The Role of Microsatellite Instability in Gastric Low- and High-Grade Lymphoma Development

Petr Starostik, Axel Greiner, Stephan Schwarz,
Jochen Patzner, Anja Schultz, and
Hans Konrad Müller-Hermelink

From the Institute of Pathology, Würzburg University,
Würzburg, Germany

DNA mismatch repair genes and their dysfunction as evidenced by microsatellite instability (MSI) play an important role in the pathogenesis of a variety of tumors, most prominently hereditary nonpolyposis colorectal cancer (HNPCC). However, their role in development of extranodal lymphomas has not been established yet. We therefore evaluated for MSI 25 gastric low-grade marginal-zone B-cell lymphomas of mucosa-associated lymphoid tissue type and 31 gastric high-grade diffuse large B-cell lymphomas (DLBCLs) with 29 and 118 microsatellites, respectively. Compared with HNPCC, the overall level of MSI was much lower with a mean of 2.6% MSI-positive repeats in the DLBCLs; the frequency of MSI showed a tendency to increase with age ($P = 0.01$), as did MSI variability ($P = 0.02$). Low-grade mucosa-associated lymphoid tissue lymphomas displayed even less MSI (sevenfold) than DLBCLs ($P = 0.009$). MSI frequency thus increases with the transition from low- to high-grade disease and with age; it does not seem to initiate lymphomagenesis. Other microsatellites than those typically mutated in HNPCC frequently revealed MSI in these lymphomas, especially dinucleotide repeats on chromosomes 3, 5, and 18. To facilitate rapid screening of lymphomas for MSI and to establish a tool for future MSI frequency comparisons, we recommend to use repeats D3S1261, D3S1530, D5S346, D17S250, D18S474, and DCC. (*Am J Pathol* 2000, 157:1129–1136)

Non-Hodgkin's lymphomas originating in the stomach constitute the most common group of primary extranodal lymphomas, lymphomas arising outside the lymph nodes.¹ Compared to nodal lymphomas, the clinicopathological features of gastric lymphomas are more closely related to the structure and function of mucosa-associated lymphoid tissue (MALT) than to peripheral lymph nodes.² The majority of these lymphomas are B-cell tumors, either a low-grade disease like marginal-zone B-cell lymphoma of MALT-type or a more aggressive high-grade diffuse large B-cell lymphoma (DLBCL).³ The high-grade lymphoma can either arise *de novo* or through transformation from a previously existing low-grade MALT disease.

The molecular basis underlying the pathogenesis of these lymphomas and the progression from low- to high-grade disease has not been elucidated yet, although some frequently occurring abnormalities, features of genomic instability, have been studied and well documented. Genomic instability is a basic property of tumor cells, it generates the diversity necessary for a cancer cell to escape from inherent restraints on growth. One form of genomic instability is the result of inactivation of tumor suppressor genes, which is the hallmark of the tumor suppressor pathway of oncogenesis. The other form results from the malfunction of the DNA mismatch repair system and leads to replication error (RER) phenotype characteristic of the mutator pathway of oncogenesis.^{4,5} Several recent findings suggest that mismatch repair system defects might be involved in the pathogenesis of extranodal MALT lymphomas. However, the range of MALT lymphoma patients found to manifest microsatellite instability (MSI) at two or more repeat loci starts at 0%⁶ and ends with >50%.⁷ This contrasts with other non-Hodgkin's lymphoma types showing frequently karyotypic but rarely MSI.⁸ Because of such conflicting results on MSI frequency in MALT lymphoma, there is no consensus regarding the role of MSI in MALT lymphoma pathogenesis and no universal MSI screening panel for lymphomas is available.

Both forms of genomic instability can be assayed for by one method, microsatellite analysis. Microsatellite markers can be used to identify genetic loci that have been lost to detect genomic alterations (losses of heterozygosity and homozygous deletions) in neoplasms. Analysis with the same markers reveals also any MSI and any novel additional alleles of different length present in the tumor. To evaluate the contribution of the mutator pathway to the gastric lymphoma pathogenesis, we analyzed 25 gastric low-grade marginal-zone B-cell lymphomas of MALT-type and 31 gastric high-grade DLBCLs, with 29 and 118 microsatellite markers, respectively, including markers from a MSI screening panel for colorectal carcinoma.⁹ We report the results of these analyses and provide an assessment of the mutator pathway role in lymphomagenesis.

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Address reprint requests to Petr Starostik, Institute of Pathology, Würzburg University, Luitpoldkrankenhaus, Josef-Schneider-Strasse 2, D-97080 Würzburg, Germany. E-mail: petr.starostik@mail.uni-wuerzburg.de.

Materials and Methods

Patients and Samples

Thirty-one consecutive extranodal gastric high-grade DLBCL patients and 25 gastric low-grade marginal-zone B-cell lymphoma of MALT-type cases from the lymph node registry at the Institute of Pathology in Wuerzburg on whom fresh frozen tissue (all high-grade and 15 low-grade lymphomas) or formalin-fixed/paraffin-embedded tissue (10 low-grade lymphomas) was available were selected for the study. The diagnosis was established according to the criteria of the Revised European-American Lymphoma Classification¹⁰ and Chan et al¹¹ by morphological and immunophenotypic analyses of paraffin-embedded and fresh-frozen tissue sections using standard staining methods as described recently.³ The high-grade lymphoma patients presented in various stages of disease (four patients were in stage EI1, 13 in stage EI2, nine in stage EII1, four in stage EII2, and one in stage EIII).¹² The tumors were localized at presentation with no clinical evidence of generalized disease. The

high-grade lymphoma patient population showed an age distribution from 31 to 79 years of age with a mean of 58. The male-to-female ratio was 2.44, there were 22 males and nine females included in the study. From the low-grade MALT-lymphoma patients, five patients were in stage EI1, five in stage EI2, 10 in stage EII1, two in stage EII2, one in stage EIII, there were no staging data available on the remaining two cases. The low-grade patient population showed an age distribution from 32 to 75 years of age with a mean of 54. The male-to-female ratio was 2.13, there were 17 males and eight females included.

Microdissection and DNA Extraction

In each case, 16 serial 10- μ m-thick tissue sections were cut. The first and last cuts were stained with hematoxylin and eosin (H&E) to assure high tumor content and as guidance for the following dissection. The fresh-frozen tissue sections were visualized under microscope and, after removal of surrounding normal tissue, an area show-

Table 1. One Hundred Eighteen-Microsatellite Markers Used in the Analysis of Gastric Lymphomas and Their Chromosomal Locations

Marker	Location
BAT-40	1p13.1
D1S237*, D1S2827*	1q32.1
BAT-26	2p21-22
D2S391*, D2S123	2p16-22
D2S2112	2p13
D3S1609, D3S1612, D3S1298, D3S1611	3p22-24.2
D3S4103*, D3S1300*	3p14.2
D3S1261*	3p14.1
D3S1206	3q21.3-25.2
D3S1212, D3S1229*	3q26.2-27
D3S1530*, D3S1580*, D3S1314*, D3S1311*	3q27-qter
D4S43	4p16.3
ABI PRISM® LMS-MD10 for chromosome 5 (22 loci)	5
D5S82*, D5S346*	5q21
ABI PRISM® LMS-MD10 for chromosome 6 (23 loci)	6
D6S1721*	6p23
D6S246, D6S268, D6S1592, D6S447*, D6S1698, D6S302, D6S261	6q21-q22.1
D6S287	6q21-q23
D6S292	6q22.3-q23
D6S310*	6q23.3-q25
D6S441*	6q24-q25.3
IGF2R-3'UTR, IGF2R-(G) ₈	6q26
D6S297*, D6S281	6q27
D7S501*, D7S523	7q31
D7S486*, D7S522	7q31.1
D7S649	7q31.1-31.2
D7S483	7q36.1
C-MYC	8q24.12-24.13
D9S2136*, D9S1748, D9S1752	9p21
MXI1-3'UTR	10q25
D11S987	11q13.3
D11S2179, D11S1356*, D11S925, D11S1345*, D11S933	11q23-24
D12S89*, D12S98, D12S70, D12S269	12p12-13
D13S152*, D13S319, D13S272, AFMa301wb5	13q14
ESF-4-(AGC) _n	16q22.1
D17S250*	17p12
TP53CA*	17p13.1
D18S474*, D18S35*, DCC.PCR1	18q21
BAX-(G) ₈	19q13
D20S110	20q22.1

Repeats used in the 29-microsatellite screening panel are marked by an asterisk (*).

ing high- or low-grade lymphoma was scraped using a blade. In a similar way, control genomic DNA was derived from separate tissue blocks not involved by the tumor. Paraffin-embedded/formalin-fixed tissue sections were additionally stained by Nuclear-Fast Red to precisely delineate tumor-containing areas and the collected tissue deparaffinized with xylene before digestion. DNA extraction was performed using proteinase K and phenol-chloroform according to routine molecular biology protocols.¹³ To exclude the possibility of tumor infiltrating the tissue used as a normal control, all low-grade lymphoma control tissue samples were confirmed by polymerase chain reaction (PCR)-based IgH clonality analysis as not being contaminated by lymphoma cells.¹⁴

Microsatellite Analysis

Microsatellite primer panels ABI PRISM LMS-MD10 for chromosomes 5 and 6 were bought from Perkin-Elmer-Cetus (Foster City, CA). Primer sequences for the amplification of the remaining microsatellite repeats listed in Table 1 were retrieved from Genome Database (<http://gdbwww.gdb.org>). PCR primers were synthesized at MWG Biotech (Munich, Germany) and one oligonucleotide of each primer pair labeled with fluorescent dye phosphoramidites FAM, TAMRA, or HEX. Paired normal and tumor DNA samples from each patient were amplified with PE AmpliTaq Gold enzyme (Perkin-Elmer-Cetus) in multiplex PCR reactions using 50 ng of genomic DNA as template under conditions specified by the Genome Database. Thirty cycles were performed in a PE-2400 thermal cycler (Perkin-Elmer-Cetus) in a total volume of 20 μ l. Aliquots of the PCR reactions were then mixed with size standard and formamide, denatured, and subjected to electrophoresis on a 373 DNA Sequencer (ABI, Foster City, CA). The automatically collected data were analyzed using GENESCAN software as described in the manufacturer's manual. Patients heterozygous and homozygous at a given locus were regarded to be informative for MSI; MSI was defined as a change of allele length caused by of either insertion or deletion of repeating units.

Immunostaining for MSH2 and MLH1

Immunohistochemical staining for MSH2 (hybridoma clone FE11; Calbiochem, Darmstadt, Germany) and MLH1 (hybridoma clone G168-15; Pharmingen, Hamburg, Germany) was performed on pressure-cooker pretreated formalin-fixed/paraffin-embedded tissue sections and visualized using standard immunoperoxidase technique. Normal tissue of the same patient served as a control. A case was considered positive for each antigen, when >80% of the tumor cells showed strong nuclear staining as compared to normal cells on the same slide.

Results

Gastric High-Grade DLBCLs Show Low-Frequency MSI (MSI-L)

We assumed that if MSI were to play a role in the pathogenesis of gastric extranodal lymphomas, then it would be more pronounced in the high-grade lymphomas than in their low-grade counterparts. We therefore screened 31 gastric high-grade DLBCLs with 118 highly polymorphic, mostly dinucleotide microsatellite markers first (Table 1) and established on that material a panel of markers with which then the low-grade disease was investigated. The 118 markers were chosen to cover chromosomal regions shown to harbor gross chromosomal aberrations detected in a previous study using a part of the same patient material,¹⁵ we also included markers showing high-level MSI in a work published previously,⁷ markers from a colorectal cancer MSI screening panel,⁹ and additionally, screening markers from the ABI PRISM LMS-MD10 panels for chromosomes 5 and 6. The overall level of MSI as detected with the 118-marker panel was low, with a mean of 2.6% MSI-positive markers and SD of 1.85%. Ninety-six (2.7%) of 3,568 genotypes revealed MSI, the majority (71%) of the novel alleles showed only one repeat difference to the original allele. Eighty-two percent were additions and 18% were deletions of one repeat from the original allele. All of the MSI cases were type II mutations (only one novel allele occurred per marker); three (10%) of 31 patients did not show any MSI and were thus microsatellite stable. None of the tumors displayed 40% or more MSI-positive markers, a level established as a cut-off for high-frequency MSI (MSI-H) in colorectal cancer.⁹ All these lymphomas showing MSI are thus MSI-L tumors. With this corresponds also our finding of sufficient levels of the MLH1 and MSH2 mismatch repair proteins in the lymphoma cells. All tumors showed strong nuclear signal when immunostained with α -MLH1 and α -MSH2 antibodies (Figure 1). The lymphomas were further analyzed for mutations at the polydeoxyadenine tract of the transforming growth factor- β type II receptor gene and polydeoxyguanine tracts of the insulin-like growth factor II receptor and BAX genes or the AGC repeat in the coding region of the E2F-4 gene, mutations that are characteristically associated with the MSI-H phenotype.¹⁶ However, none of the 31 analyzed patients revealed any MSI at these repeats.

MSI Frequency and Variability Increase with Age in DLBCLs

When the frequency of MSI in the individual patients was plotted in a diagram (Figure 2), it became obvious that the occurrence of MSI at low-frequency levels is very common in these lymphomas (90% of the tumors are MSI-positive). However, there is a considerable difference in MSI frequency as demonstrated by individual cases. One extreme was the three patients not showing any MSI at all, the other extreme the patients, case 8 and case 16, showing 10 (8.5%), and eight (6.8%) MSI-pos-

Figure 1. Immunostainings for MLH1 and MSH2, MSI at BAT-40 locus in DLBCL case 3. **A** and **B**: Representative tumor areas stained with the α -MLH1 and α -MSH2 antibodies, respectively, showing strong nuclear staining for both mismatch repair gene products MLH1 and MSH2. Original magnification, $\times 400$. **C** and **D**: Results of microsatellite analysis at the BAT-40 repeat in case 3, showing electropherograms obtained with control normal tissue and tumor, respectively. The tumor shows MSI, a new 115-bp-long allele (arrow, the highest peak surrounded by stutter bands).

itive markers, respectively. The former patient seems to be an outlier, the latter with 6.8% MSI-positive markers is very close to the border of the ± 2 standard deviations confidence interval (0 to 6.3% MSI-positive markers). Because the MSI frequency of almost all evaluated DLBCLs lies within 2 standard deviations from the mean of the population, it seems that we are dealing with a biological process generally present within the studied material and the differences in the level of MSI in individual cases are either random or depend on additional factors. A search for factors having an influence on MSI levels revealed that older age of patients seems to associate with higher MSI frequency. MSI showed a positive correlation with age ($r = 0.46$; Figure 2); indeed, the tendency to increase with age was statistically significant (Jonckheere-Terpstra test, $P = 0.012$), as was increasing MSI variability with age (F -test, $P = 0.02$). The MSI frequency difference between younger and older patients was further confirmed by comparison of two groups of patients; those ≤ 50 years of age and those ≥ 60 years revealing a significant result (Mann-Whitney U -Test, $P = 0.009$).

MSI Panel for Colorectal Carcinoma Detects MSI-H in Only One DLBCL Patient

To compare MSI frequency in extranodal lymphomas and colorectal carcinomas, we used an established panel of five microsatellite markers considered to be extremely sensitive for the detection of MSI in colorectal carcinoma¹⁷ to screen for MSI in 31 gastric high-grade DLBCLs. The panel consisted of mononucleotide repeats BAT-26 and BAT-40, and dinucleotide repeats D2S123, D5S346, and D17S250.⁹ Comparison of the tumor and normal tissue electropherograms revealed novel-length alleles characteristic of MSI in seven (5%) genotypes in six (19%) patients (Figures 1 and 3A). Only patient 11, that is one (3%) of 31 patients showed MSI with two markers, fulfilling in this way the criteria for MSI-H as defined by this marker panel.¹⁸ In contrast, the same patient had only 3.4% MSI-positive markers when investigated with the 118-marker panel and, therefore, at the end had only MSI-L, not MSI-H.⁹ Patient 3 showed MSI at the BAT-40 locus (Figure 1), which is frequently mutated in HNPCC,

Figure 2. MSI frequency increases with age in 31 gastric high-grade DLBCLs. Individual patients were plotted in a diagram according to age and number of MSI-positive repeats as detected with the 118 microsatellite marker panel (diamonds). Comparison of patients ≤ 50 years of age and ≥ 60 years revealed a significant difference in MSI frequency (Mann-Whitney U -test, $P = 0.009$) between these two groups. The MSI frequency showed a positive correlation with age ($r = 0.46$); the tendency to increase with age was statistically significant (Jonckheere-Terpstra test, $P = 0.012$), as was increasing MSI variability (F -test, $P = 0.02$).

however, that was the only MSI detected with the poly(A) markers BAT-26 and BAT-40 in this study.

Lymphoma MSI Panel as a Screening Tool for Detection of MSI in High-Grade Lymphoma

Search for MSI at individual loci of the 118-marker panel revealed 62 (52.5%) repeats to be negative for MSI in all DLBCL tumors analyzed, 30 (25.4%) markers showing only one MSI event, 18 (15.2%) with two MSI events, five (4.2%) with three MSI events, and one (0.8%) of each with either four, five, or six MSI events (Figure 4). Microsatellites showing frequent MSI were not evenly spaced across the genome, unusually many markers showing higher number of MSI events were concentrated on chromosomes 3, 5, and 18 (Figure 4). Obviously, frequently MSI-positive microsatellites should be used for screening in lymphoma. We therefore designed a new MSI screening panel for lymphoma using the following microsatellites: D3S1261, D3S1530, D5S346, D17S250, D18S474, and DCC (Figure 3B). Such a panel would detect five patients, including cases 8 and 16, as having an increased MSI frequency and is thus more sensitive in detection of possible RER⁺ phenotype.

Low-Grade Gastric Marginal-Zone B-Cell Lymphomas of MALT Type Show Significantly Lower Frequency of MSI

Twenty-nine markers (Table 1, loci marked by an asterisk), which showed any consistent MSI or allelic imbalance in the high-grade part of the study, were then used to screen 25 low-grade gastric marginal-zone B-cell lymphomas of MALT-type. Of 659 genotypes (some formalin-fixed tissue derived DNA did not give any amplificate even after repeated PCRs), only four (0.6%) showed MSI (raw data not shown). These MSI events seemed to be

A

Pat.	BAT-26	BAT-40	D2S123	D5S346	D17S250
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					

B

Pat.	D3S1261	D3S1530	D5S346	D17S250	D18S474	DCC
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
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25						
26						
27						
28						
29						
30						
31						

Figure 3. A: MSI as detected by a screening panel for colorectal carcinoma in 31 cases of gastric high-grade DLBCL. Status of each microsatellite locus is indicated as follows: **solid bars**, retention of heterozygosity; **hatched bars**, homozygosity; **vertical-striped bars**, MSI; **closed circles**, genomic amplification; and **open bars**, LOH. Markers are listed at the top of each column. Patients showing two or more loci positive for MSI are marked with a $\blacklozenge$. **B:** MSI screening panel for lymphoma, status of each locus is indicated as above.

randomly dispersed, as they occurred at loci not showing very frequent MSI in the high-grade disease and all four markers were affected only once. Twenty-two patients did not show any MSI at all, two patients had one MSI-positive marker, one patient displayed two MSI-affected markers. The overall MSI frequency in this group (LG29)

Figure 4. MSI frequency per marker in DLBCLs. Microsatellite markers showing two or more events were plotted according to the number of MSI events per marker.

was very low with mean of 0.55% MSI-positive marker and standard deviation of 1.63% (Figure 5). To perform MSI frequency comparison of the low- and high-grade lymphomas with exactly the same markers, we reevaluated the high-grade lymphomas for the same 29-marker panel the low-grade cases were screened with. With these markers, the DLBCLs showed a mean of 4.1% MSI-positive repeats with standard deviation of 5%

Figure 5. Extranodal gastric high-grade DLBCL shows a higher degree of MSI than low-grade gastric marginal-zone B-cell lymphoma of MALT type (Mann-Whitney *U*-test, $P = 0.009$). LG29 and HG29 are MSI frequencies in the low- and high-grade lymphoma cases, respectively, as detected with a set of 29 markers (listed in Table 1); LG29 displaying a mean of 0.55% MSI-positive markers and SD of 1.63%, and HG29 showing a mean of 4.1% and SD of 5%.

(HG29; Figure 5). That somewhat differs from the MSI frequency established for the DLBCL group by the use of the 118-marker panel; however, the difference is primarily because of the selection of repeats for the 29-marker panel (underrepresentation of repeats not showing any MSI). Statistical analysis of the MSI frequency data collected for the LG29 and HG29 groups confirmed a significant difference in MSI frequency between the low-grade gastric marginal-zone B-cell lymphoma of MALT type and gastric high-grade DLBCL (Mann-Whitney *U*-Test, $P = 0.009$).

Discussion

Generally, there are two pathways of carcinogenesis. The tumor suppressor pathway characterized by mutational inactivation of one allele and loss of the other allele of a tumor suppressor gene, and the mutator pathway, which involves mutational inactivation of two alleles of the same gene that is not a suppressor gene but is a mutator gene.^{4,5} The latter pathway is characterized by MSI-H and the biology of these RER⁺ tumors is fundamentally different. There is no consensus in the literature on the role of MSI in the pathogenesis of extranodal lymphomas, partially because of the absence of a clear-cut definition of the RER⁺ phenotype. Using a definition that was still accepted before the National Cancer Institute sponsored International Workshop on Microsatellite Instability and RER Phenotypes in 1997,⁹ according to which two or more markers positive for MSI equaled RER⁺ phenotype, 50% of the low-grade and 56% of the high-grade MALT lymphomas were found to be RER⁺ or 17% of the low-grade MALT lymphomas and 79% of the gastric DLBCLs showed the RER⁺ phenotype.¹⁹ In contrast, 23% of MALT lymphoma patients studied by Sol Mateo et al²⁰ were RER⁺, however, none of the patients investigated by Xu et al⁶ or Hoeve et al²¹ displayed the RER⁺ phenotype, although some of them revealed MSI with one of the studied microsatellite markers.

To determine whether MSI characterizes a subset of sporadic extranodal gastric low- and high-grade lymphomas and to investigate the contribution of the mutator pathway to lymphomagenesis, we evaluated 31 gastric high-grade DLBCLs and 25 gastric low-grade marginal-zone B-cell lymphomas of MALT-type for MSI. A thorough analysis with a panel of 118 microsatellite markers showed a low background MSI with a mean of 2.6% MSI-positive microsatellites in the high-grade lymphoma patients. Three patients were microsatellite stable and two patients showed somewhat more frequent MSI with 6.8% and 8.5% of the markers used, respectively. A direct comparison of the low- and high-grade lymphomas with a panel of 29 frequently mutated markers showed a mean of 4.1% MSI-positive markers per patient in the former group and 0.55% in the latter group. However, compared to epithelial cancers and, especially, colorectal cancer, this level of MSI is still too low to consider these tumors to be MSI-H. Only cases with 40% or more of all markers unstable are diagnosed as MSI-H or having the RER⁺ phenotype in sporadic colorectal carcinoma. A

panel of five markers has been proposed for screening purposes and defining the MSI-H colorectal cancer group.⁹ Using this reference panel on the studied high-grade lymphomas, one of our patients (case 11; Figure 3A) would be defined as MSI-H (the same patient showed only 3.4% unstable microsatellite markers with the 118-marker panel and was thus definitely MSI-L not MSI-H). However, patients displaying the most MSI in our study (cases 8 and 16) would not be detected by this MSI screening panel as having increased MSI at all. Moreover, the markers comprising this reference panel were only partly among those showing frequent MSI in the studied high-grade DLBCLs. Mononucleotide poly(A) repeats characteristically showing frequent instability in HNPCC and MSI-H colorectal cancer^{17,22} proved to be unstable in only one of the high-grade DLBCL patients. From the dinucleotide-repeat markers, only D5S346 and D17S250 revealed frequent MSI also in the lymphomas. Interestingly, of the evaluated 118-repeat panel, microsatellites showing most frequently MSI seem to be predominantly concentrated on chromosomes 3, 5, and 18 (Figure 4). This could be partly a result of clonal aberrations, because several of the patients showed trisomy 3 and there were also several trisomies 18 present as revealed by cytogenetic analysis of the material (results not shown). Nevertheless, microsatellites showing most frequently MSI thus might be tumor-specific, meaning that different markers are appropriate for MSI screening in different types of cancer and the optimal set of loci to diagnose MSI in lymphoma is different from that one for colorectal carcinoma. A MSI screening panel for lymphomas should therefore consist of different markers, microsatellites showing frequent MSI in lymphoma as opposed to a marker set appropriate for colorectal cancer MSI screening. We therefore introduce here a new lymphoma MSI screening panel composed of markers showing frequent MSI in the studied high-grade lymphomas (Figure 3B). Such a panel consisting of six markers (D3S1261, D3S1530, D5S346, D17S250, D18S474, and DCC) would detect DLBCL patients having increased level of MSI, including cases 8 and 16 with the highest MSI frequency detected in this study.

A small fraction of many tumor types, in addition to those of the colon, display some level of MSI.²³ In most of these tumors, the instability is considerably less pronounced than that observed in colon tumors and it is questionable if it is because of mismatch repair gene defects. There are several lines of evidence against a substantial role of mismatch repair genes and MSI in the pathogenesis of extranodal high-grade lymphomas. These tumors show widespread karyotypic instability and are mostly aneuploid, as revealed by recent cytogenetic^{15,24} and comparative genomic hybridization (CGH)²⁵ studies. All MSI-positive analyses in this work showed only type II mutations with a single slightly size-changed novel allele which is not typical for the RER⁺ phenotype and that can be also detected in RER-negative tumors.²⁶ Our current search for mutations characteristically associated with the RER⁺ phenotype,²⁷⁻³² like MSI at the

polydeoxyadenine tract of the transforming growth factor- β type II receptor gene and polydeoxyguanine tracts of insulin-like growth factor II receptor and BAX genes or the AGC repeat in the coding region of the E2F-4 gene did not reveal any instability at these repeats. Several studies reported close correlation between tumors displaying MSI-H and the absence of protein expression for either MSH2 or MLH1 in colorectal carcinoma.^{17,33} However, staining with α -MLH1 and α -MSH2 antibodies confirmed the presence of these mismatch repair proteins in all DLBCL patients studied. These features considered together, widespread genomic alterations in the form of losses of heterozygosity and amplifications of genetic material we saw previously on the same DLBCL material,³⁴ and the low-frequency of MSI detected in the presented study are a rather substantial evidence for the mutator pathway having only a minor role in the pathogenesis of this disease.

Nevertheless, the approximately sevenfold higher MSI frequency in the high-grade lymphomas when compared to their low-grade counterparts ($P = 0.009$) and the increase of MSI with age within the high-grade lymphoma group ($P = 0.01$) show that there is a selection pressure for MSI to increase during the transition from low- to high-grade disease on one side and with older age on the other side. Dysfunction of the mismatch repair mechanism is a relatively late phenomenon during lymphomagenesis and its role in the pathogenesis of the disease seems to be contributory, but not initiating. These lymphomas do not achieve that degree of MSI characteristic of HNPCC or MSI-H-positive colorectal carcinoma meaning that the mismatch repair system is still to some degree relatively preserved as confirmed by the detection of the MLH1 and MSH2 proteins in all DLBCLs investigated. How exactly this MSI-L contributes to lymphomagenesis is not clear. Using enough microsatellite markers for analysis, probably all tumors can be made to show MSI with one of the repeats used. The cause and effect relationships between MSI-H and mutations in mononucleotide repeats of genes like transforming growth factor- β type II receptor gene²⁷ or BAX³⁰ are well described, however, the role of MSI-L in cancerogenesis has not been established yet. To determine how it exactly contributes to the development of lymphomas will require an agreement on definition of MSI-L in lymphomas and a thorough search for target genes disabled by MSI-L. To facilitate such studies, we propose to use a marker panel sensitive enough to detect MSI-L. Our findings that different microsatellite repeats exhibit frequent MSI when DLBCLs are compared to MSI-H colorectal cancer, most striking being the minimal involvement of the mononucleotide poly(A) repeats prompted us to define a MSI screening panel for lymphomas. This panel consisting of dinucleotide repeats D3S1261, D3S1530, D5S346, D17S250, D18S474, and DCC is more sensitive in MSI detection than a previously established panel for colorectal carcinoma. Only using a sensitive common marker panel for detection of MSI in lymphomas, the controversial issues of MSI frequency and its role in the development of extranodal lymphoma can be resolved.

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