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Microsatellite Instability, Mismatch Repair Deficiency, and Genetic Defects in Human Cancer Cell Lines

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ABSTRACT

The instability of short repetitive sequences in tumor DNA can result from defective repair of replication errors due to mutations in any of several genes required for mismatch repair. Understanding this repair pathway and how defects lead to cancer is being facilitated by genetic and biochemical studies of tumor cell lines. In the present study, we describe the mismatch repair status of extracts of 22 tumor cell lines derived from several tissue types. Ten were found to be defective in strand-specific mismatch repair, including cell lines from tumors of the colon, ovary, endometrium, and prostate. The repair defects were independent of whether the signal for strand specificity, a nick, was 5′ or 3′ to the mismatch. All 10 defective cell lines exhibited microsatellite instability. Repair activity was restored to 9 of these 10 extracts by adding a second defective extract made from cell lines having known mutations in either the *hMSH2* or *hMLH1* genes. Subsequent analyses revealed mutations in *hMSH2* (4 lines) and *hMLH1* (5 lines) that could explain the observed microsatellite instability and repair defects. Overall, this study strengthens the correlation between microsatellite instability and defective mismatch repair and the suggestion that diminuition in mismatch repair activity is a step in carcinogenesis common to several types of cancer. It also provides an extensive panel of repair-proficient and repair-deficient cell lines for future studies of mismatch repair.

INTRODUCTION

Tumor cell DNA from HNPCC[2] patients and from individuals with certain sporadic cancers are characterized by instability in repetitive sequence elements (1–7). Most HNPCC kindreds have germline mutations in the human homologues of the bacterial mismatch repair genes *mutS* or *mutL* (8–14). These mutations are believed to inactivate mismatch repair, yielding a mutator phenotype that has been suggested to be required for subsequent tumor progression (15, 16). Supporting this concept are studies demonstrating that tumor cell lines mutant in *hMSH2* (17, 18), *GTBP* (19), *hMLH1* (11, 20, 21), or *hPMS2* (22, 23) exhibit microsatellite instability (17–20, 22–25), are defective in mismatch repair (17, 18, 21–26), and have high mutation rates in endogenous genes (26–29). Furthermore, mismatch repair activity can be restored to extracts of defective cell lines by complementation with complexes of hMSH2 and GTBP (Ref. 25; designated hMutSα) or *hMLH1* and *hPMS2* (Ref. 21; designated hMutLα), or by introducing a human chromosome containing a wild-type copy of *hMLH1* (30). The latter approach also corrects microsatellite instability and restores sensitivity to MNNG treatment.

Whereas progress in understanding the relationship between mismatch repair, genome stability, and cancer has been rapid, many issues remain to be resolved. These issues include the biochemical functions of mismatch repair genes in correcting a variety of DNA heteroduplexes and possibly in other DNA transactions, such as transcription-coupled nucleotide excision repair (31), recombination (32, 33), chromosomal synapsis (34), male fertility (34), and cell cycle checkpoint control (35). The number and identity of other mismatch repair genes and of other genes involved in tumor progression (36), the tissue specificity of tumor development, and the possible role of environmental influences on genome stability in cells heterozygous or homozygous for various mutator genes also remain to be addressed.

Progress on these issues is being facilitated by identifying and characterizing tumor cell lines with known mismatch repair gene mutations. To this end, we describe here the mismatch repair status of 22 cell lines and report mismatch repair gene defects in 10 lines that are repair defective and that exhibit microsatellite instability. The objectives of the study were to extend or to find exceptions to the correlation between microsatellite instability and defective repair, to determine the number of tissue types from which mismatch repair-defective tumor cell lines might be derived, to examine the hypothesis that both alleles of a mismatch repair gene need be inactivated to account for a repair defect, and to expand the number of well-characterized mismatch repair-defective cell lines for use in future studies.

MATERIALS AND METHODS

Cell Lines and Extracts. EA1 and HEC59 cells were obtained from Jeff Boyd (University of Pennsylvania, Philadelphia, PA). HeLa S3 were obtained from Thomas Kelly (Johns Hopkins, Baltimore, MD). All other human cancer cell lines were obtained from the from the American Type Culture Collection. S-MEM with 10% Cosmic calf serum was used to grow HeLa S3 cells, whereas the other lines were grown in a 1:1 mixture of D-MEM and Ham's F-12 medium supplemented with 10% Cosmic calf serum (Hyclone Laboratories, Logan, UT). All cultures were maintained at 37°C in a humidified 5% CO_2 incubator. Extracts were prepared from logarithmically growing cultures as described previously (37).

Microsatellite Instability. Single cell clones were isolated by increasing dilution into 96-well plates, harvested when the culture was nearly confluent (approximately 15 cell generations), followed by a second round of subcloning in 96-well plates. Subclones were harvested again after ~15 generations of growth. After washing the plates with PBS, 50 μl of a solution of 50 mg/ml proteinase K-1 mM EDTA-10 mM Tris (pH 7.5) were added to each well. Plates were incubated at 65°C for 2 h and then at 95°C for 15 min in a humidified atmosphere. One μl of the resulting DNA was used per PCR reaction. Microsatellite loci were amplified by PCR in reactions containing 50 mM KCl; 10 mM Tris (pH 8.3); 1.5 mM $MgCl_2$; 200 μM each of dCTP, dGTP, dATP, and dTTP; 1 μM each primer; 0.5 unit of Amplitaq polymerase (Perkin Elmer Cetus); and 10–30 ng genomic DNA in a volume of 20 μl. One primer was end labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase, using a KinAce-It kit (Stratagene), and column purified before PCR. Using a Perkin Elmer Cetus 9600 GenAmp PCR System, 30 cycles of PCR were performed, consisting of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, followed by a 7-min extension at 72°C. Products were diluted 1:1 with a loading buffer consisting of 98% formamide, 10 mM EDTA (pH 8.0), 0.02% xylene cyanol FF, and 0.2%

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[2] The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal cancer; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MMR, mismatch repair.

bromophenol blue, and then denatured for 2 min at 90°C. Approximately 5 μl were subjected to electrophoresis through 6% polyacrylamide gels containing 8.3 M urea for 2–3 h at 70 W. The gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to X-ray film at −80°C.

Mismatch Repair and DNA Replication Fidelity Assays. The sources of materials and construction of heteroduplex substrates were as described (37, 38). DNA substrates contained a nick in the minus strand either at position −264 (for the 3′-nicked substrate) or at position +276 (for the 5′-nicked substrate), where position +1 is the first transcribed base of the *lacZα* gene. Replication reactions were performed concurrently with heteroduplex repair reactions to confirm that the extracts were competent for T antigen-dependent replication. Mismatch repair and DNA replication reactions (25 μl) contained 30 mM HEPES (pH 7.8); 7 mM $MgCl_2$; 4 mM ATP; 200 μM each CTP, GTP, and UTP; 100 μM each dATP, dGTP, dTTP, and dCTP; 40 mM creatine phosphate; 100 mg/ml creatine phosphokinase; 15 mM sodium phosphate (pH 7.5); and 50 μg of extract protein. Replication reactions contained 8 fmol of duplex DNA and 1 μg of T antigen and were incubated for 2–4 h at 37°C. Mismatch repair reactions, containing 1 fmol of heteroduplex DNA but no T antigen, were incubated at 37°C for 10–30 min and then processed and introduced into *Escherichia coli* NR9162 (*mutS*) as described (38).

Mutational Analysis. Analysis of mutations in exons of the *hMSH2* and *hMLH1* genes in different cell lines was performed using the types of methods described previously (12, 14). Briefly, genomic DNA samples were prepared from each cell line using QIAamp blood kits obtained from Qiagen. All exons of each gene were amplified from samples of genomic DNA by multiplex PCR, and products were analyzed by electrophoresis through agarose gels using standard methods to determine whether each exon was present. Individual exons were then sequenced on an Applied Biosystems 373 DNA sequencer using reagents and protocols supplied by the manufacturer. Three different sequencing protocols were used: Taq DNA polymerase and Dye Terminators; Taq DNA polymerase CS and Dye Primers; and Sequenase and Dye Primers. All candidate mutations, detected as either sequence changes or the presence of heterozygous nucleotides, were verified by sequencing both strands of the region of DNA containing the mutation. One cell line, DU145, was also analyzed for the presence of mutations in the *hPMS2* gene. In this case, cDNA prepared from this cell line was sequenced using essentially the same methods, except that sequencing primers designed from the cDNA sequence were used to sequence both strands of the coding region and 5′-untranslated region of *hPMS2*.

RESULTS

Mismatch Repair in Extracts of Human Cell Lines. Table 1 lists the human cell lines used in this study, which are derived from a variety of tissue types. Extracts of all these were tested for repair of a single base mismatch using duplex M13 mp2 DNA containing a covalently closed strand (+) and a strand with a nick (−) (to direct repair to this strand) located 352 or 353 bp away from the mispair in the *lacZα* complementation coding sequence at position 88 (G·G) or 89 (A·C or A·G). The (+) strand encodes a colorless plaque phenotype, whereas the (−) strand encodes a blue plaque phenotype. If the unrepaired heteroduplex is introduced into an *E. coli* strain deficient in methyl-directed heteroduplex repair, plaques will have a mixed plaque phenotype on selective plates, due to expression of both strands of the heteroduplex. However, repair occurring during incubation of the substrate in a repair-proficient human cell extract will reduce the percentage of mixed plaques and increase the (+) strand phenotype (in this case colorless):(−) strand phenotype (blue) ratio because the nick directs repair to the (−) strand. With this assay, 12 extracts were repair proficient (Fig. 1*A*), whereas 10 extracts were repair defective (Refs. 17 and 24; Fig. 1*B*). Those lacking repair (shown in Fig. 1*B*) were defective whether the nick was 3′ or 5′ to the mismatch (not shown) and with substrates containing several other mispairs or an unpaired nucleotide (Refs. 17 and 24; see legend to Fig. 1).

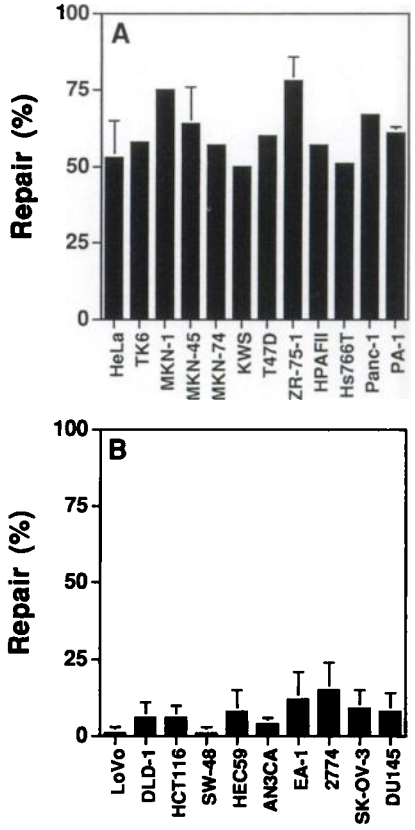

Fig. 1. Mismatch repair activity in extracts of tumor cell lines. Repair reactions were incubated for either 15 or 30 min, and the products were analyzed as described in "Materials and Methods." *Columns*, mean repair values for the G·G mismatched substrate described in the text, except for the data with KWS and T47D cell extracts, which used a A·C or A·G mismatch, respectively, at position 89. Several hundred to several thousand plaques per variable were scored. Where multiple determinations with the G·G mismatch were performed (2–10 determinations), the SDs are also shown. Extracts examined only once with the G·G mismatch (and hence lacking SDs) were all examined with one or more other substrates containing either a mismatch (A·C, G·T) or one unpaired nucleotide (A·−, C·−, −·G). In all cases, the data confirm the results shown in the figure. All extracts were active for SV40 origin-dependent DNA replication, as described (17). *A*, repair-proficient extracts. *B*, repair-deficient extracts. Because values up to ~10% repair represent experimental fluctuation in this assay (38), we cannot conclude that repair is completely absent in these extracts, only that it is reduced.

Table 1 *Human cancer cell lines used in this study*

Cell line	Tissue type (Ref.)	RER status	Reference
Mismatch repair proficient			
HeLa	Cervical (39)	–[a]	17
TK6	Lymphoblastoid (40)	–	17
MKN-1	Gastric (41)	ND	
MKN-45	Gastric (41)	ND	
MKN-74	Gastric (41)	ND	
KWS	Gastric (41)	ND	
T47D	Breast (42)	ND	
ZR-75-1	Breast (43)	ND	
HPAFII	Pancreatic (44)	ND	
Hs766T	Pancreatic (45)	ND	
Panc-1	Pancreatic (46)	ND	
PA-1	Ovarian (47)	–	This study
Mismatch repair deficient			
LoVo	Colonic (48)	+	7,20
DLD-1/HCT15	Colonic (49)	+	7
HCT 116	Colonic (50)	+	7
SW-48	Colonic (51)	+	20
HEC59	Endometrial (52)	+	17
AN_3CA	Endometrial (53)	+	17
EA1	Endometrial (54)	+	This study
2774	Ovarian (55)	+	56
SK-OV-3	Ovarian (57)	+	56
DU 145	Prostatic (58)	+	This study

[a] –, negative; +, positive; ND, not determined.

Analysis of Microsatellite Instability. Eight of the mismatch repair-defective lines have been reported by us or others to exhibit

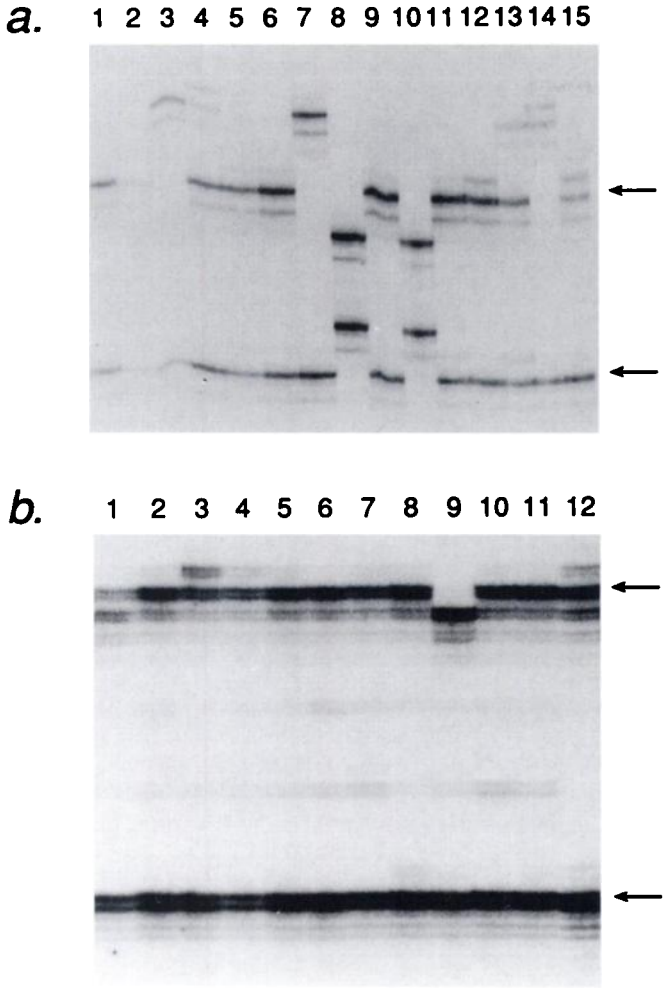

Fig. 2. Instability in single cell clones of EA-1 and DU145 cells. The analysis was performed as described in "Materials and Methods." *a*, DU145 clones; *b*, EA-1 clones. *Arrows*, positions of presumed original alleles.

microsatellite instability (Table 1). When the EA-1 and DU145 lines were examined, they too were found to exhibit microsatellite instability. Examples at the *D7S1794* locus containing CTT repeats for single cell clones of DU145 and EA-1 are shown in Fig. 2, *A* and *B*, respectively. Several other loci were also shown to be unstable in these clones. Thus, all repair-defective cell lines exhibited microsatellite instability (Table 1).

The observation that the PA-1 cell line was repair proficient was surprising because this line has been reported by others to exhibit microsatellite instability (56). We therefore examined 28 single cell clones of PA-1 cells and found no altered alleles at 3 loci (*D7S1794*, *D17S791*, and *D10S197*). We repeated the analysis with 20 independent single cell clones and again found no evidence for instability at 2 loci (*D7S1794* and *D1S158*).

Complementation of Repair-deficient Extracts. To determine the genetic defects responsible for the repair deficiencies, we performed complementation analyses (Fig. 3; Table 2) using extracts of cell lines containing previously identified mutations in either *hMSH2* or *hMLH1*. For example, HCT116 cells are hemizygous for a C→A nonsense mutation at codon 252 in *hMLH1*, yielding a truncated protein but no full-length hMLH1 protein (11). HEC59 cells are heterozygous for two mutations in hMSH2 (see below). Extracts of both cells are repair deficient, as is an extract of SK-OV-3 cells alone or mixed with an equal amount (50 μg) of HCT116 cell extract (Fig. 3). However, repair is observed in a reaction containing 50 μg each of SK-OV-3 and HEC59 cell extracts, suggesting that the defect in SK-OV-3 is not in the *hMSH2* gene. Similar analyses with other combinations provide three groups (Table 3): (*a*) those complemented by a hMLH1-defective extract; (*b*) those complemented by a hMSH2-defective extract; and (*c*) an extract (of LoVo cells) complemented by neither (see below).

Analyses of *MMR* Gene Defects. On the basis of the complementation analysis, we analyzed the *hMSH2*, *hMLH1*, and *hPMS2* genes for mutations (Table 3). As previously reported (17) LoVo was found to contain a deletion in the *hMSH2* gene, including exons 3–8 [Fig. 4, compare panels *A* (LoVo) and *B* (wild-type)].

The other lines in the first complementation group in Table 2 were

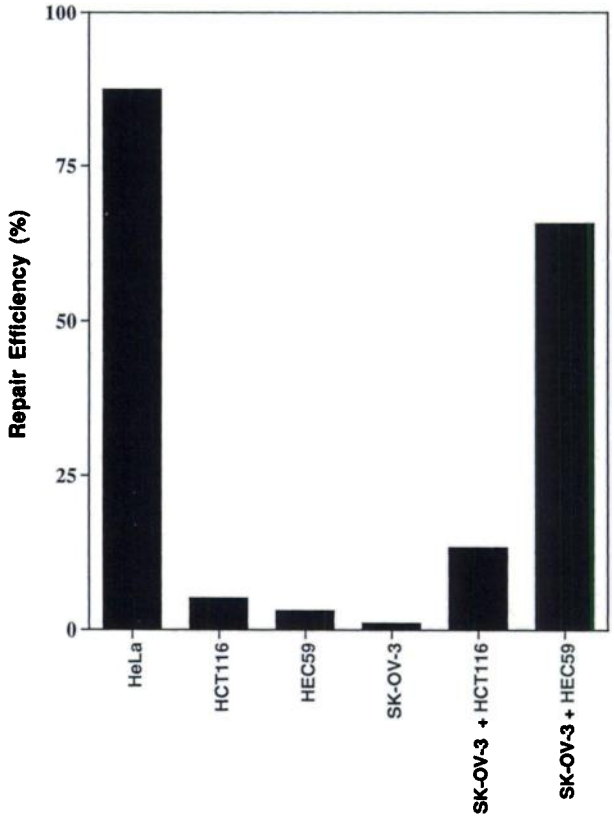

Fig. 3. Complementation of mismatch repair. The analysis was performed as described in "Materials and Methods" and "Results," using a substrate containing a single unpaired adenine at position 91. Reactions were incubated for 15 min.

Table 2 *Complementation of mismatch repair-defective extracts*

	Complementation with extract of	
	HCT116 (*hMLH1*$^-$)	HEC59 (*hMSH2*$^-$)
Complementation group 1 (not *hMLH1* defective)		
DLD-1/HCT15	+[a]	–
HEC59[b]	+	–
EA-1	+	–
2774	+	–
Complementation group 2 (not *hMSH2* defective)		
HCT116[b]	–	+
SW48	–	ND
AN_3CA[b]	–	+
SK-OV-3	–	+
DU145	–	+
No complementation		
LoVo	–	–

[a] +, positive; –, negative; ND, not determined.
[b] From Ref. 17.

Table 3 *Gene defects in mismatch repair-deficient cell lines*

Cell line	Mutation
hMSH2 defective	
LoVo[a]	Deletion of exons 3–8
HEC59	Heterozygous duplication of nucleotides 1442–1445; truncation in exon 9[a], heterozygous for CAA→TAA, truncation at codon 402 in exon 7
2774[b]	Hemizygous for CGT→CCT, Arg→Pro at codon 524 in exon 10
EA-1	Heterozygous for CGT→CCT, Arg→Pro at codon 524 in exon 10, heterozygous for CAG→TAG, truncation at codon 215 in exon 3
GTBP defective	
DLD-1 (HCT15[c])	Deletion 1 bp at codon 222, Leu→nonsense, and change of TTGATAGAGT→TTTGT at codon 1103, new termination codon 9 bp downstream
hMLH1 defective	
HCT116[d]	Hemizygous for TCA→TAA, truncation at codon 252 in exon 9
SW48	No hMLH1 RNA amplified by RT-PCR
AN_3CA	No hMLH1 RNA amplified by RT-PCR, no mutation in coding sequence
SK-OV-3	Deletion of exons 4–19
DU145	Hemizygous CAGTTT→CTGTTT splice site mutation between exons 1 and 2
hPMS2 defective	
DU145	Heterozygous for CAT→TAT, His→Tyr at codon 189

[a] From Ref. 17; also see Ref. 20.
[b] From Ref. 56.
[c] From Ref. 19.
[d] From Ref. 11.

also examined for *hMSH2* mutations. Although extracts of DLD-1 cells (and HCT15 cells, which were derived from the same tumor as DLD-1 cells) were repair defective and complemented by a hMLH-1-defective extract, sequence analysis of the complete *hMSH2* gene in DLD1 cells failed to detect a mutation that could explain the phenotype. The absence of a detectable *hMSH2* mutation in DLD-1 cells was also reported by Liu *et al.* (20). However, Papadopoulos *et al.* (19) recently reported that HCT15 cells contain frameshift mutations in both alleles of the *GTBP* gene.

As reported earlier, HEC59 cells are heterozygous for a 4-nucleotide duplication in exon 9 of *hMSH2* (Fig. 5, compare panels *C* and *D*). This frameshift mutation results in a nonsense codon just downstream (codon 482). Here, we have found a second heterozygous mutation, a C·G→T·A transition that generates a termination codon (codon 402) in exon 7 of *hMSH2* (Fig. 5, compare panels *A* and *B*). Orth *et al.* (56) have found that the ovarian tumor cell line 2774 is hemizygous for a G·C→C·G transversion, changing codon 524 in exon 10 of *hMSH2* from arginine to proline. EA1 cells were found to be heterozygous for a nonsense mutation in exon 3 of *hMSH2* (Fig. 6, compare panels *A* and *B*) and a missense mutation in exon 10 of *hMSH2* (Fig. 6, compare panels *C* and *D*). This latter mutation is the same as that reported in 2774 cells.

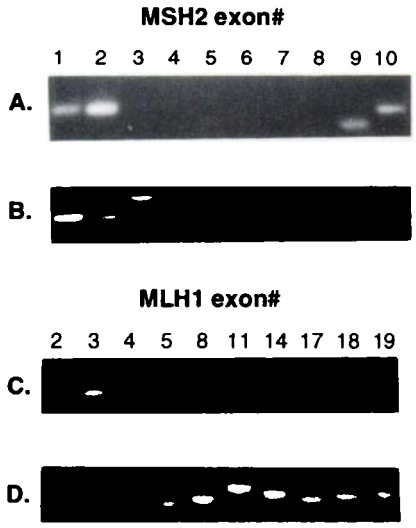

Fig. 4. PCR analysis of genomic DNAs containing deletions in *hMSH2* and *hMLH1*. All 16 exons of *hMSH2* and all 19 exons of *hMLH1* were amplified from samples of genomic DNA using first stage PCR primers as described previously (12, 14). The resulting PCR products were then analyzed by electrophoresis through agarose gels. *A*, analysis of *hMSH2* exons 1–10 from LoVo DNA. Amplification of exons 11–16 from LoVo DNA yielded the predicted wild-type sized fragments (data not shown). *B*, analysis of *hMSH2* exons 1–10 from genomic DNA from a normal individual. *C*, analysis of *hMLH1* exons 1–5, 8, 11, 14, 17, 18, and 19 from SK-OV3 DNA. Amplification of exons 6, 7, 9, 10, 12, 13, 15, and 16 from SKOV3 DNA did not yield an amplification product (data not shown). *D*, analysis of *hMLH1* exons 1–5, 8, 11, 14, 17, 18, and 19 from genomic DNA from a normal individual. Amplification of exons 6, 7, 9, 10, 12, 13, 15, and 16 from this DNA also yielded the expected wild-type sized amplification products (data not shown).

The cell lines in the second complementation group in Table 2 were analyzed for *hMLH1* mutations. As mentioned above and reported earlier (11), HCT116 cells are hemizygous for a C→A mutation generating a nonsense codon (number 252) in *hMLH1*, yielding a truncated protein but no full-length hMLH1 protein. For the SW48 and AN_3CA cell lines, no product bands were obtained from attempts to amplify mRNA by RT-PCR. RT-PCR amplification of *hMSH2* mRNA sequences did yield products, demonstrating that SW48 and AN_3CA cells lines lack wild-type *hMLH1* mRNA. Sequencing of the 19 exons and the exon-intron junctions of the *hMLH1* gene of AN_3CA did not reveal any mutations in the coding sequence. SKOV-3 cells were found to be deleted for exons 4–19 of hMLH1 [Fig. 4, compare panel *C* (SK-OV-3) to panel *D* (wild-type)]. When DU145 was analyzed for the presence of mutations in *hMLH1*, a hemizygous substitution mutation was found in the splice site between exons 1 and 2 (Fig. 7). Sequencing of *hPMS2* cDNA prepared from this cell line also revealed a heterozygous C→T substitution in *hPMS2* that changes a histidine to a tryrosine in codon 189 (data not shown).

DISCUSSION

The present study substantially increases the number of tumor cell lines exhibiting microsatellite instability that have also been demonstrated to be defective in mismatch repair (Fig. 1). This study also strengthens the correlation between microsatellite instability and defective repair. The results also increase the number of tissue types from which mismatch repair-defective tumor cell lines have been derived (Table 1). Thus, the genetic instability associated with a mismatch repair defect occurs in tumors from a variety of tissues.

One or more mutations in either of the *hMSH2, GTBP, hMLH1*, or *hPMS2* genes has been identified in 16 of the 17 known MMR-defective cell lines. A wild-type copy corresponding to the mutant gene has not been reported in any of these lines, consistent with the hypothesis that defective repair, microsatellite instability, and possibly tumor formation all require loss of function of both alleles of a particular mismatch repair gene. Four of the lines

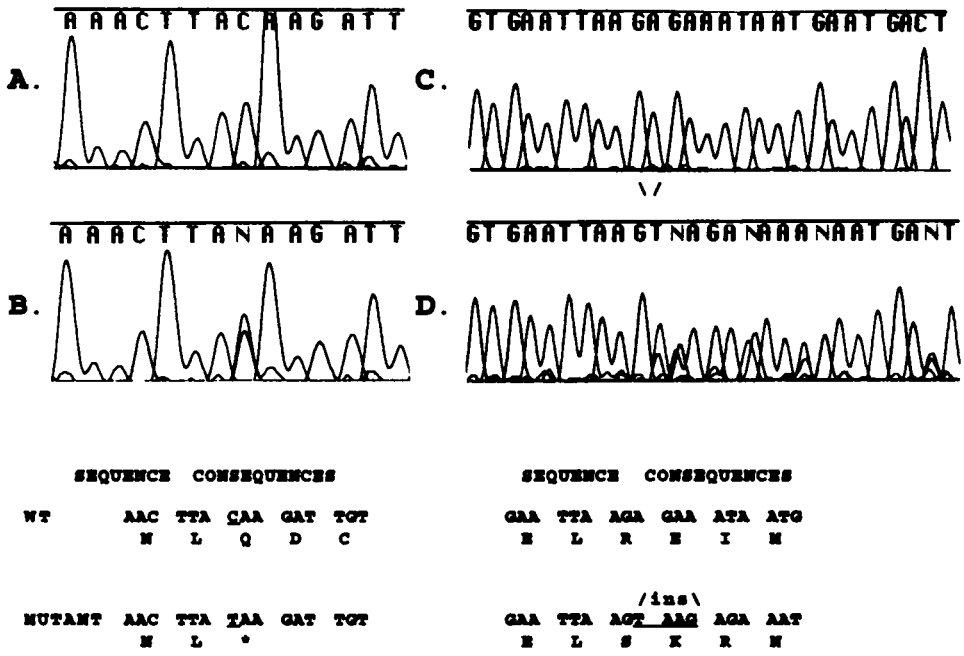

Fig. 5. Sequence analysis of *hMSH2* exons 7 and 9 amplified from HEC-59 DNA. *A*, sequence chromatogram of a portion of exon 7 from normal control DNA. *B*, sequence chromatogram of a portion of exon 7 from HEC-59 DNA showing a nucleotide position that is heterozygous for a C and a T. *C*, sequence chromatogram of a portion on exon 9 from normal control DNA. *D*, sequence chromatogram of a portion on exon 9 from HEC-59 DNA showing multiple heterozygous nucleotides downstream of the sequence GAATTAAG. The multiple heterozygous positions are consistent with sequencing of two DNAs shifted downstream out of register by 4 nucleotides indicative of a 4-base insertion mutation. The *lower panels* show the effect of the indicated nucleotide sequence changes on the MSH2 protein sequence.

examined in the present study were mutant in *hMSH2*, five were mutant in *hMLH1*, and only one had a mutation in either *hPMS2* or *GTBP*. Except for 2774 (see below), these lines are not reported to be from HNPCC paitients and can, therefore, be described as sporadic (although some of them may have had unascertained germline mutations). Nevertheless, this pattern of mutations is consistent with the finding that the majority of HNPCC tumors has a mutation in *hMSH2* or *hMLH1* (61). In contrast, only one HNPCC patient has been characterized with a mutation in the *hPMS2* gene (62), and none has been found to be mutant in the

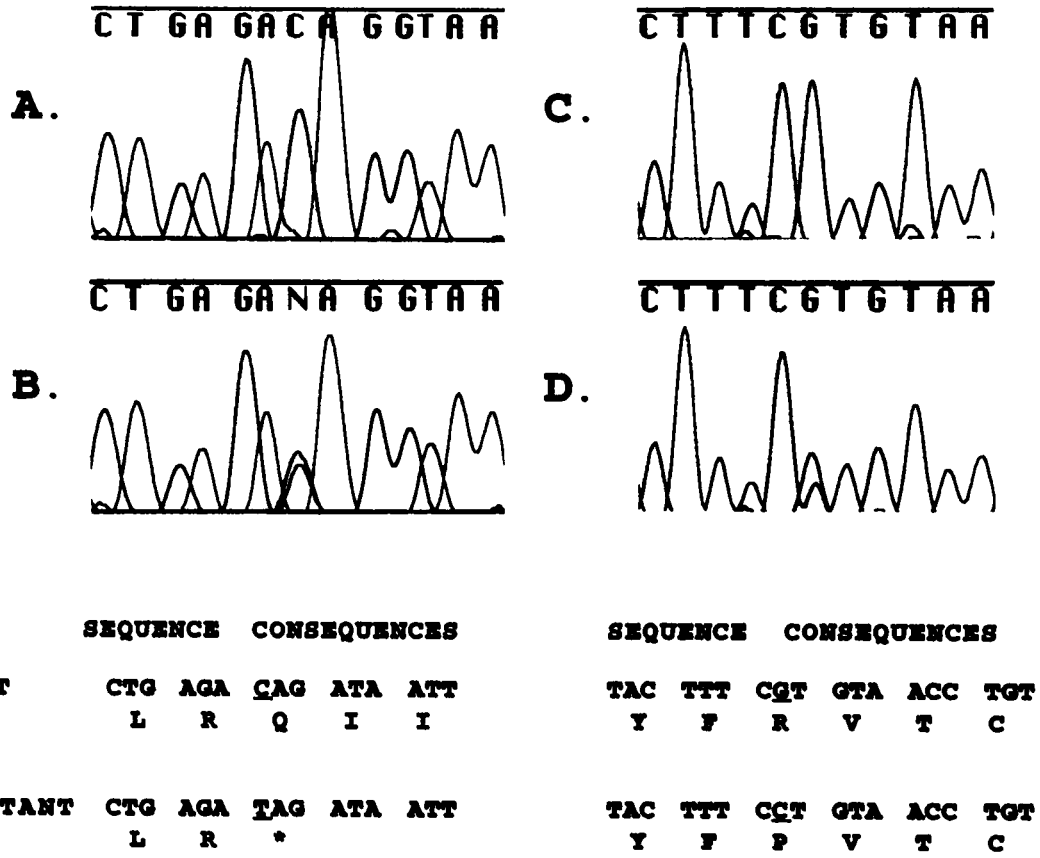

Fig. 6. Sequence analysis of *hMSH2* exons 3 and 10 amplified from EA-1 DNA. *A*, sequence chromatogram of a portion of exon 3 from normal control DNA. *B*, sequence chromatogram of a portion of exon 3 from EA-1 DNA showing a nucleotide position that is heterozygous for a C and a T. *C*, sequence chromatogram of a portion on exon 10 from normal control DNA. *D*, sequence chromatogram of a portion on exon 10 from EA-1 DNA showing a nucleotide position that is heterozygous for a G and a C. The *lower panels* show the effect of the indicated nucleotide sequence changes on the MSH2 protein sequence.

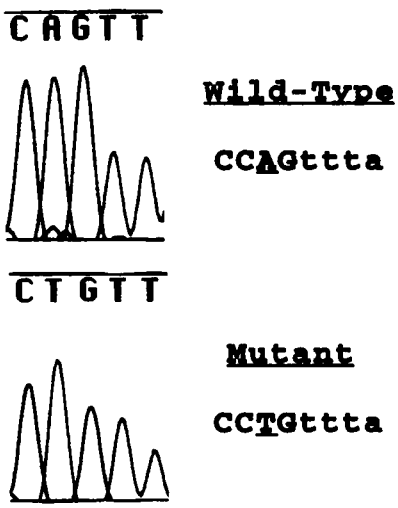

Fig. 7. Sequence analysis of *hMLH1* exon 2 amplified from DU145 DNA. *Top*, sequence chromatogram of a portion of exon 2 from normal control DNA. *Bottom*, sequence chromatogram of a portion of exon 2 from DU145 DNA showing a hemizygous change of A to T. The *right panels* show the effect of the indicated nucleotide sequence changes on the MLH1 DNA sequence. The A to T change is at conserved −2 position of the splice acceptor site. *Upper case letters*, intron sequence; *lower case letters*, exon sequence.

GTBP gene (19). We now summarize what is known about of each of the MMR-defective cell lines shown in Table 3.

hMSH2-defective Cell Lines

LoVo. This colorectal tumor cell line has an elevated mutation rate at microsatellite sequences (7) and at the *HPRT* locus (29). Extracts are defective in strand-specific repair of substrates containing mismatches or 1–16 unpaired nucleotides (17, 25, 59) and lack the ability to band-shift an oligonucleotide containing a G·T mismatch (60). Although we were not able to complement a LoVo extract with an *hMLH1*-defective extract (Table 2), Drummond *et al.* (25) did achieve successful complementation with an extract made from H6, a clonal derivative of HCT116 that is *hMLH1* defective. They also obtained complementation by a purified protein fraction designated hMutSα, which is composed of the *hMSH2* and *GTBP* gene products (25). Exons 3–8 of the *hMSH2* gene are deleted, and no wild-type gene copy is detectable (Refs. 17 and 20; Fig. 4).

HEC59. This endometrial tumor cell line has an elevated mutation rate at microsatellite sequences (17) and is defective in strand-specific repair of substrates containing mismatches (Fig. 1) or an unpaired nucleotide (17). Two heterozygous nonsense mutations are present in the *hMSH2* gene (Fig. 5).

2774. This line is derived from an HNPCC patient who died of ovarian cancer at an early age (56 years). The line has an elevated mutation rate at microsatellite sequences (56) and harbors only one *hMSH2* allele (56), a mutant that encodes a proline rather than an arginine at codon 524. This arginine is conserved in the yeast *MSH2* gene, suggesting functional significance. Its replacement by proline is a nonconservative substitution that is inferred to impair function(s) of the hMSH2 protein. Consistent with this, an extract of 2774 cells is defective in repair of a G·G mismatch (Fig. 1) or a substrate containing an unpaired nucleotide, and the deficiency can be complemented with an *hMLH1*-defective cell line (Table 2). Considering the nature of the mutation, it is worth noting that although the 2774 extract is clearly defective in mismatch repair, given the sensitivity of the repair assay and the repair value shown in Fig. 1, we cannot conclude that this cell line is completely devoid of all mismatch repair activity.

EA1. This endometrial tumor cell line has an elevated mutation rate at microsatellite sequences (Fig. 2). An extract is defective in strand-specific repair of substrates containing mismatches (Fig. 1) or an unpaired nucleotide. Two heterozygous mutations are present in the *hMSH2* gene, one resulting in a nonsense codon at residue 215, the other identical to the missense mutation reported in 2774 cells.

In addition to the lines described above, we have recently characterized (18) two *hMSH2*-defective, MMR-defective cell lines derived from a uterine mixed mesodermal tumor with similar characteristics. This brings to six the total number of *hMSH2*-defective, MMR-defective cell lines.

GTBP-defective Cell Lines

DLD-1/HCT15. The characteristics of these two lines will be considered together because, although they were derived from a colon carcinoma independently by two investigators, they are genetically indistinguishable (49). The most interesting feature of these cells is their mutational and repair specificity. Both DLD-1 and HCT15 have mutation rates at microsatellite sequences that are substantially elevated, but not as high as in several other unstable lines (7). Analysis of microsatellite alleles in HCT15 showed greater instability at homopolymeric A tracts than at $(CA)_n$ microsatellites, and, even at the (A)n tracts, the length variations were less than those in tumors with defects in *hMSH2*, *hMLH1*, or *hPMS2* (19). These cell lines also have strongly elevated mutation rates at the *HPRT* locus (27, 60). It was of interest that, in one study (27), the *HPRT* mutation rates in DLD-1 and HCT15 cells were 6- and 2-fold lower, respectively, than in *hMLH1*-defective HCT116 cells.

Having originally found that extracts of DLD-1 cells are defective in repair of substrates containing mismatches or an unpaired nucleotide (17) (Fig. 1), we later observed that DLD-1 and HCT15 extracts are in fact repair proficient for substrates with five unpaired nucleotides (59). This differential repair defect has also been observed in studies of HCT15 extracts, which lack repair of mismatches and single-unpaired nucleotides but do repair substrates with 2, 3, or 4 unpaired nucleotides (25), albeit with somewhat reduced efficiency. These specificities can now be interpreted in light of the discovery of the *GTBP* gene (19). This gene encodes a M_r 160,000 protein that associates with hMSH2 to form the hMutSα complex that binds a G·T mismatch (63) and restores repair of mismatched substrates to an extract of *hMSH2*-defective LoVo cells (25). The capacity of *GTBP*-defective cell extracts to repair substrates with several unpaired nucleotides is consistent with the ability of purified hMSH2 protein to bind to substrates with up to 14 unpaired nucleotides (64–66).

HCT15 cells contain two mutations in the *GTBP* coding sequence that potentially inactivate the gene (19). [The MT1 lymphoblastoid cell line is also mutant in the *GTBP* gene (19) and has properties similar to those of HCT15 cells.] Taken together, these data are consistent with the possibility that the GTBP gene product is required for repair of mismatches and single unpaired nucleotides but is not absolutely required for repair of heteroduplexes containing 2–5 unpaired nucleotides. Other possibilities have also been discussed (25), as have possible explanations for why mutations in the *GTBP* gene have not yet been found to be associated with HNPCC kindreds (19).

DLD-1 cells have been shown to be much less sensitive to the cytotoxic effect of treatment with *N*-methyl-*N*-nitrosurea (29). DLD-1 and HCT15 cells also harbor a G→A transition mutation in the DNA polymerase δ gene (67). This results in a nonconservative amino acid substitution (R to H) in a conserved motif for the proofreading exonuclease. Although the functional significance of this mutation is not known, it could result in diminished proofreading during replication, thus elevating mutation rates in these cells.

hMLH1-defective Cell Lines

HCT116. These colonic tumor cells have an elevated mutation rate at endogenous (7) and exogenous (24) microsatellite sequences and at the *HPRT* locus (27–29). HCT116 cells are resistant to the cytotoxic effect of treatment with MNNG (30), *N*-methyl-*N*-nitrosurea (29), or 6-thioguanine (35). In contrast to wild-type cells, 6-thioguanine-treated HCT116 cells readily progress through G_2 (35). HCT116 cells contain a hemizygous C→A mutation that yields a nonsense codon at amino acid 252 in *hMLH1* (10). A truncated hMLH1 protein can be detected by an *in vitro* synthesized protein assay, but no full-length protein is detected (10). An extract of a clonal derivative of this colon tumor cell line (designated H6) was the first to be demonstrated to be defective in strand-specific mismatch repair (24). Additional experiments have revealed a defect in repair of several substrates containing 1–4 unpaired nucleotides (17, 24) but efficient repair of substrates containing 5, 8, or 16 unpaired nucleotides, and 1 of 2 substrates with a 4-nucleotide loop (59). An HCT116 extract is complemented by an extract of an *hMSH2*-defective cell line (Refs. 17 and 25; Table 2) or by a purified protein fraction designated hMutLα, which is composed of hMLH1 and a second protein that is probably the product of the *hPMS2* gene (21). The repair defect, microsatellite instability, resistance to MNNG treatment, and progression of cells through G_2 after 6-thioguanine-treatment can all be reversed by introduction of a single wild-type copy of human chromosome 3, containing the *hMLH1* gene, to HCT116 cells (30, 35).

SW48. This colonic tumor cell line has an elevated mutation rate at microsatellite sequences (20). It was of interest that the mutation rate at the *HPRT* gene is 24-fold lower than for *hMLH-1*-defective HCT116 cells (29), despite the fact that no *hMLH1* mRNA (Ref. 30; Table 3) or protein (20) has been detected in SW48 cells. These cells are resistant to the cytotoxic effect of treatment with MNNG (29). Extracts of SW48 cells are defective in repair of substrates containing a G·G mismatch (Fig. 1) or one unpaired nucleotide (not shown).

AN_3CA and SK-OV-3. These endometrial and ovarian tumor cell lines have elevated mutation rates at microsatellite sequences (17, 56) and are defective in repair of substrates containing mismatches or an unpaired nucleotide (Ref. 17; Fig. 1). The repair defects are complemented by an *hMSH2*-defective extract (Table 2), consistent with the inability to amplify *hMLH1* mRNA in AN_3CA cells or the deletion of exons 4–19 in SK-OV-3 cells (Fig. 4; Table 3).

DU145. This prostatic tumor cell line exhibits substantial microsatellite instability (Fig. 2) and is deficient in repair of substrates containing a G·G mismatch (Fig. 1) or an unpaired nucleotide (not shown). The observation that the repair activity defect is complemented by an *hMSH2*-defective extract (Table 2) led us to examine the *hMLH1* and *hPMS2* genes for mutations. Mutations in both genes were found that are consistent with some loss of function: the *hMLH1* splice site mutation via an altered transcript and the *hPMS2* missense mutation because it is a nonconservative amino acid substitution at a residue conserved between yeast and human *PMS* genes. In addition to DU145, other mismatch repair-defective cell lines have been identified that have *hPMS2* mutations (22, 23).

Because the first 12 cell lines listed in Table 1 were all observed to be proficient in mismatch repair (Fig. 1), we did not examine microsatellite stability in most of these lines. However, we did examine PA-1 cells because we found this line to be repair-proficient, despite the fact that it had been reported to be RER^+ (56). We did not detect microsatellite instability in our analysis of single cell clones. Although we cannot exclude some degree of instability at some loci in this cell line, this lack of detectable microsatellite instability is consistent with the mismatch repair proficiency of the extract.

Although a relationship between certain types of cancer and defects in the general strand-specific mismatch repair process is now clearly established, this repair defect is not likely to be associated with all types of cancer. Thus, the repair-proficient lines shown in Fig. 1 may prove useful in the search for other gene defects that could initiate tumor formation.

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