Check for updates

Mutations and Loss of Expression of a Mismatch Repair Gene, hMLH1, in Leukemia and Lymphoma Cell Lines

By Akira Hangaishi, Seishi Ogawa, Kinuko Mitani, Noriko Hosoya, Shigeru Chiba, Yoshio Yazaki, and Hisamaru Hirai

Defects in genes involved in DNA mismatch repair have been detected in both hereditary and sporadic tumors of colon, endometrium, and ovary and suggested to be associated with tumorigenesis. To investigate disruptions of the mismatch repair system in hematological malignancies, we examined alterations of the human mutL homologue 1 (hMLH1) gene, a member of the mismatch repair gene family, in a total of 43 human leukemia and lymphoma cell lines, by polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) and sequencing analyses. Mutations of the hMLH1 gene were detected in three cell lines established from lymphoid leukemias. Moreover, Northern and Western blot analyses showed that expression of hMLH1 transcript or protein was abrogated in these three leukemia cell lines. Further studies for microsatellite loci showed that these cell lines without hMLH1 expression showed microsatellite instability. This is the first report that describes mutations and inactivation of the hMLH1 gene in human leukemia cells, suggesting that disruption of DNA mismatch repair system may play an important role in the development of human lymphoid leukemias.
© 1997 by The American Society of Hematology.

SEVERAL MECHANISMS through which cells are subjected to malignant transformation have been identified. Among these, defects in DNA mismatch repair systems have been recently described in colon tumorigenesis related to hereditary nonpolyposis colorectal cancer (HNPCC).[1-5] HNPCC is an autosomal dominant disorder and one of the most common genetic conditions that determines susceptibility to colorectal cancers and tumors in other organs, including endometrium, stomach, ovary, ureter, renal pelvis, pancreas, biliary tree, skin, larynx, and bone marrow.[6] Recently, in more than 90% of HNPCC kindreds, a germ-line mutation was shown to occur in one of a group of genes involved in DNA nucleotide mismatch repair, including human mutS homologue 2 (hMSH2),[7-9] human mutL homologue 1 (hMLH1),[10,11] and human postmeiotic segregation 1 (hPMS1) and 2 (hPMS2).[12] Examinations on mismatch repair genes in tumors of the HNPCC patients have shown abnormalities in both parental alleles, implying that inactivation occurs in the wild-type allele in addition to the germline mutation.[8,12-14]

Tumors in HNPCC patients show a particular form of genetic instability, also termed replication error (RER) or microsatellite instability, which is generally characteristic of a mismatch repair defect.[2,4,15,16] This results in accumulation of changes in the length of microsatellite and other short-repeat sequences and possibly of single-base changes due to failure to correct mistakes that may occur during DNA replication,[17] implying that defects in the mismatch repair system may cause the accumulation of mutations in key oncogenes or tumor suppressor genes, thus contributing to the development of tumors.[2,7,8]

In addition to HNPCC,[2,15,18] microsatellite instabilities have also been observed in a variety of sporadic cancers, including colon, gastric, pancreatic, endometrial, ovarian, and small cell lung carcinomas,[19] and in hematopoietic malignancies such as blastic crisis of chronic myelogenous leukemia (CML),[20] myelodysplastic syndrome (MDS),[21] chronic lymphocytic leukemia (CLL),[22] Burkitt's lymphoma and human immunodeficiency virus (HIV)-associated lymphomas.[23,24] On the other hand, it is known that lymphoid leukemias and lymphomas sometimes arise in the HNPCC patients.[25-27] Of interest, one branch of HNPCC families has developed an excess of hematopoietic tumors.[28] From these observations, it is likely that a common defect in the mismatch repair system could affect hematopoietic cells and contribute to the development of leukemias and/or lymphomas.

In this study, we performed mutation analyses of the hMLH1 gene in human hematopoietic tumor cell lines and analysis for microsatellite instability to clarify a possible involvement of aberrant DNA mismatch repair mechanisms in the generation of human leukemias and/or lymphomas.

MATERIALS AND METHODS

Cell lines and preparation of samples. Forty-eight human leukemia and lymphoma cell lines were included in this study consisting of 11 myelocytic/monocytic (HL60, SKH1, KG1, KU812, ME-F2, JOSK-K, JOSK-S, P39 TSU, THP1, U937, and ML1), 4 erythroid (F36E, K562, HEL, and TF1), 5 megakaryocytic (UT7, CMK, MegJ, MOLM1, and SUM90-7), 17 B-lymphocytic (BALL-1, Daudi, Raji, IM9, M5, HA, P32 ISH, SCMCL-L1, SCMCL-L3, SCMCL-L4, KPMM1, KCL22, Ramos, BALM1, Ri-1, RPMI8226, and DND39), and 11 T-lymphocytic cell lines (Jurkat, A3/KAW, MOLT4, MOLT16, P30OHK, SKW3, CEM, MT1, HUT78, Peer, and TALL1). The cells were grown in suspension culture in RPMI medium 1640 supplemented with 10% fetal calf or bovine serum. TF1, UT7, CMK, and F36E are factor-dependent cell lines and were cultured in the presence of 5 ng/mL recombinant human granulocyte-monocyte colony-stimulating factor (rhGM-CSF). Genomic DNA and total RNA of cells was extracted as described previously.[29]

Polymerase chain reaction-single–strand conformation polymorphism (PCR-SSCP) and sequencing of the hMLH1 gene. We analyzed the status of the four exons (exons 2, 9, 15, and 16) of the hMLH1 gene in which more than two mutations had been described in previous reports.[30-32] Point mutations, small nucleotide deletions, and insertions in these exons were examined by PCR-SSCP and sequencing analyses according to the previously described methodology.[33] Nucleotide sequences of the primers used to amplify exons 2, 9, and 16 and conditions of PCR-SSCP analysis were referred to the literature.[32] The sequences of the primers used to amplify exon 15 are as follows: 5′-CAGTGAAGAACTGTTCTACCAG-3′ and 5′-GATATTAGTGGAGAGCTACTAT-3′. PCR products that showed polymorphic bands were subcloned into the pCRTMII Vec-

From the Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Submitted June 17, 1996; accepted October 18, 1996.

Address reprint requests to Hisamaru Hirai, MD, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked ''advertisement'' *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

© 1997 by The American Society of Hematology.
0006-4971/97/8905-0030$3.00/0

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

tor (Invitrogene, San Diego, CA) and eight clones were sequenced in both directions to confirm mutations.[33]

Northern blot analysis of hMLH1. Total RNA prepared from cultured cells was separated by electrophoresis through 1.2% agarose-formaldehyde gels (10 μg/lane), blotted onto a nylon membrane, and hybridized with combined hMLH1 probes obtained by PCR amplification of exons 2 and 19 of the gene. Nucleotide sequences of the primers used to amplify exon 19 were also referred to the literature.[32] As an internal gauge of RNA level, the blot was also probed with a full-length glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

Immunoblotting. Cells were lysed on ice with the lysis buffer containing 50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.05% sodium dodecyl sulfate (SDS), 1% deoxycholate, 1% Triton X-100, 10 U/mL aprotinin and 2 mmol/L phenylmethylsulfonyl fluoride. One hundred micrograms of protein was separated through SDS-polyacrylamide gel electrophoresis (PAGE) and electrotransferred onto polyvinylidene difluoride filters (Millipore, Bedford, MA). Filters were probed with both anti-hMLH1 (PharMingen, San Diego, CA) and anti-Hsp 70 (BioMakor, Rehovot, Israel), the latter being used for confirmation of integrity of the protein samples, as previously described.[33] The membrane was then washed three times with the Tris-buffered saline with 0.1% Triton X-100 (TBST) buffer containing 10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.1% Triton X-100, and incubated at room temperature with an antimouse (IgG1) antibody conjugated to alkaline phosphatase. The specific bands were visualized by calorigenic substrates, nitroblue tetrazolium, and 5-bromo–4-chloro–3-indoyl phosphatase (Promega, Madison, WI).

Assay for microsatellite instability. The techniques used for determining RER status of cell lines are the same as described previously.[1-3,19] Briefly, the four microsatellite regions containing repeat sequences, D2S123, D18S58, BAT25, and BAT26, were PCR-amplified using genomic DNAs of the cell lines with hMLH1 alterations. The primers used for these microsatellite markers and conditions for PCR were referred to the literature.[34] After PCR amplification, the products were denatured and analyzed on a 6% polyacrylamide denaturing gel containing 8.3 mol/L urea. We tested both DNAs extracted from original cell lines and those from single cell-derived subclones of each cell line isolated by limiting dilution in 96-well microtiter plates.[1,19]

RESULTS

Mutations of the hMLH1 gene. We screened the 43 cell lines for mutations in regions containing exons 2, 9, 15, and 16 of the hMLH1 gene by PCR-SSCP and sequencing analyses and confirmed loss of hMLH1 expression by Northern and/or Western blot analyses for those cell lines that had a mutation of the hMLH1 gene. As for mutations of the exon 9-containing region, abnormally migrating bands were detected on PCR-SSCP analysis in three cell lines; CEM, KCL22, and P30OHK (Fig 1). Sequencing analysis showed nucleotide alterations of the hMLH1 gene in all three cell lines (Fig 2). CEM and KCL22 were thought to be heterozygous for the mutations because both the normal and the mutated sequences of the exon 9–containing region were obtained (data not shown), confirming to the results from the PCR-SSCP analysis, in which normally migrating bands, as well as abnormally migrating bands, were also detected (Fig 1). On the other hand, in P30OHK, the PCR-SSCP analysis showed exclusively abnormally migrating bands and only the abnormal sequence of the exon 9-containing region was obtained from sequencing of the PCR product, suggesting that an allelic loss of the exon 9-containing region

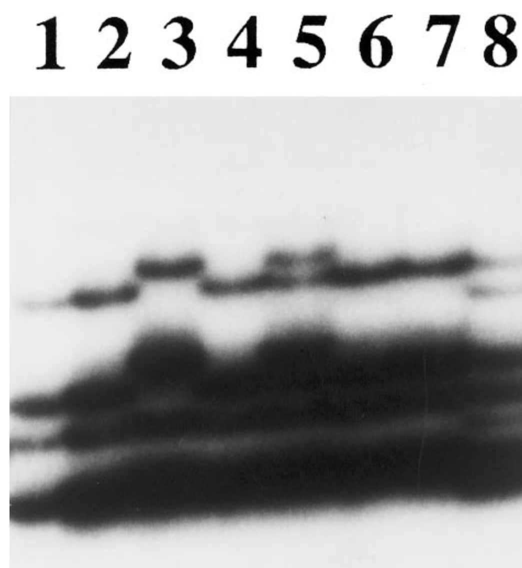

Fig 1. PCR-SSCP analysis of exon 9 of the hMLH1 gene. Lanes 1 through 8 were DNA samples from ML1, TALL1, P30OHK, A3/KAW, CEM, M5, SUM90-7, and KCL22 cell lines, respectively. Lanes 3, 5, and 8 showed abnormal migrating bands.

exists in this cell line. P30OHK and CEM had the same splicing-site mutation in splicing donor site of intron 9 (Fig 2 and Table 1), while KCL22 had a missense mutation at codon 259 (TTA to TCA) (Fig 2 and Table 1). Results are summarized in Table 1. All of these three cell lines were established from leukemias of lymphoid origin; CEM from T-lineage acute lymphocytic leukemia (T-ALL),[35] P30OHK from preT-lineage ALL (PreT-ALL),[36] and KCL22 from B-lymphoid crisis of CML.[37,38] As summarized in Table 2, mutations were found in 1 of the 12 B-lymphocytic cell lines (8.3%), 2 of the 11 T-lymphocytic cell lines (18.2%), and none of the 20 cell lines of nonlymphoid origins. Mutations were not detected in the regions containing exons 2, 15, and 16 of the hMLH1 gene we examined.

Expression of the hMLH1 gene. To evaluate expression of hMLH1 transcript and protein, we performed Northern and Western blot analyses, respectively. As shown in Fig 3, a 2.7-kb transcript was detected in all cell lines, except P30OHK and KCL22, in which the hMLH1 message was undetectable despite normal expression of GAPDH. Next, we studied expression of hMLH1 protein in the three cell lines having mutations of the hMLH1 gene. Representative blots are shown in Fig 4. hMLH1 and Hsp70 proteins from normal lymphocytes were observed around 85 and 70 kD, respectively (Fig 4, lane 1). Expression of Hsp70 was used as a control for the amount and integrity of the sample protein. P30OHK, KCL22, and CEM, showed loss of or remarkably low levels of hMLH1 protein expression despite normal expression of Hsp70. The results of expression analyses of hMLH1 are summarized in Table 2.

Microsatellite instability in cell lines with loss of hMLH1 expression. To confirm functional loss of hMLH1, the cell lines without hMLH1 protein expression were analyzed for microsatellite instability at four different polymorphic loci. First, we screened P30OHK, KCL22, and CEM for heteroge-

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

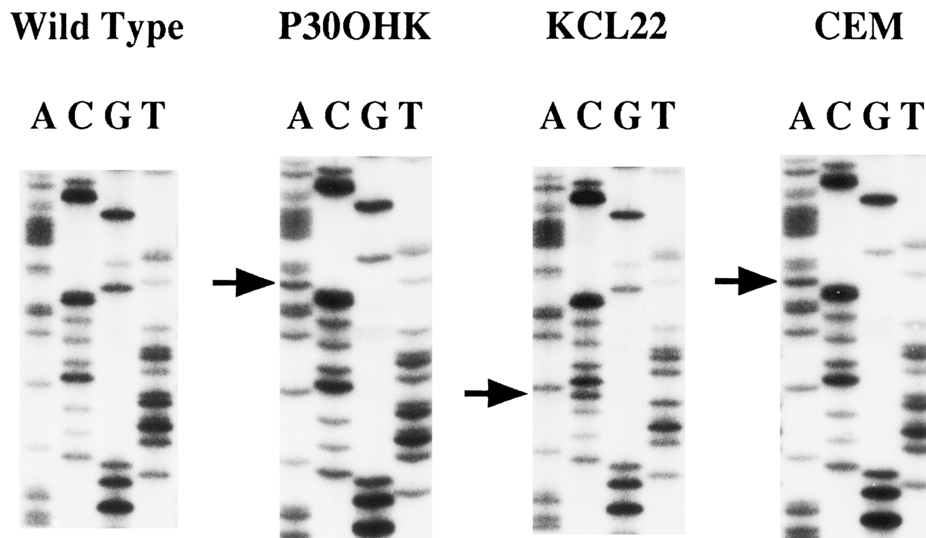

Fig 2. Sequencing analysis of the hMLH1 mutations. The left panel represents the normal sequence of the hMLH1 exon 9. The arrows show the positions of point mutations. A nucleotide change G to A in consensus splice donor site is seen in both P30OHK and CEM. A transition of T to C at codon 259 is found in KCL22.

neity of microsatellite structure. As shown in Fig 5, multiple alleles of microsatellite locus were evident in these three cell lines at D18S58. PCR-amplified allelic markers of P30OHK, KCL22, and CEM all have radiated into tightly bunched ladders of bands that are similar to those originally described in tumors arising in affected members of HNPCC families,[2] at a minimum of three of four microsatellite loci. Secondly, to examine microsatellite instability in greater detail, we isolated various single cell clones of KCL22 and CEM. Each single cell clone of KCL22 and CEM was found to exhibit expansions and/or contractions of microsatellite length at all four microsatellite loci. Representative results of microsatellite analysis for single cell clones of KCL22 at D2S123 and D18S58 are shown in Fig 6A and B, respectively (data not shown for CEM). A single cell of P30OHK cell line did not grow well in culture.

DISCUSSION

We have shown mutations and loss of expression of the hMLH1 gene in three of 43 human hematopoietic tumors cell lines. All three cell lines had mutations in exon 9 or in the boundary of exon 9 and intron 9 and showed loss of hMLH1 protein. Two of the three cell lines, CEM and P30OHK, had the same mutation at 5′-splicing donor site in intron 9. P30OHK showed exclusively abnormal sequence in this position, which most probably resulted from an allelic loss of exon 9 and a point mutation on the residual allele. Because no hMLH1 transcript could be observed in this cell line, it is suggested that the mutation at 5′-splicing donor site of hMLH1 intron 9 might cause abnormal splicing of the transcript and lead to loss or remarkably low level of mature hMLH1 messages. On the other hand, CEM was found to be heterozygous for this splicing donor site mutation; it had both the normal and the mutated sequences of the exon 9 containing region. Because the mutation was the same as that found in P30OHK, no mature hMLH1 transcript could be transcribed from this mutated allele. In addition, the remaining allele is also presumed to become inactivated, because hMLH1 protein was not expressed in CEM. Considering that a full-length hMLH1 message was apparently expressed in CEM, the loss of hMLH1 protein might be ascribed to another mutation in the other exon that we examined.

The other cell line, KCL22, lost hMLH1 transcript. It had an allelic mutation in exon 9, a TTA to TTC transversion. This is a novel mutation that might potentially accompany an amino acid substitution of Leu to Ser. None of the DNA samples from 150 unrelated individuals we examined showed the same nucleic acid substitution. Nevertheless, it might represent either a polymorphism or a mutation of no consequence, because this single mutation itself is not likely to result in total loss of hMLH1 transcript, as shown in KCL22. The loss of hMLH1 transcript in this cell line should thus be ascribed to biallelic mutations somewhere else in the hMLH1 gene.

Mutations and inactivation of mismatch repair genes have been considered as the cause of microsatellite instability phenotype and proposed to foster a mutator phenotype im-

Table 1. Summary of Mutations in hMLH1 Gene

Cell Line	Mutation Analysis		mRNA	Protein
	Site	Nucleotide (amino acid)		
P30OHK	Splicing donor site of intron 9	AACCgtaa→AACCataa	−	−
CEM	Splicing donor site of intron 9	AACCgtaa→AACCataa	+	−
KCL22	Codon 259 in exon 9	TTA(Leu)→TCA(Ser)	−	−

Exonic sequences are upper case and intronic sequences are lowercase letters.

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

Table 2. hMLH1 Alterations in Human Leukemia and Lymphoma Cell Lines

	Mutation of the Gene					
	Exon 2	Exon 9	Exon 15	Exon 16	mRNA	Protein
Myeloid/monocytic						
HL60	W	W	W	W	+	+
SKH1	W	W	W	W	ND	ND
KG1	W	W	W	W	ND	ND
KU812	W	W	W	W	ND	ND
ME-F2	W	W	W	W	ND	ND
JOSK-K	W	W	W	W	ND	ND
JOSK-S	W	W	W	W	ND	ND
P39 TSU	W	W	W	W	ND	ND
THP1	W	W	W	W	+	ND
U937	W	W	W	W	ND	ND
ML1	W	W	W	W	+	ND
Erythroid						
F36E	W	W	W	W	ND	ND
K562	W	W	W	W	ND	ND
HEL	W	W	W	W	+	ND
TF1	W	W	W	W	+	ND
Megakaryocytic						
UT7	W	W	W	W	ND	ND
CMK	W	W	W	W	ND	ND
MegJ	W	W	W	W	+	ND
MOLM1	W	W	W	W	ND	ND
SUM90-7	W	W	W	W	ND	ND
B-lymphocytic						
BALL-1	W	W	W	W	+	ND
Daudi	W	W	W	W	+	+
Raji	W	W	W	W	+	+
IM9	W	W	W	W	ND	ND
M5	W	W	W	W	ND	ND
HA	W	W	W	W	+	+
P32 ISH	W	W	W	W	ND	ND
SCMCL-L1	W	W	W	W	ND	ND
SCMCL-L3	W	W	W	W	ND	ND
SCMCL-L4	W	W	W	W	ND	ND
KPMM1	W	W	W	W	ND	ND
KCL22	W	M	W	W	−	−
Ramos	ND	ND	ND	ND	+	ND
BALM1	ND	ND	ND	ND	+	ND
Ri-1	ND	ND	ND	ND	+	ND
RPMI8226	ND	ND	ND	ND	+	ND
DND39	ND	ND	ND	ND	+	ND
T-lymphocytic						
Jurkat	W	W	W	W	+	ND
A3/KAW	W	W	W	W	ND	+
MOLT4	W	W	W	W	+	+
MOLT16	W	W	W	W	+	+
P30OHK	W	M	W	W	−	−
SKW3	W	W	W	W	+	ND
CEM	W	M	W	W	+	−
MT1	W	W	W	W	ND	ND
HUT78	W	W	W	W	ND	ND
Peer	W	W	W	W	ND	ND
TALL1	W	W	W	W	ND	ND

Abbreviations: W, wild type; M, mutation; ND, not done; +, normal expression; −, loss.

portant in the development of cancers.[7,8,11,12] Mismatch repair gene defects including hMLH1 and hMSH2 were reported in a part of both sporadic and hereditary colorectal cancers and tumor cell lines with microsatellite instability.[13,34,39] Moreover, recent studies have shown that mice engineered to be deficient in MLH1, MSH2, or PMS2 showed microsatellite instability.[40-44] These studies clearly show that the RER phenotype is the result of the lack of mismatch repair proficiency. In our analysis, the three cell lines with hMLH1 inactivation showed microsatellite instability, which seems

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

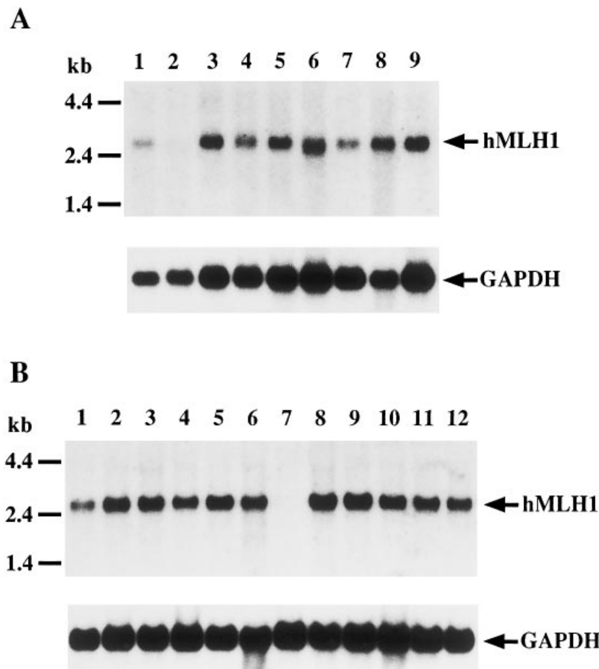

Fig 3. Northern blot analysis of hMLH1 gene transcript. Filters were hybridized with either hMLH1 probes (upper panel) or GAPDH probe (lower panel) as a control. (A) Lanes 1 through 9 are MegJ, P30OHK, BALL-1, MOLT4, MOLT16, CEM, Raji, THP1, and SKW3, respectively. (B) Lanes 1 through 12 show Ramos, Jurkat, HA, DND39, BALM1, ML1, KCL22, Ri-1, Daudi, HL60, RPMI8226, and TF1, respectively. A 2.7-kb transcript is detectable in all lanes except lane 2 of (A) and lane 7 of (B). No hMLH1 mRNA expression is found in P30OHK and KCL22.

Fig 5. Analysis of microsatellite instability of hematopoietic tumor cell lines. DNA purified from each cell line was analyzed for heterogeneity at the microsatellite locus D18S58. Lanes 1 through 8 are normal lymphocytes, U937, KCL22, CEM, P30OHK, HEL, HL60, and SKH1, respectively. Multiple alleles were particularly evident in KCL22, but could also have appeared in CEM and P30OHK.

consistent with the above observations. Although there is the possibility of further defects in other mismatch repair genes that we have not studied, inactivation of the hMLH1 gene would result in microsatellite instability in these cell lines.

Several studies showed that microsatellite instability was associated with the development, rather than the initiation, of the carcinoma because microsatellite instability was frequently detected in advanced stage, rather than in the early stage, of colon and gastric cancers.[1,4,45-48] In hematological tumors, microsatellite instability in CML patients was observed in cases of the blastic crisis and accelerated phase, but no cases in the chronic phase.[20] Viewed in this light, our result that the KCL22 cells, derived from patients in the blastic crisis of CML showed RER phenotype may be of interest.

In our study, mutations of the hMLH1 gene were detected in lymphoid leukemias only. Mutations have not been found in nonlymphoid tumor cell lines, although we could not rule out a possibility that there may be other mutations in some exons of the hMLH1 gene, which we have not studied. With respect to this point, development of hematological malig-

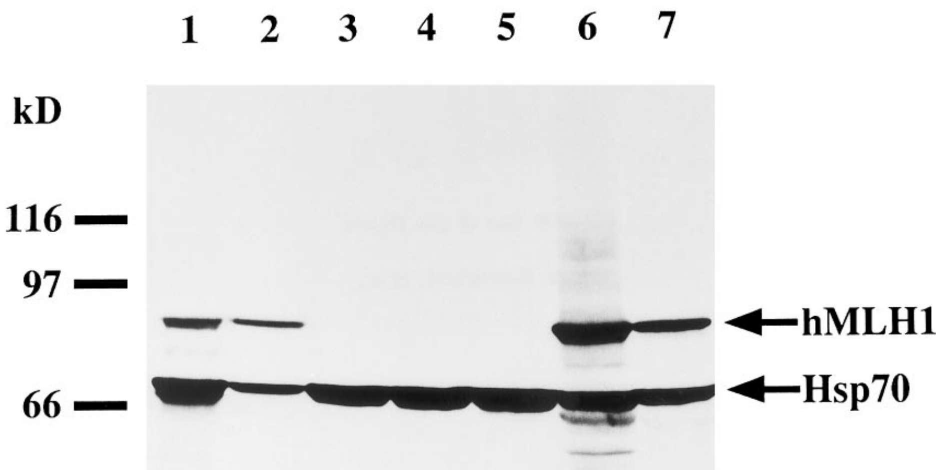

Fig 4. Western blot analysis of the hMLH1 protein. hMLH1 protein is observed around 85 kD. The lower bands detected around 70 kD shows expression of Hsp70, as controls for the amounts and integrity of protein. Lane 1 represents normal lymphocytes derived from a normal volunteer used as a normal control. Lanes 2 through 7 are cell lysates prepared from cell lines MOLT16 (lane 2), P30OHK (lane 3), CEM (lane 4), KCL22 (lane 5), BALL1 (lane 6), and U937 (lane 7). P30OHK, CEM, and KCL22 had loss of hMLH1 protein expression with normal expression levels of Hsp70.

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

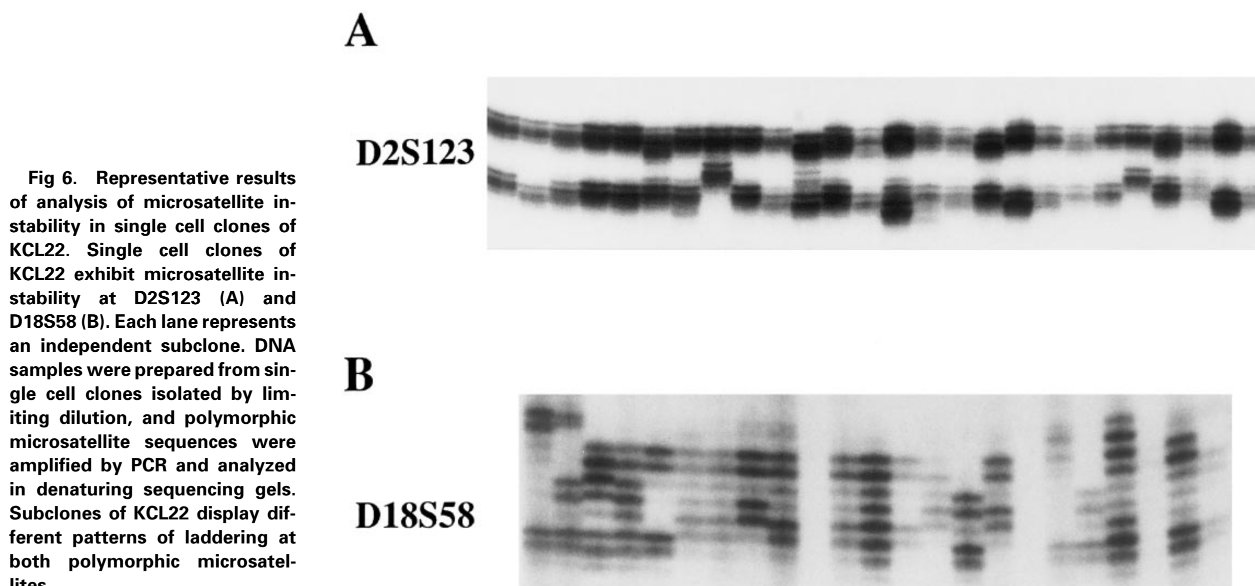

Fig 6. Representative results of analysis of microsatellite instability in single cell clones of KCL22. Single cell clones of KCL22 exhibit microsatellite instability at D2S123 (A) and D18S58 (B). Each lane represents an independent subclone. DNA samples were prepared from single cell clones isolated by limiting dilution, and polymorphic microsatellite sequences were amplified by PCR and analyzed in denaturing sequencing gels. Subclones of KCL22 display different patterns of laddering at both polymorphic microsatellites.

nancies in human HNPCC kindreds was also reported exclusively in lymphoid malignancies including acute and chronic lymphocytic leukemias and malignant lymphomas.[25-28] Moreover, the predisposition to lymphoid tumors was reported in MSH2- and PMS2-knockout mice.[42-44] Although MLH1-deficient mice were not yet old enough to determine cancer susceptibility and were not fully analyzed, one group recently reported that an MLH1-deficient mouse had developed a lymphoma.[40] These observations suggest that defects in these mismatch repair genes, including hMLH1, may be associated with lymphoid malignancies.

In summary, we have detected inactivations of the hMLH1 gene in three of 43 hematopoietic tumor cell lines. This is the first report describing mutations of the hMLH1 gene in human leukemias. Our findings imply that inactivations of the hMLH1 gene may play an important role in the development of human lymphoid tumors. Further studies of hMLH1 and other mismatch repair genes in primary hematological malignancies would be of interest.

ACKNOWLEDGMENT

We gratefully acknowledge Drs Hideaki Mizoguchi, Department of Medicine, Tokyo Women's Medical College, Tokyo, Japan for providing MegJ cell line, Yasuhide Hayashi, Department of Pediatrics, University of Tokyo, Tokyo, Japan for providing SCMCL-L1, SCMCL-L3, and SCMC-L4, Chihiro Shimazaki, Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan for providing KPMM1, Shigeru Fujita, Department of Medicine, Ehime University, Ehime, Japan for providing ME-F2, and Jun Minowada, Fujisaki Cell Center, Hayashibara Biochemical Laboratories Inc, Okayama, Japan for providing MOLM1, Ramos, BALM1, Ri-1, RPMI8226, DND39, A3/KAW, MOLT16, and SKW3.

REFERENCES

1. Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M: Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. Nat Genet 6:273, 1994

2. Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A: Clues to the pathogenesis of familial colorectal cancer. Science 260:812, 1993

3. Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P: Hypermutability and mismatch repair deficiency in RER+ tumor cells. Cell 75:1227, 1993

4. Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, Lynch HT, Watson P, Tallqvist G, Juhola M, Sistonen P, Hamilton SR, Kinzler KW, Vogelstein B, de la Chapelle A: Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 54:1645, 1994

5. Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom LM, Pylkkanen L, Heimdal K, Andersen TI, Moller P, Rognum TO, Fossa SD, Haldorsen T, Langmark F, Brogger A, de la Chapelle A, Borresen AL: Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Res 53:5849, 1993

6. Rustgi AK: Hereditary gastrointestinal polyposis and nonpolyposis syndromes. N Engl J Med 331:1694, 1994

7. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R: The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75:1027, 1993

8. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom LM, Guan XY, Zhang J, Meltzer PS, Yu JW, Kao FT, Chen DJ, Cerosaletti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin JP, Jarvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch HT, Trent JM, de la Chapelle A, Kinzler KW, Vogelstein B: Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215, 1993

9. Liu B, Parsons RE, Hamilton SR, Petersen GM, Lynch HT, Watson P, Markowitz S, Willson JK, Green J, de la Chapelle A, Kinzler KW, Vogelstein B: hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. Cancer Res 54:4590, 1994

10. Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomaki P, Mecklin JP, de la Chapelle A, Kinzler KW, Vogelstein

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

B: Mutation of a mutL homolog in hereditary colon cancer. Science 263:1625, 1994

11. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjold M, Fishel R, Kolodner R, Liskay RM: Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary nonpolyposis colon cancer. Nature 368:258, 1994

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

13. Boyer JC, Umar A, Risinger JI, Lipford JR, Kane M, Yin S, Barrett JC, Kolodner RD, Kunkel TA: Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res 55:6063, 1995

14. Borresen AL, Lothe RA, Meling GI, Lystad S, Morrison P, Lipford J, Kane MF, Rognum TO, Kolodner RD: Somatic mutations in the hMSH2 gene in microsatellite unstable colorectal carcinomas. Hum Mol Genet 4:2065, 1995

15. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT, Boyd J: Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 53:5100, 1993

16. Honchel R, Halling KC, Schaid DJ, Pittelkow M, Thibodeau SN: Microsatellite instability in Muir-Torre syndrome. Cancer Res 54:1159, 1994

17. Strand M, Prolla TA, Liskay RM, Petes TD: Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 365:274, 1993

18. Lindblom A, Tannergard P, Werelius B, Nordenskjold M: Genetic mapping of a second locus predisposing to hereditary nonpolyposis colon cancer. Nat Genet 5:279, 1993

19. Orth K, Hung J, Gazdar A, Bowcock A, Mathis JM, Sambrook J: Genetic instability in human ovarian cancer cell lines. Proc Natl Acad Sci USA 91:9495, 1994

20. Wada C, Shionoya S, Fujino Y, Tokuhiro H, Akahoshi T, Uchida T, Ohtani H: Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia. Blood 83:3449, 1994

21. Kaneko H, Horiike S, Inazawa J, Nakai H, Misawa S: Microsatellite instability is an early genetic event in myelodysplastic syndrome. Blood 86:1236, 1995

22. Gartenhaus R, Johns III MM, Wang P, Rai K, Sidransky D: Mutator phenotype in a subset of chronic lymphocytic leukemia. Blood 87:38, 1996

23. Robledo M, Martinez B, Arranz E, Trujillo MJ, Gonzalez AA, Rivas C, Benitez J: Genetic instability of microsatellites in hematological neoplasms. Leukemia 9:960, 1995

24. Bedi GC, Westra WH, Farzadegan H, Pitha PM, Sidransky D: Microsatellite instability in primary neoplasms from HIV+ patients. Nat Med 1:65, 1995

25. Love RR: Small bowel cancers, B-cell lymphatic leukemia, and six primary cancers with metastases and prolonged survival in the cancer family syndrome of Lynch. Cancer 55:499, 1985

26. Law IP, Hollinshead AC, Whang PJ, Dean JH, Oldham RK, Herberman RB, Rhode MC: Familial occurrence of colon and uterine carcinoma and of lymphoproliferative malignancies. II. Chromosomal and immunologic abnormalities. Cancer 39:1229, 1977

27. Law IP, Herberman RB, Oldham RK, Bouzoukis J, Hanson SM, Rhode MC: Familial occurrence of colon and uterine carcinoma and of lymphoproliferative malignancies: Clinical description. Cancer 39:1224, 1977

28. Lynch HT, Krush AJ: Cancer family ''G'' revisited: 1895-1970. Cancer 27:1505, 1971

29. Ogawa S, Hirano N, Sato N, Takahashi T, Hangaishi A, Tanaka K, Kurokawa M, Tanaka T, Mitani K, Yazaki Y, Hirai H: Homozygous loss of the cyclin-dependent kinase 4-inhibitor (p16) gene in human leukemias. Blood 84:2431, 1994

30. Wijnen J, Khan PM, Vasen H, Menko F, van der Klift H, van den Broek M, van Leeuwen-Cornelisse I, Nagengast F, Meijers-Heijboer EJ, Lindhout D, Griffioen G, Cats A, Kleibeuker J, Varesco L, Bertario L, Bisgaard ML, Mohr J, Kolodner R, Fodde R: Majority of hMLH1 mutations responsible for hereditary nonpolyposis colorectal cancer cluster at the exonic region 15-16. Am J Hum Genet 58:300, 1996

31. Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Linch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, de la Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW: Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med 2:169, 1996

32. Han HJ, Maruyama M, Baba S, Park JG, Nakamura Y: Genomic structure of human mismatch repair gene, hMLH1, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). Hum Mol Genet 4:237, 1995

33. Hangaishi A, Ogawa S, Imamura N, Miyawaki S, Miura Y, Uike N, Shimazaki C, Emi N, Takeyama K, Hirosawa S, Kamada N, Kobayashi Y, Takemoto Y, Kitani T, Toyama K, Ohtake S, Yazaki Y, Ueda R, Hirai H: Inactivation of multiple tumor-suppressor genes involved in negative regulation of the cell cycle, MTS1/p16INK4A/CDKN2, MTS2/p15INK4B, p53, and Rb genes in primary lymphoid malignancies. Blood 87:4949, 1996

34. Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, Papadopoulos N, Fujiwara T, Jen J, Kinzler KW, Wyllie AH, Vogelstein B, Dunlop MG: Genetic instability occurs in the majority of young patients with colorectal cancer. Nat Med 1:348, 1995

35. Foley G, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE: Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer 18:522, 1965

36. Hirose M, Minato K, Tobinai K, Ohira M, Ise T, Watanabe S, Shimoyama M, Taniwaki M, Abe T: A novel pre-T cell line derived from acute lymphoblastic leukemia. Gann 73:600, 1982

37. Kubonishi I, Miyoshi I: Establishment of a Ph1 chromosome-positive cell line from chronic myelogenous leukemia in blast crisis. Int J Cell Cloning 1:105, 1983

38. Berenson J, Koeffler HP: Rearrangement and expression of beta-T-cell receptor and immunoglobulin genes in established Ph1 chronic myelogenous leukemia cell lines. Hematol Pathol 3:125, 1989

39. Liu B, Nicolaides NC, Markowitz S, Willson JK, Parsons RE, Jen J, Papadopolous N, Peltomaki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B: Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nat Genet 9:48, 1995

40. Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM: Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13:336, 1996

41. Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Kolodner RD, Kucherlapati R: Meiotic pachytene arrest in MLH1-deficient mice. Cell 85:1125, 1996

42. Reitmair AH, Schmits R, Ewel A, Bapat B, Redston M, Mitri A, Waterhouse P, Mittrucker HW, Wakeham A, Liu B, Thomason A, Griesser H, Gallinger S, Ballhausen WG, Fishel R, Mak TW: MSH2 deficient mice are viable and susceptible to lymphoid tumours. Nat Genet 11:64, 1995

43. de Wind N, Dekker M, Berns A, Radman M, te Riele H: Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell 82:321, 1995

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024

44. Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM: Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. Cell 82:309, 1995

45. Young J, Leggett B, Gustafson C, Ward M, Searle J, Thomas L, Buttenshaw R, Chenevix TG: Genomic instability occurs in colorectal carcinomas but not in adenomas. Hum Mutat 2:351, 1993

46. Chong JM, Fukayama M, Hayashi Y, Takizawa T, Koike M, Konishi M, Kikuchi YR, Miyaki M: Microsatellite instability in the progression of gastric carcinoma. Cancer Res 54:4595, 1994

47. Jass JR, Stewart SM, Stewart J, Lane MR: Hereditary nonpolyposis colorectal cancer—Morphologies, genes and mutations. Mutat Res 310:125, 1994

48. Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, Cavalieri RJ, Boland CR: Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: An updated review. Gastroenterology 104:1535, 1993

Downloaded from http://ashpublications.net/blood/article-pdf/89/5/1740/1410531/1740.pdf by guest on 24 May 2024