Characterization of Distinct Human Endometrial Carcinoma Cell Lines Deficient in Mismatch Repair That Originated from a Single Tumor*

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The role of specific mismatch repair (MMR) gene products was examined by observing several phenotypic end points in two MMR-deficient human endometrial carcinoma cell lines that were originally isolated from the same tumor. The first cell line, HEC-1-A, contains a nonsense mutation in the hPMS2 gene, which results in premature termination and a truncated hPMS2 protein. In addition, HEC-1-A cells carry a splice mutation in the hMSH6 gene and lack wild-type hMSH6 protein. The second cell line, HEC-1-B, possesses the same defective hMSH6 locus. However, HEC-1-B cells are heterozygous at the hMSH6 locus; that is, along with carrying the same nonsense mutation in hPMS2 as in HEC-1-A, HEC-1-B cells also contain a wild-type hPMS2 gene. Initial recognition of mismatches in DNA requires either the hMSH2/hMSH6 or hMSH2/hMSH3 heterodimer, with hPMS2 functioning downstream of damage recognition. Therefore, cells defective in hPMS2 should completely lack MMR (HEC-1-A), whereas cells mutant in hMSH6 only (HEC-1-B) can potentially repair damage via the hMSH2/hMSH3 heterodimer. The data presented here in HEC-1-B cells illustrate (i) the reduction of instability at microsatellite sequences, (ii) a significant decrease in frameshift mutation rate at HPRT, and (iii) the in vitro repair of looped substrates, relative to HEC-1-A cells, illustrating the repair of frameshift intermediates by hMSH2/hMSH3 heterodimer. Furthermore, the role of hMSH2/hMSH3 heterodimer in the repair of basebase mismatches is supported by observing the reduction in base substitution mutation rate at HPRT in HEC-1-B cells (hMSH6-defective but possessing wild-type hPMS2), as compared with HEC-1-A (hMSH6/hPMS2-defective) cells. These data support a critical role for hPMS2 in human MMR, while further defining the role of the hMSH2/hMSH3 heterodimer in maintaining genomic stability in the absence of a wild-type hMSH2/hMSH6 heterodimer.

Deficiencies in human mismatch repair (MMR) have been illustrated in a variety of sporadic and hereditary neoplasia in recent years (reviewed in Refs. 1–3). This observation has led to a wealth of studies focusing on the intricacies of human MMR. However, the proteins and specific biochemistry within the MMR pathway are still currently being defined. The list of functional human MMR genes now includes hMSH2, hMSH3, hMSH6 (GTBP), hMLH1, and hPMS2, which are believed to function in MMR as depicted in Fig. 1. Several additional homologues of the bacterial MMR proteins MutS and MutL have been identified in human cells (4–7); however, their function in the human pathway is yet undefined. Nonetheless, their presence suggests that the aforementioned list of functional human MMR genes may be incomplete.

Mismatch recognition in human MMR involves homologues of the bacterial protein MutS. These proteins appear to function in at least two heterodimers, designated hMutSα (hMSH2/hMSH6) and hMutSβ (hMSH2/hMSH3) (8–12), which appear to have overlapping and redundant repair capabilities with respect to the type of DNA mismatch or loop substrate (12). A redundancy in mismatch recognition by the human heterodimers hMSH2/hMSH6 and hMSH2/hMSH3 was first predicted by overlapping in vitro binding affinities (10, 11). Recently, a human cell line, HHUA, defective in both hMSH6 and hMSH3, has been defined (4), and the predicted redundancy in human mismatch recognition was supported by complementing either defect by transfer of a specific human chromosome (12). These hMSH6/hMSH3-defective cells have an elevated mutation rate at the HPRT locus and a high rate of instability at microsatellite sequences, both of which were significantly reduced by complementing either the hMSH6 or hMSH3 defect by chromosome transfer. This overlapping phenotypic restoration illustrates the redundancy of function by either hMSH2/hMSH6 or hMSH2/hMSH3 for base-base mismatch repair or frameshift intermediate repair (12). In yeast, genetic studies also suggest that the MSH2 gene product forms a heterodimer with either MSH6 or MSH3 (13–15), with each heterodimer complementing the function of the other. This redundancy in yeast mismatch recognition was first indicated by the observation that mutation rates are only slightly elevated at several loci for either MSH6- or MSH3-deficient yeast strains. However, a strain with mutations in both MSH6 and MSH3 has significantly elevated mutation rates, comparable with the mutation rates observed in MSH2-deficient strains (14–17). Although the complexities observed with initial mismatch recognition in the human MMR pathway appear analogous to that observed in yeast, there has not been any evidence that yeast MSH2/MSH3 is capable of base-base mismatch repair such as that illustrated by hMSH2/hMSH3 in humans (12).

Following recognition of DNA mismatches in human cells, an additional heterodimer of hMLH1/hPMS2, designated hMutLα (18), functions in repair complex formation (8, 18, 19). The
proteins that comprise this heterodimer are homologues of the bacterial protein MutL. Although only two MutL homologues have been implicated in the process of repair complex formation, at least eleven additional homologues belonging to the PMS gene family have been characterized in human cells (5, 6). It would seem possible that hMLH1/hPMS2 function may be complemented to some extent by any of these PMS homologues, perhaps by a second hMutL heterodimer consisting of two presently undefined proteins, or an additional protein complexed with either of the subunits in hMLH1/hPMS2. One such PMS gene homologue, hPMS1, has been implicated in human mismatch repair by the observation of a mutant hPMS1 allele isolated from a family predisposed to colon cancer (21), although a function for hPMS1 has yet to be determined. A transgenic mouse mutant in either hMLH1 or hPMS2 has differing phenotypes (22, 23), with respect to cancer predisposition and meiotic phenotypes, suggesting that the roles of each specific gene are not exclusive to the hMLH1/hPMS2 heterodimer. Taken together, the proteins involved in the formation of a repair complex are not as well defined as those involved in mismatch detection. Therefore, additional MutL homologues or perhaps additional biochemical roles for either hMLH1 or hPMS2 other than in the hMLH1/hPMS2 heterodimer may be involved in human MMR.

To further investigate the biochemical complexities involved in human MMR, several phenotypes were determined in two cell lines originating from the same endometrial carcinoma. These cell lines, HEC-1-A and HEC-1-B, both contain a mutation in the hMSH6 gene and a hPMS2 allele that contains a premature nonsense codon; however, they differ by either the lack or presence of a wild-type copy of hPMS2, respectively. Fig. 1 illustrates these differences within the MMR pathway between HEC-1-A and HEC-1-B as compared with the current model of MMR. The roles of individual MMR heterodimers can then be examined by comparing differences in mismatch repair in vitro, microsatellite instability, and the mutation rate and mutational specificity at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus. The data presented here support the notion that hPMS2 plays a critical role in maintaining genomic stability in human cells, as illustrated by the significant instability observed in HEC-1-A cells. Furthermore, the data suggest the biochemical role of the hMutSβ (hMSH2/ hMSH3) heterodimer, as illustrated by observing the differences in phenotypes between the HEC-1-B cells (hMSH6-defective) and the HEC-1-A cells (hMSH6hPMS2-defective).

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—Human endometrial carcinoma cell lines HEC-1-A and HEC-1-B were from the American Type Culture Collection. Both cell lines were maintained in DF medium (Dulbecco's modified Eagle's medium/Ham's F-12 (1:1)+ 10% dialyzed fetal bovine serum; HyClone). Analysis of Mismatch Repair Gene Mutations in HEC-1-A and HEC-1-B Cell Lines—The mutation of the mismatch repair gene hPMS2 in HEC-1-A has been previously described (24). The status of hPMS2 in HEC-1-B was determined by a protein truncation test as described (24). Briefly, total RNA was prepared from cells using a single-step procedure. cDNA was prepared and used as a template for coupled transcription and translation using the TNTTM system (Promega). The resulting protein products were then analyzed. Upon noting a wild-type copy of hPMS2 protein in HEC-1-B, the cDNA was then reamplified and sequenced (as described below for HPRT cDNA). Other MMR genes in both cell lines were analyzed in a similar manner.

Mismatch Repair Assay—Extracts from cell lines were prepared as described previously (25). Procedures for substrate preparation and for measuring mismatch repair activity were as described (26). Briefly, reaction mixtures (25 μl contained 30 mM Hepes (pH 7.8), 7 mM MgCl₂, 4 mM ATP, 200 μM each CTP, GTP, and UTP, 100 μM each dATP, dGTP, dTTP, and dCTP, 40 μM creatine phosphate, 100 μg/ml creatine phosphokinase, 15 μg sodium phosphate, 1 nM of the indicated heteroduplex DNA, and 50 μg of the cell extract protein. After incubating at 37 °C for 15 min, samples were processed and introduced into Escherichia coli NR0162 (mutS) via electroporation. Cells were plated, M13mp2 plaque colors were scored, and the repair efficiency was calculated (26).

**Microsatellite Instability Analysis in HEC-1-A and HEC-1-B Cell Lines**—Single cell clones of the HEC-1-A and HEC-1-B cell lines were isolated by limiting dilution. DNA was isolated from independent single cell clones and amplified by PCR as described (24). Several microsatellite loci examined were then examined for variations in allele length.

**Mutation Frequency and Mutation Rate at HPRT**—Mutation frequency and mutation rate determinations at the HPRT locus were determined as described previously (28). Mutation frequencies were obtained by plating 10⁶ cells in 40 μM 6-thioguanine (6-TG; Sigma) at a density of 5 × 10⁴ per 10-cm dish. Cells were incubated 12–14 days and 6-TG-resistant (6-TG') colonies were visualized by staining with 0.5% crystal violet (in 50% methanol, v/v; Sigma). Mutation rate determinations were performed using cell populations cleansed of pre-existing HPRT mutants by culturing in HAT medium (DF medium + 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine; Sigma). Following HAT removal, the initial HPRT mutation frequency was determined and 2–3 × 10⁵ cells were subcultured. Several additional mutant frequencies were obtained by maintaining the cells in logarithmic growth. Population doublings were determined by counting colony frequency determinations. After obtaining 5–6 subsequent mutant frequencies, mutation rate was then obtained by plotting the observed mutant frequency as a function of population doubling and calculating the slope by linear regression. The slope of the curve yields the mutation rate (mutations/cell/generation).

**HPRT cDNA Amplification—** Amplification of HPRT cDNA from mutant clones was performed by a procedure modified from Yang et al. (9). Primers of primer pairs used for HPRT cDNA amplification and sequencing and their annealing sites relative to the A of the ATG initiation codon (30) are as follows: HP1, 5'-CTCTCTCTGCGCCACCGCGGTCCTCC-3' (65 to −41); HP2, 5'-AACATTGATAATTATTTGGCGGATGTCTC-3' (701 to 727); HP3, 5'-CTCTCTCTGCGCCACCGCGGTCCTCC-3' (64 to −41); and HP4, 5'-CGATGTAATGGCACCGGAGCTTTG-3' (683 to 707). PCR products were cloned into plasmids containing ice-cold phosphate-buffered saline, resuspended at a density of approximately 500 cells/μl and stored as 1-μl aliquots at −80 °C. 1-μl aliquots were quickly thawed, and 9 μl of cDNA synthesis mixture was added. This mixture contained 500 μM dNTPs (Amersham Pharmacia Biotech), 1 μg of bovine serum albumin, 10 mM dithiothreitol, 2.5% (v/v) Nonidet P-40, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 25 μM of Superscript II RNaseH reverse transcriptase (Life Technologies), 10 units RNasin RNase inhibitor (Promega), and 100 ng of HP2 primer. cDNA synthesis was performed at 37 °C for 1 h. PCR1 mixture was then added directly to the cDNA synthesis mixture, which contained 300 μM dNTPs, 15 μM Tris-HCl (pH 8.5), 60 mM KCl, 3.5 mM MgCl₂, 2.5 units Taq polymerase (Perkin-Elmer), 100 ng of HP1 primer, and 50 ng of HP2 primer. PCR1 was performed as follows: 1 cycle of 94 °C for 4 min, 65 °C for 1 min, 72 °C for 2.5 min, followed by 29 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 2 min. Nested primer PCR was then performed by adding 5 μl of PCR1 to PCR2 mixture (PCR mixture with 100 ng each of HP3 and HP4 primers substituted for HP1 and HP2 primers). PCR2 was performed under the same PCR conditions as PCR1. PCR2 products were checked on an agarose gel, and full-length cDNA was visualized on the gel. A dideoxy terminator cycle sequencing reaction was performed using a dye terminator ready mix with Taq polymerase.

**Sequencing of HPRT cDNA**—The entire HPRT coding region from each mutant was sequenced utilizing automated sequencing with both the HP3 and HP4 primers. A dioxy terminator cycle sequencing reaction was performed using a dye terminator ready mix with Taq polymerase.
polymerase (Perkin-Elmer) as follows: 94 °C for 30 s, 60 °C for 4 min for 25 cycles. DNA sequences were obtained on Applied Biosystems automated DNA sequencer model 377. Each sequencing reaction allowed for analysis of the entire 657 base coding region of \( \text{HPRT} \), and therefore, each mutation determined was confirmed by sequence analysis in the opposite direction.

RESULTS

Characterization of MMR Gene Defects in HEC-1-A and HEC-1-B Cell Lines—The endometrial carcinoma HEC-1-A cell line and the substrain HEC-1-B cell line were isolated from the same adenocarcinoma (31). Although originating from the same tumor, the individual cell lines differ in their karyotype: the HEC-1-A cell line is near diploid (modal chromosome number of 49), whereas the HEC-1-B cell line is near triploid to tetraploid (modal chromosome number of 82). There are a few cell line-specific markers present, such as a deletion on chromosome 9 (p13p22) in HEC-1-B. However, most markers are shared between the HEC-1-A and HEC-1-B cell lines, such as a deletion on chromosome 7 (q32) and a deletion on chromosome 6 (p21.3–p23). There are two X chromosomes present in both the HEC-1-A and HEC-1-B cell lines.

The defect in the \( \text{hPMS2} \) gene in HEC-1-A cells has been previously defined (24). This cell line contains a C to T mutation at codon 802 in the \( \text{hPMS2} \) gene, which results in a TGA termination codon. The same truncated protein product present in HEC-1-A was observed in HEC-1-B, as determined by protein truncation test, which upon sequencing revealed the same C to T mutation at codon 802. But in contrast to HEC-1-A, the \( \text{hPMS2} \) protein truncation test in HEC-1-B cells yielded two gene products (Fig. 2). The additional polypeptide was a full-length product, and DNA sequencing of the cDNA used in the analysis determined it to be wild-type \( \text{hPMS2} \). Thus, HEC-1-B cells contain both wild-type \( \text{hPMS2} \) protein, as well as truncated protein. These results were confirmed by Western analysis of \( \text{hPMS2} \) protein (data not shown).

Recent work involving the HEC-1-A cell line suggested the presence of a second MMR gene mutation (32). Analyses of other MMR genes in HEC-1-A were conducted by Western analysis, protein truncation tests, and by direct DNA sequencing. Both the \( \text{hMLH1} \) and \( \text{hPMS1} \) genes were investigated and appear to be wild type at the molecular level by DNA sequencing of the entire coding region. However, analysis of the \( \text{hMSH6} \) gene did not reveal a wild-type sequence. Sequencing of the cDNA illustrated a 199-base deletion in the 3' region of the cDNA, corresponding to nucleotides 3802–4001 (32). The presence of a wild-type copy of the cDNA was not observed. Therefore, HEC-1-A cells are mutant in both \( \text{hPMS2} \) and \( \text{hMSH6} \). Similar analysis of the HEC-1-B cell line indicated the same mutation in the \( \text{hMSH6} \) gene, that of a deletion of base 3802–4001. No wild-type copy of \( \text{hMSH6} \) cDNA was observed in HEC-1-B. By observing these MMR gene mutations, the cell lines were then defined as mutant in both \( \text{hPMS2} \) and \( \text{hMSH6} \) (HEC-1-A) or mutant in only \( \text{hMSH6} \) (HEC-1-B). Fig. 1 demonstrates the defects in both cell lines and how these defects relate to the current model of MMR.

Mismatch Repair Activity in HEC-1-A and HEC-1-B Cell Lines—The ability of HEC-1-A and HEC-1-B cells to repair various DNA mismatches or loops \textit{in vitro} was examined in
cellular extracts derived from either cell line. This repair activity was determined using a substrate located within a lacZ gene sequence. The repair of the substrate can be assessed colorimetrically dependent upon α-complementation. The in vitro repair efficiencies for HEC-1-A and HEC-1-B are presented in Fig. 3. These repair activities are compared with the repair activities observed in MMR-proficient HeLa cell extracts. This figure illustrates repair at a representative base: base mismatch, and at a 1-, 2-, and 4-base DNA loop. For the repair of single base:base mismatches, both cell lines were defective relative to HeLa cells. However, there was differential repair of DNA looped intermediates. HEC-1-B cells were proficient in the repair of 1, 2, and 4 bases, whereas HEC-1-A cells were deficient. This in vitro repair assay suggests that the hMSH2/hMSH3 heterodimer is functional in HEC-1-B.

**Microsatellite Instability in HEC-1-A and HEC-1-B Cell Lines**—The instability at microsatellite sequences was examined in both HEC-1-A and HEC-1-B cells at several loci by determining microsatellite tract length in single-cell clones. These repetitive tracts of DNA are prone to slippage during DNA synthesis, and the looped intermediates are subsequently repaired by MMR proteins. Lack of mismatch repair predisposes these repetitive tracts of DNA to instability, which is observed as expansion or contraction of the tract length. The instability at several microsatellite loci examined in HEC-1-A were highly unstable, indicative of cells deficient in MMR. Table I also presents the instability at microsatellite sequences for HEC-1-B cells. These sequences are relatively stable in comparison with HEC-1-A. For instance, 8 of 24 clones were unstable at the D17S791 marker in HEC-1-A, whereas only 2 of 24 were unstable in HEC-1-B. In total, 22% of the clones examined in HEC-1-A were unstable compared with only 4% in HEC-1-B. The instability associated with HEC-1-B cells is only slightly elevated from MMR-proficient cells, similar to that reported previously for cells defective in hMSH6 (12, 33, 34). These findings suggest that repair of 2–4-base loops in DNA is only slightly compromised in HEC-1-B, whereas HEC-1-A cells appear deficient in 2–4-base-loop repair.

**Mutation Rate at the HPRT Locus in HEC-1-A and HEC-1-B Cell Lines**—A substantial increase in the rate of spontaneous mutations has been observed in cells defective in MMR. To observe potential differences in the rate of mutations arising spontaneously between HEC-1-A and HEC-1-B cells, the mutation rate at HPRT was determined for both cell lines. These rates are presented in Table II. The elevated spontaneous mutation rate in HEC-1-A cells has been previously reported (28). The rate of mutation at the HPRT locus in the HEC-1-A and HEC-1-B cell lines was 3.1 × 10⁻⁶ and 5.1 × 10⁻⁶ mutations/cell/generation, respectively. These spontaneous mutation rates are increased by 660-fold for HEC-1-A and 110-fold for HEC-1-B cells relative to a nontransformed normal human fibroblast line, NHF-1. These rates correspond to a 6-fold difference between HEC-1-A and HEC-1-B cells, illustrating increased instability present in the HEC-1-A cells.

![Figure 3](image-url)
Mismatch Repair Defects in HEC-1-A and HEC-1-B Cell Lines

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**Mutational Specificity at HPRT in HEC-1-A and HEC-1-B Cell Lines**—The molecular nature of mutations arising spontaneously in HEC-1-A and HEC-1-B cells was determined by sequencing independent HPRT mutations and compiling a mutational spectrum. Pre-existing HPRT mutants were eliminated by growing cultures in HAT medium, followed by plating 100-cell inocula. Unique mutations within the same 100-cell inocula or the same mutation arising from different 100-cell clones were defined as independent. A total of 107 independent HPRT mutants were isolated and sequenced; 52 mutants were analyzed for HEC-1-A and 55 for HEC-1-B. The obtained spectra at HPRT for both lines is presented in Table III, whereas the type and position of the molecular defects are presented in Table IV. Additionally, the determined mutational spectra was used to derive the mutation rate per type of mutation by multiplying a specific incidence by the HPRT mutation rate presented in Table II. The calculated mutation rate per type of mutation was then normalized to that of a normal spontaneous mutation rate (obtained in a similar manner: spectra of normal spontaneous mutations from the human HPRT data base (35) multiplied by the mutation rate observed in the normal human fibroblast line, NHF-1, resulting in a background, fractional spontaneous mutation rate). In comparison with the increased rate of frameshifts in HEC-1-A, the rate is reduced 28-fold (19,000-fold above background in HEC-1-A versus 670-fold above background in HEC-1-B; Table V). Considering all frameshifts in HEC-1-B, the relative rate was 150-fold above normal spontaneous frameshift rates, which is also a 28-fold reduction from HEC-1-A rates.

Both the HEC-1-A and HEC-1-B cell lines exhibited similar specificity for base substitutions, as well as for the positions of mutations at HPRT (Table IV). Several mutational events were common among both cell lines, including two hotspot base substitution mutations at position 134 (G to T) and at position 508 (C to T). These two hotspot mutations accounted for 19% of all base substitution mutations (14 of 75) for both HEC-1-A and HEC-1-B cells. The C to T mutation at 508 was slightly favored (8 of 14). The presence of these hotspots was not cell line-specific because they occurred in both cell lines at approximately the same incidence (six in HEC-1-A and eight in HEC-1-B). The remaining base substitutions were distributed evenly throughout the 657-base coding region of HPRT. Considering the similar specificity among base substitutions between the HEC-1-A and HEC-1-B cell lines, it can be stated that the most striking difference in the mutation spectra is the proportion and relative rate of frameshift mutation, specifically those occurring in the run of six guanines at positions 207–212.

**DISCUSSION**

The finding that mismatch repair deficiencies contribute to human carcinogenesis has launched intense investigations into the biochemistry of the proteins involved. Several human cancer cell lines have been identified with specific MMR gene

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### Table II

| Cell line | Mutant frequency $\times 10^{-5}$ | Mutation rate $\times 10^{-7}$ | Std error $\times 10^{-7}$ | Average mutation rate $\times 10^{-7}$ | Relative mutation rate $\times 10^{-7}$ |
|-----------|----------------------------------|------------------------------|----------------------------|--------------------------------------|----------------------------------------|
| HEC-1-A   | 47 ± 3.8                         | 290                          | 19                        | 310                                  | 660                                    |
| HEC-1-B   | 36 ± 6.9                         | 49                           | 6.6                       | 51                                   | 110                                    |
| NHF-1     | ≤0.3                             | 53                           | <0.5                      | <0.5                                 | 1                                      |

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**TABLE III**

| HEC-1-A | HEC-1-B |
|---------|---------|
| Base substitutions $^a$ | 26 (50) | 49 (89) |
| Transitions: | | |
| G:C → A:T | 10 (19) | 12 (22) |
| A:T → G:C | 4 (8) | 6 (11) |
| Transversions: | | |
| G:C → C:G | 0 | 0 |
| G:C → T:A | 7 (14) | 19 (35) |
| A:T → C:G | 2 (4) | 2 (4) |
| A:T → T:A | 0 | 5 (9) |
| Putative splice $^c$ | 3 (6) | 5 (9) |
| Frameshifts $^b$ | 26 (50) | 6 (11) |
| +1 bp | 22 (42) | 5 (9) |
| −1 bp | 4 (8) | 1 (2) |
| Total number of mutants | 52 (100) | 55 (100) |

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$^a$ Incidence (percent of total).
$^b$ Includes putative splice mutations as base substitutions.
$^c$ Either exon deletions or deletion of several bases at exon junctions.
Mismatch Repair Defects in HEC-1-A and HEC-1-B Cell Lines

TABLE IV
Position and type of spontaneous mutation at the HPRT locus in HEC-1-A and HEC-1-B cell lines

| Position | Mutation | 5' to 3' Sequence | Result | HEC-1-A | HEC-1-B |
|----------|----------|-------------------|--------|---------|---------|
| 1        | A → G    | gtt ATG GCG       | M      | 1/52    | 1/55    |
| 3        | G → T    | gtt ATG GCG       | M      | 1/52    | 1/55    |
| 46       | G → T    | CCA GGT TAT       | M      | 1/55    | 1/55    |
| 50       | A → G    | GGT TAT GAC       | M      | 1/55    | 1/55    |
| 59       | A → T    | CTT GAT TTA       | M      | 1/55    | 1/55    |
| 113      | C → A    | ATT CCT CAT       | M      | 1/55    | 1/55    |
| 118      | G → T    | CAT GGA CTA       | N      | 2/55    | 2/55    |
| 119      | G → T    | CAT GGA CTA       | M      | 1/55    | 1/55    |
| 125      | T → G    | CTA ATT ATG       | M      | 1/52    | 1/52    |
| 131      | A → G    | ATG GAC AGG       | M      | 1/52    | 1/52    |
| 134      | G → T    | GAC AGC ACT       | M      | 1/52    | 4/55    |
| 143      | G → A    | GAA CGT CTT       | M      | 1/52    | 1/55    |
| 148      | G → A    | CTT GGT CAG       | M      | 1/52    | 1/55    |
| 149      | C → T    | CTT GGT CAG       | M      | 1/52    | 1/55    |
| 154      | G → T    | CGA GAT TGT       | M      | 1/55    | 1/55    |
| 155      | A → G    | CGA CAT GTC       | M      | 1/52    | 1/55    |
| 158      | A → C    | GAT GTC ATG       | M      | 1/55    | 1/55    |
| 194      | T → A    | GCC TCT TGT       | M      | 1/55    | 1/55    |
| 197      | G → A    | CTC GTG TGT       | M      | 1/55    | 1/55    |
| 200      | T → G    | TGT GTG CTC       | M      | 1/55    | 1/55    |
| 205      | A → G    | CTC AAG GGG       | M      | 1/52    | 1/55    |
| 207      | -G       | AAG GGG GGC       | F      | 2/52    | 1/55    |
| 209      | G → A    | AAG GGG GGC       | M      | 1/55    | 1/55    |
| 212      | G → A    | GGG GGC TAT       | M      | 1/55    | 1/55    |
| 212      | G → T    | GGG GGC TAT       | M      | 2/55    | 2/55    |
| 249      | A → C    | ATC AAG GCA       | M      | 1/52    | 1/52    |
| 342      | -A       | ATA AAA GTA       | F      | 1/52    | 2/55    |
| 409      | A → T    | ATG ATT GAC       | M      | 1/52    | 1/55    |
| 416      | C → A    | GAC ACT GGC       | M      | 1/52    | 1/55    |
| 416      | C → T    | GAC ACT GGC       | M      | 1/52    | 1/55    |
| 419      | G → A    | ACT GGC AAA       | M      | 1/52    | 1/55    |
| 485      | G → T    | GCA GTC ATT       | M      | 1/52    | 3/55    |
| 496      | -A       | GTC AAA AGG       | F      | 1/52    | 1/52    |
| 498      | A → G    | GTG AAA AGG       | M      | 1/55    | 1/55    |
| 500      | G → T    | AAA AGG ACC       | M      | 2/55    | 2/55    |
| 508      | C → T    | CCA GGA ACT       | N      | 4/52    | 4/55    |
| 527      | C → A    | AAG CCA GAC       | M      | 1/55    | 1/55    |
| 530      | A → G    | CCA GAC TTT       | M      | 1/55    | 1/55    |
| 531      | C → T    | CCA GGA TTT       | S      | 1/52    | 1/55    |
| 568      | G → T    | GTA GGA TAT       | N      | 2/52    | 1/55    |
| 595      | T → G    | TAC TGT AGG       | M      | 1/55    | 1/55    |
| 598      | A → G    | TGC AGG GAT       | M      | 2/52    | 1/55    |
| 610      | C → T    | AAT CAT GTT       | M      | 1/55    | 1/55    |
| 620      | T → A    | TGT GGC ATT       | M      | 1/55    | 1/55    |

a Relative to the A of ATG initiation codon (30).
b Surrounding sequence.
c Result: F, frameshift; M, missense; N, nonsense; S, silent.
d Silent mutation observed in a double mutant.

defects, which have aided these investigations. However, these studies have been limited by virtue of the fact that the commonly studied cell lines are not isogenic, and furthermore, cell lines defective in MMR possess a mutator phenotype (34). One technique that has proven useful for the study of MMR is the complementation of a specific gene defect in the parent cell line by transferring a normal human chromosome that carries a wild-type copy of the gene in question (reviewed in Ref. 36). These chromosome transfer cell lines complement the MMR gene defective in the parent line by introducing a wild-type copy of the gene. Thus, the specific MMR gene can be implicated as causative in the genetic instability observed in the parental cells. This procedure has proven invaluable with regard to providing insights into the roles that specific MMR genes play in maintaining genomic stability (4, 12, 27, 37).

With HEC-1-A and HEC-1-B cell lines, we are provided with a unique opportunity: two endometrial carcinoma cell lines originally isolated from the same tumor that differ in the presence of a particular wild-type MMR gene. By comparing the phenotypes observed in each individual cell line, the roles of specific MMR gene products, or their respective heterodimers, can be investigated. The HEC-1-A cell line lacks full-length protein for either the hPMS2 or the hMSH6 gene. This cell line is therefore potentially defective in both the hMutLa (hMLH1/hPMS2) repair complex heterodimer and the hMutSa (hMSH2/hMSH6) mismatch recognition heterodimer. According to the current model, MMR processes initiated by either hMutSa (hMSH2/hMSH6) or hMutSβ (hMSH2/hMSH3) should be compromised by the defect in hMutLa (hMLH1/hPMS2) because both are proposed to process mismatched DNA through the same hMutLa heterodimer. The HEC-1-B cell line, however, retains a wild-type copy of the hPMS2 gene. The only apparent MMR defect in the cells is in the hMSH6 gene, resulting in a defective hMutSa (hMSH2/hMSH6) heterodimer. This cell line has a wild-type hPMS2 gene, and in light of the proficiency in vitro repair of looped intermediates (Fig. 3), it can be inferred that the second mismatch recognition complex, hMutSβ (hMSH2/hMSH3), is functional in HEC-1-B cells. Thus, phenotypic differences observed between the HEC-1-A and HEC-1-B cell lines can be directly attributed to this hMSH2/hMSH3 heterodimer, assuming no other defect exists within the pathway, as illustrated in Fig. 1.

The HEC-1-A cell line is defective in both hMSH6 and hPMS2 and lacks repair of all MMR substrates examined. Fig.
Mismatch Repair Defects in HEC-1-A and HEC-1-B Cell Lines

3 illustrates that HEC-1-A cells are deficient in MMR in vitro for a variety of mismatched intermediates. This lack of MMR in HEC-1-A leads to high levels of microsatellite instability (Table I) and a substantially elevated HPRT mutation rate (660-fold; Table II). The specificity of mutation determined by sequencing independent HPRT mutants shows an approximately equal incidence of frameshift and base substitution mutations (Table III), which corresponds to a relative rate of 4,100-fold and 650-fold above spontaneous background mutation rates for frameshift and base substitution mutations. This significantly elevated relative rate of frameshift mutations clearly illustrates the crucial role MMR plays in the recognition and repair of frameshift intermediates. Taken together, the phenotypes observed for HEC-1-A cells are hallmarks of genomic instability observed as a result of the total loss of MMR.

In HEC-1-B cells, there is a wild-type hPMS2 allele present, as well as the mutant hPMS2 allele found in HEC-1-A cells. The remaining defect in hMSH6 provides an interesting situation: phenotypic differences observed in HEC-1-B cells when compared with HEC-1-A cells may directly implicate the role of hMSH3, or more correctly, hMutSβ (hMSH2/hMSH3) (Fig. 1). The first observed phenotypic difference in the HEC-1-B cell line relative to HEC-1-A cells is illustrated by the HPRT mutation rates presented in Table II. Here, HEC-1-B cells have a 6-fold lower rate than HEC-1-A cells, suggesting a substantial contribution by hMSH2/hMSH3 in the repair of single-base mismatches. Furthermore, the specificity of mutation presented in Table III suggests that hMSH3 may be more efficient in the repair of 1-base loops versus base-base mismatches. This suggestion is supported by the drastic shift in the incidence of frameshift mutations from 50% in HEC-1-A to 11% in HEC-1-B. Furthermore, the relative rate of frameshift mutation in HEC-1-B is 150-fold above normal spontaneous background (Table V), in comparison with 4,100-fold above background for HEC-1-A. This 28-fold decrease in the relative rate of frameshift mutation corresponds to an approximately 96% reduction in the rate of frameshifts in HEC-1-B cells relative to HEC-1-A cells. In comparison, the relative rate of base substitution mutation is 190-fold above normal spontaneous, whereas the rate of base substitution mutation in HEC-1-A is 650-fold above background. This 3.5-fold reduction equates to a 70% reduction in the rate of base substitution mutations. These data suggest that the hMutSβ heterodimer is capable of repairing base-base mismatches.

Further evidence for hMutSβ's involvement in the repair of single-base mismatches is provided by the in vitro repair activity (Fig. 3) observed in the HEC-1-B cells. Although base-base mismatch repair is not detected, HEC-1-B cells are proficient in the repair of 1-base-looped intermediates, suggesting that hMutSβ (hMSH2/hMSH3) is capable of repairing single-base mismatches. These findings support a redundancy for repair between hMSH2/hMSH3 and hMSH2/hMSH6, the heterodimer originally suspected to be chiefly involved in the repair of single-base mismatches. It should be noted, however, that this observed redundancy may only have implications in cells defective in hMSH6, and although the repair of single-base mismatches is obvious from the above results, the biochemical contribution of hMutSβ (hMSH2/hMSH3) under normal conditions for the repair of single-base mismatches may be minimal.

The data presented here obtained from two endometrial carcinoma cell lines that originated from the same tumor provide a means to implicate specific MMR genes in the roles of human MMR. The differing genotypes for each cell line and comparisons between determined phenotypes allow concluding remarks with regards to overlapping function. Interestingly, the data presented here parallel that obtained in the HHUA cell line and a respective chromosome transfer line (12). The phenotypes associated with the double-mutant HHUA cell line (hMSH6/hMSH3-defective) are similar to that obtained for HEC-1-A (hPMS2/hMSH6-defective). These include a significantly elevated mutation rate at HPRT predominated by frameshifts, elevated microsatellite instability, and lack of in vitro MMR activity for a wide range of substrates. By complementing the hMSH3 mutant in HHUA by transferring a normal chromosome 5, which potentially restores function to the hMutSβ (hMSH2/hMSH3) heterodimer while remaining defective in hMutSα (hMSH2/hMSH6), the observed phenotypes are quite similar to HEC-1-B. These similarities include the proficient repair of 1- to 4-base-looped DNA intermediates in vitro, the relative stability of microsatellite sequences, and the substantial decrease in the rate of mutation at HPRT for both base substitution and frameshift mutation. Thus, HEC-1-A and HEC-1-B compared with HHUA and HHUA + chromosome 5
Mismatch Repair Defects in HEC-1-A and HEC-1-B Cell Lines

(wild-type hMSH3), respectively, appear phenotypically analogous. These observations support conclusions made here. That is, the complete loss of MMR, such as that observed in HEC-1-A cells (hMSH6/hPMS2-defective), results in substantial genomic instability and that frameshift intermediates appear to be an important component of such instability. Additionally, the data collected for HEC-1-B cells (hMSH6-defective), illustrate the role of hMutSβ in the repair of single-base mismatches, as indicated by the substantial reduction in phenotypes indicative of such repair. These results support the functional overlap of hMSH6 and hMSH3 recently illustrated (12).

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