

Mismatch repair deficiency in hematological malignancies with microsatellite instability

Liya Gu^{*1}, Brandee Cline-Brown¹, Fujian Zhang³, Lu Qiu¹ and Guo-Min Li^{1,2,3}

¹Department of Pathology and Laboratory Medicine, University of Kentucky Medical Center, Lexington, Kentucky, KY 40536, USA; ²Lucille P. Markey Cancer Center, University of Kentucky Medical Center, Lexington, Kentucky, KY 40536, USA;

³Graduate Center for Nutritional Sciences, University of Kentucky Medical Center, Lexington, Kentucky, KY 40536, USA

Mutations in human mismatch repair (MMR) genes are the genetic basis for certain types of solid tumors displaying microsatellite instability (MSI). MSI has also been observed in hematological malignancies, but whether these hematological malignancies are associated with MMR deficiency is still unclear. Using both biochemical and genetic approaches, this study analysed MMR proficiency in 11 cell lines derived from patients with hematological malignancies and demonstrated that six out of seven hematological cancer cell lines with MSI were defective in strand-specific MMR. *In vitro* complementation experiments, using characterized MMR mutant extracts or purified proteins, showed that these hematological cancer cells were defective in either hMutS α (a heterodimer of hMSH2 and hMSH6) or hMutL α (a heterodimer of hMLH1 and hPMS2). Furthermore, cell lines deficient in hMutS α showed large deletions or point mutations in *hMSH2*, while those deficient in hMutL α exhibited point mutations in *hMLH1* or a lack of expression of *hPMS2*. From these results, we conclude that, as in solid tumors, hematological malignancies with MSI are also associated with MMR deficiency, and that the cause of MMR deficiency in these cell lines is due to a defective MutS α or MutL α . We also report here, for the first time, that an MSI-positive cell line derived from Burkitt's lymphoma is proficient in MMR.

Oncogene (2002) 21, 5758–5764. doi:10.1038/sj.onc.1205695

Keywords: hMSH2; hMLH1; microsatellite instability; hematological malignancy

Introduction

DNA mismatch repair (MMR) maintains genomic stability by correcting chromosomal errors associated with DNA replication and recombination (Kolodner, 1996; Modrich and Lahue, 1996) and by mediating

DNA damage-induced apoptosis (Li, 1999). Mutations in human MMR genes are the genetic basis for certain types of hereditary and sporadic tumors, including hereditary nonpolyposis colorectal cancer (HNPCC) (for reviews see Buermeier *et al.*, 1999; Hsieh, 2001; Jiricny, 1998; Kolodner and Marsischky, 1999; Modrich and Lahue, 1996). HNPCC and sporadic tumors with mutations of MMR genes exhibit frequent alterations in simple repetitive DNA sequences called microsatellites (Aaltonen *et al.*, 1993; Boland *et al.*, 1998; Eshleman and Markowitz, 1995; Thibodeau *et al.*, 1993). This form of alteration is referred to as microsatellite instability (MSI) and is regarded as a hallmark of MMR deficiency.

In addition to its demonstration in solid tumors, MSI has also been observed in hematological malignancies (De Vita *et al.*, 1997; Hayami *et al.*, 1999; Kaneko *et al.*, 1996; Kodera *et al.*, 1999; Larson *et al.*, 1997). However, whether these hematological malignancies are associated with MMR defects is still controversial. For example, Molenaar *et al.* (1998) studied genomic instability in DNA isolated from the blasts or cell lines derived from lymphoblastic leukemia patients, and provided evidence suggesting there was no association between MMR defects and leukemia. In contrast, other reports have identified alterations of MMR genes in leukemia cells displaying MSI (Hang-aishi *et al.*, 1997; Levati *et al.*, 1998; Zhu *et al.*, 1999). These conflicts could be due to the complex nature of the human MMR system and the methods used in previous studies that were unable to accurately determine MMR proficiency in cells.

In HNPCC patients, mutations have been identified in the genes that code for the human MutS (hMutS) and MutL (hMutL) homologs, which are an absolute requirement for the human MMR. Unlike in *Escherichia coli*, where MutS and MutL are a functional homodimer, human cells possess multiple forms of hMutS and hMutL, each of which is a heterodimer. hMSH2 interacts with hMSH6 or hMSH3 to form the hMutS α heterodimer (Drummond *et al.*, 1995; Palombo *et al.*, 1995) or the hMutS β heterodimer (Genschel *et al.*, 1998; Palombo *et al.*, 1996), respectively. hMLH1 interacts with hPMS2, hPMS1, or hMLH3 to form three distinct hMutL heterodimers (Flores-Rozas and Kolodner, 1998; Leung *et al.*, 2000; Li and Modrich, 1995; Lipkin *et al.*, 2000). These hMutS and

*Correspondence: L Gu, Department of Pathology and Laboratory Medicine, University of Kentucky Medical Center, 800 Rose Street, Lexington, KY 40536, USA; E-mail: lgu0@uky.edu
Received 26 March 2002; revised 15 May 2002; accepted 20 May 2002

hMutL heterodimers are functionally redundant (reviewed in Nakagawa *et al.*, 1999). For example, although hMutS α (hMSH2–hMSH6) specifically recognizes base–base mismatches, it can also bind to small insertion/deletion mispairs, which are also recognized by hMutS β (hMSH2–hMSH3). Thus, defects in *hMSH6* or *hMSH3* alone will not result in a mutator phenotype that is as severe as that observed in an *hMSH2* mutant. Presently, it is not known whether the identified alterations of MMR genes in leukemia cells have any effects on MMR function. Additionally, while mutations of hMutS α and hMutL α genes alone may be the sole reason for potential defects of MMR in these cell lines, it is conceivable that defects in unidentified MMR genes may also be responsible for the MSI phenotype in hematological cancer cells.

To accurately determine if hematological malignancies are associated with MMR defects, this study utilized a functional assay to analyse MMR proficiency in cell lines derived from hematological malignancies. Our results demonstrate that hematological malignant cells with MSI are defective in strand-specific MMR. *In vitro* complementation experiments using the known MMR mutant extracts or purified proteins demonstrate that the cause of MMR defects in these cancer cell lines is due to a defective hMutS α or hMutL α . Genetic analysis revealed that most of these hematological malignancies harbor alterations in *hMSH2* and *hMLH1*. Therefore, hematological malignancies with MSI are also associated with MMR deficiency.

Results

Hematological malignant cell lines with MSI are defective in MMR

MSI status of most hematological cancer cell lines used in this study was well documented (see Table 1 for details). To examine the MMR capability of these cell lines, a G-T (base–base mismatch) heteroduplex with a strand break 5' to the mismatch, designated as 5' G-T, and an unpaired CA insertion/deletion heteroduplex with a strand break 3' to the heterology, designated as 3' γ CA \setminus , were constructed (Parsons *et al.*, 1993) (also see Figure 1). These two heteroduplex substrates allow determination of MMR proficiency for error-specificity and orientation-specificity of the repair (Drummond *et al.*, 1995). We tested MMR capability of ten leukemia cell lines and a Burkitt's lymphoma cell line using these two substrates.

As shown in Table 1, nuclear extracts of all four MSI-negative cell lines (CCRF-SB, 697, WI-L2-NS, and TK6) were capable of correcting both the 5' G-T and the 3' γ CA \setminus substrates, but nuclear extracts derived from all MSI-positive cell lines, except NAMALWA (see below for details), did not support the repair of either substrate. Repair assays mixing the individual defective extracts and the MMR-proficient TK6 extract (Table 1, also see Kat *et al.*, 1993) ruled out the presence of a diffusible inhibitor as the basis of the

Table 1 Mismatch repair proficiency in hematological cancer lines

| Cell line | MSI | Repair (fmol/15 min) | |
|------------|------------------|----------------------|----------------------------|
| | | 5' G-T | 3' γ CA \setminus |
| CCRF-CEM | + ^{a,b} | <0.3 | <0.3 |
| CCRF-HSB-2 | + ^a | <0.3 | <0.3 |
| NALM-6 | + ^{a,b} | <0.3 | <0.3 |
| NAMALWA | + ^{b,c} | 10.3 | 9.8 |
| REH | + ^{a,b} | <0.3 | <0.3 |
| MOLT4 | + ^b | <0.3 | <0.3 |
| MOLT14 | + ^a | <0.3 | <0.3 |
| CCRF-SB | - ^a | 6.2 | 3.2 |
| 697 | - ^a | 4.4 | 4.1 |
| WI-L2-NS | - ^c | 8.7 | 7.6 |
| TK6 | - ^d | 9.7 | 7.9 |

Repair assays were performed in reactions containing 50 μ g of nuclear extract and 24 fmol of heteroduplex DNA. The reaction mixtures were incubated at 37°C for 15 min. DNA samples were recovered and digested with restriction endonucleases (*HindIII* and *Bsp106* for 5' G-T substrate and *XcmI* and *Bsp106* for 3' γ CA \setminus substrate) to score the repair as described in Materials and methods. Each value represents the average of two determinations. ^aMolenaar *et al.*, 1998. ^bKodera *et al.*, 1999. ^cThis study. DNA was purified from independent single-cell clones and used to amplify microsatellite markers BAT25, BAT26, D2S123, and D5S346 as described (Parsons *et al.*, 1993; Umar *et al.*, 1994). Different microsatellite patterns were observed among subclones derived from NAMALWA cells in all four markers, but not in those from WI-L2-NS cells (data not shown). ^dUmar *et al.*, 1994

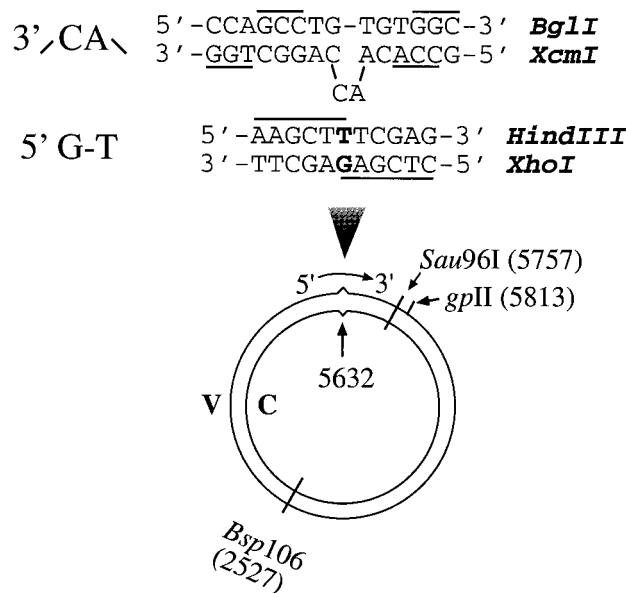


Figure 1 DNA heteroduplex substrates used in MMR assay. The heteroduplexes were constructed from the f1MR phage series (Su *et al.*, 1988) to contain (A) a G-T mismatch and a strand break (at the *Sau96I* site) in the complementary strand (C) 125 bp 5' to the mismatch (5' G-T) or (B) a γ CA \setminus dinucleotide insertion/deletion mismatch with a strand break (at the *gpII* site) in the viral strand (V) 181 bp 3' to the mispair (3' γ CA \setminus substrate). Both substrates are resistant to restriction enzyme hydrolysis at the site of the mismatch; however, repair of the substrate forms a homoduplex product that is sensitive to *HindIII* (for the 5' G-T substrate) or *XcmI* (for the 3' γ CA \setminus substrate) hydrolysis

repair deficiency in these cells (results not shown). This was also confirmed by complementation experiments

among these defective extracts (see below). These results suggest that, like the MSI-positive cell lines derived from solid tumors (Boyer *et al.*, 1995; Parsons *et al.*, 1993; Umar *et al.*, 1994), hematological malignant cells with MSI are also defective in strand-specific MMR.

Leukemia cell lines are defective in either hMutS α or hMutL α

To determine the molecular basis for the repair deficiencies in the mutant leukemia cell lines, we performed complementation analyses (Table 2) by mixing nuclear extracts of these mutant cells with those of the known MMR mutants, hMutL α -deficient H6 (Li and Modrich, 1995) and hMutS α -deficient MT1 (Drummond *et al.*, 1995). Based on these experiments, these mutant leukemia cell lines can be divided into two complementation groups: one that could be complemented by the H6 extracts (the H6-complementation group) and the other that could be complemented by the MT1 extracts (the MT1-complementation group). As shown in Table 2, nuclear extracts of H6 cells could complement extracts of NALM-6, CCRF-HSB-2, and MOLT14 in repair of the 5' G-T substrate, but could not correct the defects in MOLT4 and CCRF-CEM cells, suggesting that the defect(s) in the former lines are not in hMutL α , and that the latter lines harbor the same defects as in the H6 cell line (i.e., hMutL α). Conversely, nuclear extracts of MT1 could restore MMR to cell lines MOLT4 and CCRF-CEM, but could not do so to cell lines MOLT14, CCRF-HSB-2, and NALM-6, indicating that cell lines MOLT14, CCRF-HSB-2, and NALM-6 are defective in hMutS α . These assumptions were confirmed when purified hMutS α or hMutL α were used in the complementation assay. It was found that hMutS α restored MMR to extracts of MOLT14 (data not shown), CCRF-HSB-2 (Figure 2, lane 6), and NALM-6 cells (Figure 2, lane 9); and that hMutL α corrected the defect in CCRF-CEM (Figure 2, lane 4) and MOLT4 (data not shown) cells. As expected, complementation experiments among these mutant cell lines revealed that nuclear extracts derived from any individual cell lines of the H6-complementation group could complement repair to those of any cell lines in

Table 2 Complementation groups of leukemia cell lines

| | MT1 | H6 | MOLT4 | CEM | MOLT14 | HSB | NALM-6 |
|--------|----------------------|------|-------|------|--------|------|--------|
| | Repair (fmol/15 min) | | | | | | |
| MOLT4 | 3.8 | <0.3 | - | <0.3 | 3.9 | 3.8 | 3.6 |
| CEM | 3.2 | <0.3 | - | - | 10.5 | 6.0 | 4.6 |
| MOLT14 | <0.3 | 4.2 | - | - | - | <0.3 | <0.3 |
| HSB | <0.3 | 3.2 | - | - | - | - | <0.3 |
| NALM-6 | <0.3 | 4.3 | - | - | - | - | - |

Repair assays were performed in reactions containing 50 μ g of each nuclear extract and 24 fmol of 5' G-T substrate. After incubation at 37°C for 15 min, DNA samples were recovered and digested with *Hind*III and *Bsp*106 to score the repair as described in Materials and methods. Each value represents the average of two determinations

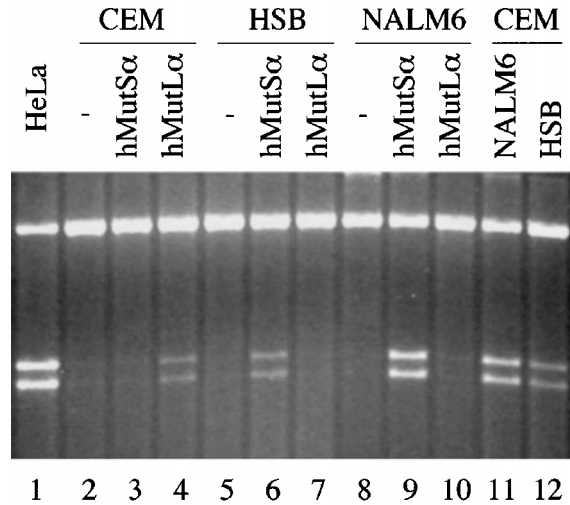


Figure 2 Restoration of MMR to leukemia cell lines by exogenous hMutS α or hMutL α . MMR assays were performed in 15- μ l reactions containing 50 μ g of nuclear extract from each cell line, 24 fmol 5' G-T (6.4 kb in size), and 50 ng of purified hMutS α or hMutL α (when present). After incubation at 37°C for 15 min, DNA was recovered, digested with restriction enzymes *Hind*III (scoring enzyme) and *Bsp*106, and fractionated by agarose gel electrophoresis. The top band in each reaction represents unrepaired substrate (6.4 kb in size) and the two smaller fragments (3.3-kb and 3.1-kb in size) are repaired products. CEM, cell line CCRF-CEM; HSB, cell line CCRF-HSB-2

the MT1-complementation group, and the reverse is also true (Table 2 and Figure 2, lanes 11 and 12). These results further confirm that MMR deficiency in these leukemia cell lines is not due to an inhibitory factor, but due to defective repair component(s). However, repair was not observed in reactions containing two extracts derived from cell lines in the same complementation group (Table 2), indicating that all members of the same complementation group have an identical defect, i.e., hMutL α in the MT1-complementation group and hMutS α in the H6-complementation group.

Alterations of hMSH2, hMLH1, or hPMS2 in leukemia cell lines

Both hMutS α (hMSH2-hMSH6) and hMutL α (hMLH1-hPMS2) are functional heterodimers (Drummond *et al.*, 1995; Li and Modrich, 1995). To determine which subunit is defective in the hMutS α - and hMutL α -deficient leukemia cell lines, antibodies against the individual subunits of each heterodimer were utilized for Western blot analysis of leukemia cell extracts. For the H6-complementation group, little (MOLT14) or no (NALM-6 and CCRF-HSB-2) hMSH2 could be detected, although significant amounts of hMLH1 and hPMS2 were observed (Figure 3). Conversely, in the MT1-complementation group, the expression of hMSH2 and hMSH6 were apparently normal, but the lack of expression of hMLH1 or hPMS2 was evident. For example, while REH cells did not express hMLH1 and hPMS2, MOLT4 lacked

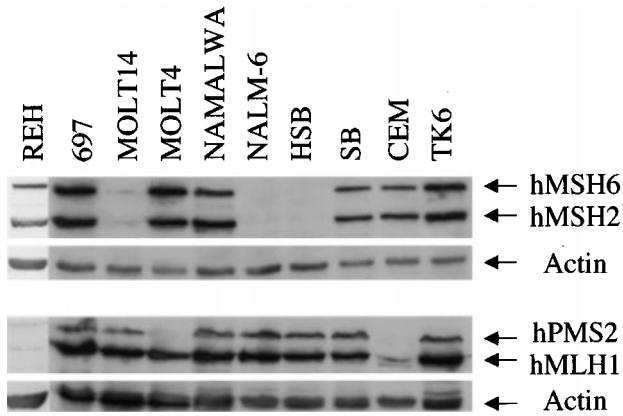


Figure 3 Western blot analysis of MMR proteins in hematological cancer cell lines. Nuclear extracts (50 μ g) were electrophoresed through SDS-polyacrylamide gels and electro-transferred to nitrocellulose membranes. The membranes were blotted with antibodies against hMSH2 and hMSH6 (top panel) or hMLH1 and hPMS2 (bottom panel), and bound antibodies were detected by chemiluminescence using a secondary antibody conjugated with horseradish peroxidase. Actin was used to monitor the level of proteins loaded in each reaction. CEM, cell line CCRF-CEM; HSB, cell line CCRF-HSB-2

hPMS2 only, and CCRF-CEM lacked hPMS2 and expressed very limited hMLH1 (Figure 3). Given the fact that hMLH1 and hMSH2 are the obligatory components for all hMutL and hMutS heterodimers, respectively, lost or significantly reduced expression of either of these two components likely leads to destabilization of their partners. Therefore, cell lines in the H6-complementing group (NALM-6, MOLT14, and CCRF-HSB-2) are probably defective in hMSH2 and those in the MT1-complementing group (except MOLT4, which is defective in hPMS2), are most likely defective in hMLH1.

On the basis of the complementation and the Western blot experiments, we analysed the *hMLH1* gene for mutations in CCRF-CEM and REH cells and the *hMSH2* gene for mutations in NALM-6 and CCRF-HSB-2 cells. Primers were designed to amplify all *hMSH2* exons and *hMLH1* exons. All *hMSH2* exons could be amplified from genomic DNA isolated from MMR proficient cells (e.g., CCRF-SB) or hMutL α -deficient cells (e.g., CCRF-CEM and REH), but not all *hMSH2* exons could be detected in hMutS α -deficient NALM-6 and CCRF-HSB-2 cells (Figure 4). In NALM-6 cells, PCR products were observed for exons 3, 6 and 10 of *hMSH2*, while repeated attempts to amplify the remaining *hMSH2* exons were unsuccessful. These observations suggest the presence of deletions or rearrangements of the *hMSH2* gene in the NALM-6 cell line. Despite the fact that all hMLH1 exons were detected (results not shown) in the CCRF-HSB-2 cell line, no single *hMSH2* exon in CCRF-HSB-2 could be amplified by PCR compared with wild-type controls (Figure 4). Therefore, cell line CCRF-HSB-2 also appears to exhibit deletions/rearrangements in the *hMSH2* gene.

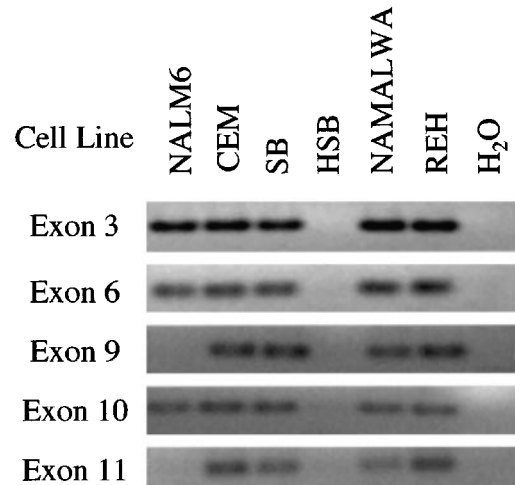


Figure 4 Deletion of *hMSH2* exons in leukemia cell lines. Individual *hMSH2* exons were amplified by PCR reactions in the presence or absence of 100 ng of genomic DNA from individual cell lines as described in Materials and methods. PCR products were analysed by agarose gel electrophoresis and visualized by UV illumination in the presence of ethidium bromide. Only exons 3, 6, 9, 10, and 11 are shown. CEM, cell line CCRF-CEM; HSB, cell line CCRF-HSB-2

Similar analysis was performed for the *hMLH1* gene in cell lines CCRF-CEM and REH cells. PCR products of the individual *hMLH1* exons exhibited expected sizes from both cell lines (data not shown), suggesting that point mutations rather than deletions of the *hMLH1* gene may be responsible for repair deficiency in these cell lines. We therefore conducted single-strand conformation polymorphism (SSCP) analysis to determine mutations of *hMLH1* in CCRF-CEM and REH cells. An abnormal PCR product was observed in exon 9 of *hMLH1* for both cell lines (data not shown). DNA sequencing analysis of these abnormal PCR products revealed that both cell lines harbored a guanine to adenine base substitution in the consensus splicing donor site (gtaa) of intron 9, which converts the splicing donor sequence from AACCGtaa to AACCCataa (see Figure 5). This G to A substitution in REH cells is a homozygous mutation (Figure 5), but is heterozygous mutation in CCRF-CEM cells (data not shown). Our observation in CCRF-CEM is consistent with a previous report (Hangaishi *et al.*, 1997). This mutation has been shown to block the splicing of the *hMLH1* mRNA and lead to an altered hMLH1 protein (Hangaishi *et al.*, 1997).

MSI-positive Burkitt's lymphoma cell line NAMALWA is proficient in MMR

Previous studies in solid tumor cells (Boyer *et al.*, 1995; Parsons *et al.*, 1993; Umar *et al.*, 1994), and this study in leukemia cells, have demonstrated that MSI is closely associated with MMR deficiency. However, this correlation is challenged by studies of a Burkitt's lymphoma cell line, NAMALWA. NAMALWA cells clearly display MSI, as judged by their frequent

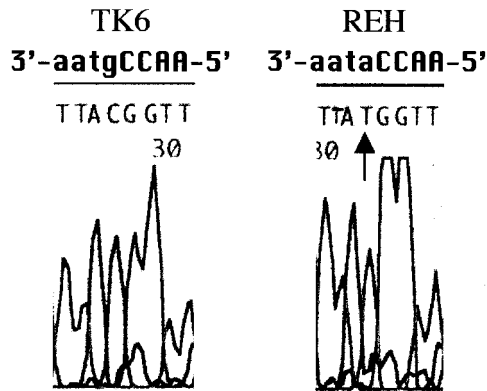


Figure 5 Mutations of *hMLH1* in leukemia cells. Genomic DNA (100 ng) was used to amplify individual *hMLH1* exons in the presence of dNTPs and [α - 32 P]-dCTP. PCR products were fractionated on a 0.5X MED SSCP gel and detected by autoradiography or by phosphorimaging. Abnormal SSCP bands (not shown) were excised and reamplified by PCR, and the products were then purified and sequenced with an ABI sequencer, as described in Materials and methods. A guanine to adenine transition (arrow) in the splicing site of intron 9 was identified in cell lines CCRF-CEM (not shown) and REH, as compared with the wild-type sequence in TK6 cells

alterations in microsatellite markers tested, including BAT25, BAT26 (Kodera *et al.*, 1999), D2S123, and D5S346 (results not shown in this study). When the cell line was tested for *in vitro* MMR, it corrected heteroduplexes 5' G-T and 3' CA as efficiently as MSI-negative and MMR-proficient cells tested, such as TK6 and WI-L2-NS (see Table 1). This finding suggests that NAMALWA cells may be defective in a non-MMR pathway that is also responsible for genomic stability as described in yeast (Myung *et al.*, 2001a,b) or defective in a downstream component of MMR that cannot be scored by the current assay.

Discussion

Hematological malignancies have been reported to display microsatellite instability (De Vita *et al.*, 1997; Hayami *et al.*, 1999; Kaneko *et al.*, 1996; Kodera *et al.*, 1999; Larson *et al.*, 1997), but whether they are also deficient in MMR was still a matter of debate. To help resolve this issue, we studied MMR proficiency in several hematological cancer cell lines, using both biochemical and genetic analyses, and our results suggest that a significant portion of hematological cancer cells with MSI are MMR defective.

We found that among seven MSI-positive hematological cancer cell lines tested, six of them are defective in repair of heteroduplexes containing a strand break. As summarized in Table 3, defects in specific MMR components for these cell lines were identified. Our work indicate that these cell lines can be classified into two complementation groups; cell lines whose deficient MMR is rescued by exogenous hMutS α , or cell lines whose deficient MMR is rescued by exogenous hMutL α .

Table 3 Mutations of MMR genes in hematological cancer cell lines

| Cell line | Mutation |
|------------|--|
| CCRF-CEM | Heterozygous G to A transition at exon 9 splicing site |
| CCRF-HSB-2 | <i>hMSH2</i> gene deletion (entire gene) |
| NALM-6 | <i>hMSH2</i> gene deletion (exon 1-5, 7-9, and 11-16) |
| NAMALWA | ? |
| REH | Homozygous G to A transition at exon 9 splicing site |
| MOLT4 | No expression of hPMS2, but mutation(s) not determined |
| MOLT14 | No expression of hMSH2, but mutation(s) not determined |

It is interesting to note that almost all MMR mutant cell lines identified to date (Boyer *et al.*, 1995; Drummond *et al.*, 1995, 1996, 1997; Levati *et al.*, 1998; Marra *et al.*, 1998; Parsons *et al.*, 1993; Umar *et al.*, 1994), regardless of origin, belong to these two complementation groups. It is known that *E. coli*, in addition to MutS or MutL mutants, mutations in MutH, uvrD (helicase II), and exonucleases also lead to MMR deficiency. Why has the identification of MMR-deficient cancer cell lines that have mutations in these later homologous components not yet occurred? One possibility is that for some of these components, human cells may have multiple forms, which are functionally redundant. Thus, defects in one form may not be sufficient to block MMR. Secondly, some of these components may be essential for DNA metabolism so that their defects are lethal to cells. Thirdly, the *in vitro* MMR assays that are used by many laboratories are not sufficiently sensitive to detect some required MMR components. For example, the assay used in this work may not be able to score the involvement of a human MutH (hMutH) homolog if it is required, since a nicked substrate bypasses the requirements for MutH in prokaryotic MMR (Lahue *et al.*, 1989). Taken together, all these may explain why only two (hMutS and hMutL) MMR complementation groups have been identified in human cells.

There are multiple hMutS homolog genes (*hMSH2*, *hMSH3*, and *hMSH6*) and hMutL homolog genes (*hMLH1*, *hMLH3*, *hPMS1*, and *hPMS2*). However, five out of six MMR deficient-hematological cancer cell lines harbor mutations in either *hMSH2* or *hMLH1*, and only one is defective in *hPMS2*. Previous studies in HNPCC and other solid MSI-positive tumor cells also showed that most of mutations occur in *hMSH2* and *hMLH1* (reviewed in Boyer *et al.*, 1995; Kolodner and Marsischky, 1999; Modrich and Lahue, 1996). These observations are consistent with the obligatory roles of hMSH2 and hMLH1 in hMutS and hMutL heterodimers, respectively. We found that when hMSH2 or hMLH1 cannot be detected in a cell line, their corresponding partners are also missing (see Figure 3). In contrast to this, defects in a partner of hMSH2 or hMLH1 usually have no effect on the presence of the obligatory component. For example, lack of expression of hPMS2 in MOLT4 cells did not affect

the level of hMLH1 (see Figure 3), since hMLH1 can still form a dimer with hPMS1 and/or hMLH3. These results suggest that interaction of hMSH2 and hMLH1 with their partners is very important for stabilizing the later.

It is generally accepted that MSI is a hallmark of MMR deficiency. However, we show here that this is not true for the Burkitt's lymphoma cell line NAMALWA. While positive in MSI, NAMALWA cells are capable of processing both base-base and insertion/deletion mismatches with high efficiency (Table 1). To our knowledge, this is the first MSI-positive cell line reported to date that does not have an associated MMR deficiency in the current MMR assay. In addition to displaying MSI, the NAMALWA cell line has a mutator phenotype similar to most of the known MMR mutant cell lines. For example, the cell line is highly resistant to *N*-methyl *N'*-nitro *N*-nitrosoguanidine and hypermutable at the *HPRT* locus (Zhang and Li, unpublished results). These observations suggest that the NAMALWA cell line may still be defective in MMR. If this is true, the cell line could be defective in an activity conferring strand-specificity (including a MutH-like activity) or responsible for a downstream repair event. Alternatively, the uncoupling of MSI with MMR deficiency in NAMALWA cells may suggest that the cell line is deficient in a DNA repair pathway rather than MMR that is also responsible for stabilizing simple repetitive sequences. Myung *et al.* (2001a,b) have recently identified multiple pathways or group of proteins that suppress genomic instability, including those involving in S phase checkpoint function, homologous recombination, and telomere metabolism. The NAMALWA cell line is likely defective in one of these pathways. Therefore, uncovering the basis of MSI in NAMALWA cells will help to characterize pathway(s) suppressing genomic instability.

Materials and methods

Materials

Cell lines used in this study were purchased either from the Deutsche Sammlung Von Mikroorganism und Zellkulturen (697, REH, MOLT4, MOLT14, and NALM-6), Braunschweig, Germany or from the American Type Culture Collection (CCRF-CEM, CCRF-HSB-2, CCRF-SB and WI-L2-NS), Rockville, MD, USA. They were derived from patients with either B-lymphocyte leukemia (697, REH, CCRF-CEM, CCRF-HSB-2, and CCRF-SB), T-lymphocyte leukemia (MOLT4, MOLT14, WI-L2-NS), or Burkitt's lymphoma (NAMALWA). All cell lines were grown in RPMI 1640 supplemented with 10–20% fetal bovine serum (Hyclone). Nuclear extracts were prepared as described previously (Holmes *et al.*, 1990). hMutS α and hMutL α were purified to near homogeneity (>99% purity as judged by Coomassie Brilliant Blue staining, data not shown) as previously described (Drummond *et al.*, 1995; Li and Modrich, 1995).

Western blot analysis

Nuclear extracts (50 μ g) were electrophoresed through SDS-polyacrylamide gels and electrotransferred to nitrocellulose membranes (Fisher). The membranes were blotted with antibodies against hMSH2, hMSH6, hMLH1, and hPMS2, and the bound antibodies were detected by chemiluminescence using a secondary antibody conjugated with horseradish peroxidase (Amersham). The hMSH2 and hPMS2 antibodies were purchased from Oncogene Sciences (Boston, MA, USA), and the hMLH1 and hMSH6 antibodies were obtained from BD Transduction Laboratory (Lexington, KY, USA).

Heteroduplex preparation and MMR assay

DNA heteroduplexes used in this study were 6.4-kb double-stranded circular molecules (Figure 1) containing either a G-T mismatch with a strand break 125 bp 5' to the mismatch (5' G-T substrate) or a CA dinucleotide insertion/deletion mispair with a strand break 181 bp 3' to the mispair (3' CA substrate). DNA substrates were constructed as described utilizing the fIMR series DNA (Su *et al.*, 1988). Unless otherwise specified, MMR assays were performed (Holmes *et al.*, 1990) in a 15 μ l reaction mixture containing 24 fmol (100 ng) heteroduplex DNA, 50 μ g of nuclear extract protein, 10 mM Tris-HCl (pH 7.6), 5 mM MgCl₂, 1.5 mM ATP, and 0.1 mM each of the four dNTPs. For complementation repair assays, 50 ng of purified hMutS α or hMutL α were added to the reaction mixture. After incubation at 37°C for 15 min, DNA samples were recovered by phenol extraction and ethanol precipitation and double-digested with *Bsp106/HindIII* (5' G-T substrate) or *Bsp106/XcmI* (3' CA substrate). Reaction products were separated on a 1% agarose gel and visualized by UV-illumination in the presence of ethidium bromide.

Single-strand conformation polymorphism, and DNA sequencing analyses

Genomic DNA isolated from individual cell lines was used as a template to amplify exons of the *hMSH2* and the *hMLH1* genes by PCR. For amplification, 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 50–55°C), and extension (1.5 min at 72°C) were performed in 25- μ l reaction mixtures containing 0.1 μ g of DNA, 10 mM Tris-HCl (pH 7.6), 200 μ M dATP, dTTP, dGTP, and dCTP each, along with 1 μ Ci of [α -³²P]dCTP (3000 Ci/mmol, New England Nuclear), 1.0–3.0 mM MgCl₂, 0.5 μ M of each primer, and 1.0 U of Taq DNA polymerase. PCR products were separated by electrophoresis on 0.5 X MDE (Mutation Detection Enhancement) gel (BioWhittaker Molecular Applications, Rockland, ME) with or without 10% glycerol, and visualized by autoradiography or a Phosphor Imager (Molecular Dynamics). Primer sequences for these amplifications are available upon request. Products with abnormal bands in single-strand conformation polymorphism (SSCP) gels were purified using a gel purification kit (Qiagen) and then were sequenced using the primers that generated the PCR products. For determination of deletions of the *hMSH2* and *hMLH1* genes, PCR amplification of individual exons of each gene was performed as described for SSCP analyses, but with no ³²P-labeling. PCR products were analysed on 2% agarose gels and detected by UV illumination in the presence of ethidium bromide.

Acknowledgments

We thank Steve Presnell and Cecilia Ramilo for helpful comments on the manuscript. This work was supported in part by grants CA85377 (from the National Cancer

Institute) and GMC-98538 (from the American Cancer Society) to G-M Li, and IRG-85-001-16 (from the American Cancer Society through Lucille P. Markey Cancer Center) to L Gu.

References

- Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B and de la Chapelle A. (1993). *Science*, **260**, 812–816.
- Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S. (1998). *Cancer Res.*, **58**, 5248–5257.
- Boyer JC, Umar A, Risinger JI, Lipford JR, Kane M, Yin S, Barrett JC, Kolodner RD and Kunkel TA. (1995). *Cancer Res.*, **55**, 6063–6070.
- Buermeyer AB, Deschenes SM, Baker SM and Liskay RM. (1999). *Annu. Rev. Genet.*, **33**, 533–564.
- De Vita S, Gasparotto D, Pivetta B, Vukosavljevic T, Zagonel V, Carbone A and Boiocchi M. (1997). *Br. J. Haematol.*, **97**, 463–465.
- Drummond JT, Anthony A, Brown R and Modrich P. (1996). *J. Biol. Chem.*, **271**, 19645–19648.
- Drummond JT, Genschel J, Wolf E and Modrich P. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 10144–10149.
- Drummond JT, Li GM, Longley MJ and Modrich P. (1995). *Science*, **268**, 1909–1912.
- Eshleman JR and Markowitz SD. (1995). *Curr. Opin. Oncol.*, **7**, 83–89.
- Flores-Rozas H and Kolodner RD. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 12404–12409.
- Genschel J, Littman SJ, Drummond JT and Modrich P. (1998). *J. Biol. Chem.*, **273**, 19895–19901.
- Hangaishi A, Ogawa S, Mitani K, Hosoya N, Chiba S, Yazaki Y and Hirai H. (1997). *Blood*, **89**, 1740–1747.
- Hayami Y, Komatsu H, Iida S, Utsunomiya A, Hanada S, Hua XJ, Huiping N, Harada S, Tsuboi K, Banno S, Wakita A, Kato T and Ueda R. (1999). *Leuk. Lymphoma*, **32**, 345–349.
- Holmes J, Clark S and Modrich P. (1990). *Proc. Natl. Acad. Sci. USA*, **87**, 5837–5841.
- Hsieh P. (2001). *Mutat. Res.*, **486**, 71–87.
- Jiricny J. (1998). *Mut. Res.*, **409**, 107–121.
- Kaneko H, Inoue R, Yamada Y, Sukegawa K, Fukao T, Tashita H, Teramoto T, Kasahara K, Takami T and Kondo N. (1996). *Int. J. Cancer*, **69**, 480–483.
- Kat A, Thilly WG, Fang WH, Longley MJ, Li GM and Modrich P. (1993). *Proc. Natl. Acad. Sci. USA*, **90**, 6424–6428.
- Kodera T, Kohno T, Takakura S, Morishita K, Hamaguchi H, Hayashi Y, Sasaki T and Yokota J. (1999). *Genes Chromosomes Cancer*, **26**, 267–279.
- Kolodner R. (1996). *Genes Dev.*, **10**, 1433–1442.
- Kolodner RD and Marsischky GT. (1999). *Curr. Opin. Genet. Dev.*, **9**, 89–96.
- Lahue RS, Au KG and Modrich P. (1989). *Science*, **245**, 160–164.
- Larson RS, Manning S, Macon WR and Vnencak-Jones C. (1997). *Blood*, **89**, 1114–1115.
- Leung WK, Kim JJ, Wu L, Sepulveda JL and Sepulveda AR. (2000). *J. Biol. Chem.*, **275**, 15728–15732.
- Levati L, Marra G, Lettieri T, D'Atri S, Vernole P, Tentori L, Lacal PM, Pagani E, Bonmassar E, Jiricny J and Graziani G. (1998). *Genes Chromosomes Cancer*, **23**, 159–166.
- Li G-M. (1999). *Oncol. Res.*, **11**, 393–400.
- Li GM and Modrich P. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 1950–1954.
- Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM and Collins FS. (2000). *Nat. Genet.*, **24**, 27–35.
- Marra G, Iaccarino I, Lettieri T, Roscilli G, Delmastro P and Jiricny J. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 8568–8573.
- Modrich P and Lahue R. (1996). *Annu. Rev. Biochem.*, **65**, 101–133.
- Molenaar JJ, Gerard B, Chambon-Pautas C, Cave H, Duval M, Vilmer E and Grandchamp B. (1998). *Blood*, **92**, 230–233.
- Myung K, Chen C and Kolodner RD. (2001a). *Nature*, **411**, 1073–1076.
- Myung K, Datta A and Kolodner RD. (2001b). *Cell*, **104**, 397–408.
- Nakagawa T, Datta A and Kolodner RD. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 14186–14188.
- Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, D'Arrigo A, Truong O, Hsuan JJ and Jiricny J. (1995). *Science*, **268**, 1912–1914.
- Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T and Jiricny J. (1996). *Curr. Biol.*, **6**, 1181–1184.
- Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B and Modrich P. (1993). *Cell*, **75**, 1227–1236.
- Su S-S, Lahue RS, Au KG and Modrich P. (1988). *J. Biol. Chem.*, **263**, 6829–6835.
- Thibodeau SN, Bren G and Schaid D. (1993). *Science*, **260**, 816–819.
- Umar A, Boyer JC, Thomas DC, Nguyen DC, Risinger JI, Boyd J, Ionov Y, Perucho M and Kunkel TA. (1994). *J. Biol. Chem.*, **269**, 14367–14370.
- Zhu YM, Das-Gupta EP and Russell NH. (1999). *Blood*, **94**, 733–740.