Sinnett D, Richer C, Mitchell G, Vanasse M and Labuda D (1992) Single-strand conformational polymorphism (SSCP): detection of useful polymorphisms at the dystrophin locus. Hum Genet 89: 453–456

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