The t(14;18)(q32;q21)/*IGH-MALT1* translocation in MALT lymphomas contains templated nucleotide insertions and a major breakpoint region similar to follicular and mantle cell lymphoma

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The t(14;18)(q32;q21) involving the immunoglobulin heavy chain locus (*IGH*) and the *MALT1* gene is a recurrent abnormality in mucosa-associated lymphoid tissue (MALT) lymphomas. However, the nucleotide sequence of only one t(14;18)–positive MALT lymphoma has been reported so far. We here report the molecular characterization of the *IGH-MALT1* fusion products in 5 new cases of t(14;18)–positive MALT lymphomas. Similar to the *IGH*-associated translocations in follicular and mantle cell lymphomas, the *IGH-MALT1* junctions in MALT lymphoma showed all features of a recombination signal sequence–guided V(D)J-mediated translocation at the *IGH* locus. Furthermore, analogous to follicular and mantle cell lymphoma, templated nucleotides (T-nucleotides) were identified at the t(14;18)/*IGH-MALT1* breakpoint junctions. On chromosome 18, we identified a novel major breakpoint region in *MALT1* upstream of its coding region. Moreover, the presence of duplications of *MALT1* nucleotides in one case suggests an underlying staggered DNA-break process not consistent with V(D)J-mediated recombination. The molecular characteristics of the t(14;18)/*IGH-MALT1* resemble those found in the t(14;18)/*IGH-BCL2* in follicular lymphoma and t(11;14)/*CCND1-IGH* in mantle cell lymphoma, suggesting that these translocations could be generated by common pathomechanisms involving illegitimate V(D)J-mediated recombination on *IGH* as well as new synthesis of T-nucleotides and nonhomologous end joining (NHEJ) or alternative NHEJ repair pathways on the *IGH*-translocation partner. (Blood. 2010;115:2214-2219)

Introduction

Four recurrent translocations, t(1;14)(p22;q32), t(3;14)(p14.1;q32), t(11;18)(q21;q21), and t(14;18)(q32;q21), have been described in marginal zone B-cell lymphomas of the mucosa-associated lymphoid tissue (MALT) type.[1-6] The 2 latter translocations involved the *MALT1* gene and lead to an *API2-MALT1* gene fusion in the t(11;18)(q21;q21) and an *IGH-MALT1* fusion in the t(14;18)(q32;q21).[1-3] The molecular analyses of the t(11;18)/*API2-MALT1* have led to its complete characterization, facilitating the development of detection assays based on fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction (PCR).

In contrast, less is known about the molecular genetic features of the t(14;18)/*IGH-MALT1* in MALT lymphoma because only one case has been described molecularly, so far.[7] The scarcity of data could be due to the relative low incidence of this translocation, because only up to 10% of MALT lymphomas are t(14;18)-positive, to the difficulty in obtaining densely infiltrated tumor tissue, and to the low proliferation rate of the neoplastic cells. In addition, t(14;18)/*IGH-MALT1*–positive MALT lymphomas originate at sites such as the liver, skin, ocular adnexa, or salivary glands.[1,3,8]

Nevertheless, the molecular characterization of t(14;18)/*IGH-MALT1* is important to understand the underlying pathogenetic mechanisms and to develop detection methods for the diagnostic workup. In this context it is important to note that the t(14;18)/*IGH-MALT1* in MALT lymphomas and the t(14;18)(q21;q32)/*IGH-BCL2* in follicular lymphomas (FLs) are indistinguishable by conventional cytogenetic analysis.[7]

In other B-cell lymphomas, such as FL and mantle cell lymphoma (MCL), the analyses of the immunoglobulin heavy chain locus (*IGH*) breakpoint junctions have led to important insights in the mechanisms leading to the *IGH*-associated translocations in these lymphoma subtypes.[9-13]

The t(14;18)/*IGH-BCL2* in FL and the t(11;14)/*CCND1-IGH* in MCL result from an illegitimate V(D)J recombination with insertions of templated nucleotides (T-nucleotides) at the junction sites.[9,10] Moreover, the study of the breakpoints of translocations involving the *IGH* locus has been used to understand the cellular origin of B-cell lymphomas. The presence of D_H segments carrying somatic mutations at the direct J_H-*BCL2* and *CCND1*-J_H junctions has suggested that these translocations can also emerge in germinal/

Table 1. Clinical and cytogenetic data of patients with MALT lymphoma with t(14;18)/*IGH-MALT1*

Case no.	Sex/age	Diagnosis	Therapy/response	Karyotype
1	F/71	Low-grade MALT lymphoma of the conjunctiva	RT/CR	No mitosis
2	M/53	Low-grade MALT lymphoma of the lung	Lobectomy, CT (Chloramb)/CR	49,XY,+3,+5,+12,t(14;18)(q32;q21)[4]/ 50,idem,+8[13]/46,XY[3]
3	M/57	Low-grade MALT lymphoma of the orbit	NA	NA
4	F/65	Low-grade MALT lymphoma of the skin	NA	NA
5	M/78	Low-grade MALT lymphoma of the skin	NA	NA
6*	M/76	Low-grade MALT lymphoma of the orbit	RT/CR	47,XY,+3,t(14;18)(q32;q21)[6]/46,XY[3]

The status for all cases was diagnosis at the time the sample was taken.
RT indicates radiation therapy; CR, complete remission; CT, chemotherapy; Chloramb, chlorambucil; and NA, not available.
*Case 6 corresponds to case 1 of the study of Sánchez-Izquierdo et al.[7]

postgerminal center B cells and not only at early stages in the B-cell development.[9,10,12]

We herein report the molecular genetic characterization of the direct *MALT1-J_H* and reciprocal *D_H-MALT1* junctions in 5 new MALT lymphomas harboring the t(14;18)/*IGH-MALT1*.

Methods

Clinical and cytogenetic data

Five cases with the diagnosis of an extranodal marginal zone B-cell lymphoma of the MALT type, the presence of the t(14;18)/*IGH-MALT1* as shown by interphase FISH, and available frozen or paraffin-embedded tissue were collected from different genetic institutes (University Medical Center Hamburg-Eppendorf, Germany [n = 3]; Center Hospitalier Lyon Sud, France [n = 1]; University of Cambridge, United Kingdom [n = 1]) and included in the present study. The clinical and cytogenetic data of these cases are described in Table 1.

Interphase FISH

Four of 5 MALT lymphomas originating from the skin (n = 2), the lung (n = 1), and the conjunctiva (n = 1) were analyzed by interphase FISH as previously described with the use of probes for the *MALT1* gene (PAC117B5 and PAC59N7) and the *IGH* locus (cosmid $C_{\alpha1}$).[1] One further MALT lymphoma of the orbit (case 3) was analyzed with the LSI MALT1 (18q21) Dual Color, Break Apart Rearrangement Probe, the LSI IGH (14q32) Dual Color, Break Apart Rearrangement Probe, and the LSI IGH/MALT1 t(14;18)(q32;q21) Dual Color, Dual Fusion Translocation Probe (all from Abbott Vysis).

Amplification of the direct and reciprocal *IGH-MALT1* products on frozen tissue and statistical analysis

The direct *IGH-MALT1* fusions (*MALT1-J_H*) were confirmed in cases 1 and 2 by long-distance PCR with the use of the GeneAmp XL PCR system (Applied Biosystems) on patients' DNA with nested primers MALT1-R1a and MALT1-R1b in combination with JH6-R1a and JH5-R1a.

The reciprocal *IGH-MALT1* fusion products (*D_H-MALT1*) were amplified with the use of the AccuPrime *Pfx* DNA Polymerase (Invitrogen) and the *MALT1*-specific primer MALT1-F01a in combination with 1 of a set of 7 specific *D_H* primers D1 to D7,[9] followed by a nested PCR with MALT1-F01b with one of the nested D1N1 to D7N1 specific primers, as previously described.[9]

The products were amplified, sequenced twice in both directions, and analyzed with the use of the BLAST algorithm at National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast).

The presence of T-nucleotides at the *IGH-MALT1* junctions was investigated by using the statistical approach described by Jäger et al.[9] The de novo nucleotide additions found at the breakpoints were analyzed by using a binomial test. A *P* value of no more than .05 was selected as the point of high statistical significance.[9,10]

Amplification of the *IGH-MALT1* products on paraffin-embedded tissue

For the amplification of the *IGH-MALT1* fusion products in cases 3 to 5, DNA was isolated from formalin-fixed, paraffin-embedded sections, and PCR was performed with the use of the AccuPrime *Pfx* DNA Polymerase (Invitrogen). Pairs of nested *MALT1* primers were designed to hybridize close to the *MALT1* breakpoints detected in cases 1 and 2 and to enable the amplification of small-sized DNA fragments. For the detection of the *MALT1-J_H* fusion products, the nested primers MALT1-R2a and MALT1-R2b (in cases 3 and 4) and the nested primers MALT1-R3a and MALT1-R3b (in case 5) were used combined with *IGH* primers J_HEx-B and J_HCo-B, described elsewhere.[9]

The products were amplified, sequenced, and analyzed as described above. For the amplification of the *D_H-MALT1* junctions different primer combinations were tested, but no fusion product could be amplified in these patients (cases 3-5).

Primer sequences

MALT1-J_H fusion products were as follows: MALT1-R1a, 5′-CAGGGGCG-GAAGAACAAATC-3′; MALT1-R1b, 5′-CCAATTAGACAGGTTTGC-CGAG-3′; MALT1-R2a, 5′-CTTCTTAGAAAAGTCTGCCATGTA-3′; MALT1-R2b, 5′-AGAAAAGTCTGCCATGTACAAAG-3′; MALT1-R3a, 5′-GTCTTTACTGGAATCCTGC-3′; MALT1-R3b, 5′-TTACTGGAATCC-TGCCTTT-3′; JH6-R1a, 5′-CATTCTTACCTGAGGAGACGGTG-3′; JH5-R1a, 5′-CTTTCTTTCCTGACCTCCAAAATG-3′.

D_H-MALT1 fusion products were as follows: MALT1-F01a, 5′-ATGCCCATAAGAGAACCACAGG-3′; MALT1-F01b, 5′-GCGGGTAG-TGACTGGATAGG-3′.

Results

We herein report the molecular characterization of the t(14;18)/*IGH-MALT1* fusions in 5 new cases of MALT lymphomas: 2 originating from the skin, 1 from the conjunctiva, 1 from the lung, and 1 from the orbit. So far, molecular genetic data of one case representing a MALT lymphoma of the conjunctiva harboring the t(14;18)/*IGH-MALT1* has been published.[7]

Cytogenetic data were available in one of our cases (case 2) and showed the t(14;18)/*IGH-MALT1* accompanied by trisomies of chromosomes 3, 5, 8, and 12. In the case reported by Sánchez-Izquierdo et al,[7] the t(14;18)/*IGH-MALT1* occurred together with a trisomy 3 (case 6 in Table 2).

The presence of the t(14;18)/*IGH-MALT1* fusion was confirmed by interphase FISH in all of our 5 cases. The number of *MALT1/IGH*-positive cells ranged from 6.6% to 70% (case 1, 50%; case 2, 54%; case 3, 70%; case 4, 7%; case 5, 8%).

In all 5 cases, we could confirm the presence of the *IGH-MALT1* fusion by PCR. Even in DNA extracted from paraffin-embedded tissues (cases 3-5) and in cases with a low tumor cell infiltration

Table 2. Sequences at the direct breakpoint junctions of the MALT1-J_H fusion products

Cases (organ)*	Processing of ends†	MALT1 sequence (18q21)‡	De novo nucleotide additions (D regions)	J_H sequence (14q32)‡	J_H	Processing of ends
Germline		27462TTTACTGGAATCCTGCCTTTTAATTTAAAATTCAAC		ACTACTTTGACTACTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H4	
Germline		27541CAAAGCGTTGCTTATGCAACCTCTCGTGTGCATAC		ACAACTGGTTCGACCCCTGGGGCCAGGAACCCTGGTCACCGTCTCCTCATAC	J_H5	
Case 1 (conjunctiva)	+8 bp	27541CAAAGCGTTGCTTATGCAACCTCTCGTGTGCAT	CCTAAGGGTTCGTGGGCGGGGA-GCCCAGGTC	GACTACTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H4	−8 bp
Case 2 (lung)	−4 bp	27541CAAAGCGTTGCTTATGCAACCTtTCG		ACTACTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H4	−9 bp
Case 3 (orbit)	Unknown	27541CAAtGCGTTGCTTATGCAACCTCTC	AGTCGGTCTCGCGCGCGA	TTGACTACTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H4	−6 bp
Case 4 (skin)	Unknown	27541CAAAGCGTTGCTTATGCAACCTCT	ATGGTTTTGGC	CTCGTTCGACCCCTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H5	−4 bp
Case 5 (skin)	Unknown	27462TTTACTGGAATCCTGCCTTTTAATTT	CAGCGCGCCGCGCGCCAGCCG-GCCTATTA	AACCCaGGTCACCGTCTCCTCAGGT	J_H5	−28 bp
Case 6 (orbit)	Unknown	27541CAAAGCGTTGCTTATGCA	GAGT	AACTgGGTTCGACCCCTGGGGCCAGGAACCCTGGTCACCGTCTCCTCAGGT	J_H5	−2 bp

*Case 6 corresponds to case 1 of the study of Sánchez-Izquierdo et al.[7]

†Status of coding ends at IGH locus or MALT1 end processing (− bp indicates deletion; + bp, addition).

‡Numbers in superscript in the MALT1 sequence indicate the position of the first nucleotide in BAC clone RP11-12601 according to GenBank sequence AC104971. Mismatches to the germline are in lowercase letters. Point mutations are underlined and nucleotide insertions are specified above the line. These sequences are deposited at GenBank, accession nos. GQ406056, GQ406058, GQ406060, GQ406061, and GQ406062.

(cases 4 and 5), we were able to amplify the *IGH-MALT1* fusion products.

Characteristics of the breakpoints at the *IGH* locus

The sequencing of the PCR products showed a fusion of sequences upstream of the coding region of *MALT1* to the J_H segment of the *IGH* locus in all 5 cases. These results and the data of one other case published by Sánchez-Izquierdo et al[7] are summarized in Table 2.

Our detailed analyses of the direct *MALT1-J_H* breakpoints showed the typical features of a V(D)J-mediated recombination at the *IGH* locus. In our 5 cases and in the previously reported case, the breakage and rejoining occurred at the recombination signal sequence (RSS) adjacent to the join coding segments J_H4 (cases 1, 2, and 3) and J_H5 (cases 4, 5, and 6; Table 2). In these 6 cases, the t(14;18)/*IGH-MALT1* occurred probably during the D_H-to-J_H joining before the complete $V_H D J_H$ assembly.

In addition to the direct *MALT1-J_H* products, we amplified and sequenced the reciprocal D_H-*MALT1* junctions in 2 patients (cases 1 and 2; Table 3; Figure 1). The sequencing showed a juxtaposition of *MALT1* sequences to the diversity gene segments *D2-15* in case 1 and *D2-21* in case 2. Because the coding sequence of the D_H segment present in case 1 consisted of a fragment of 2 nucleotides, the assignment to the *D2-15* segment was performed according to the intronic sequence.

Further features at the *MALT1-J_H* and D_H-*MALT1* junctions indicate that the V(D)J recombination machinery is responsible for the breaks at the *IGH* locus in the t(14;18)/*IGH-MALT1*. In the processing steps during the V(D)J recombination, antigen variability is introduced by nucleotide deletions at the coding ends and addition of nucleotides at the point of joining that were not present in the germline.[14] Certainly, nucleotide deletions and/or de novo nucleotide additions (N-nucleotides) were also observed in the direct *MALT1-J_H* junctions of all cases (Table 2). Similar to the direct breakpoints, the reciprocal D_H-*MALT1* breakpoints in cases 1 and 2 showed also deletions and additions of residues, resembling the processing of coding ends of V(D)J recombination products (Table 3).

T-nucleotides are present in the t(14;18)/*IGH-MALT1* fusion products

T-nucleotides have recently been identified in the translocations t(14;18)/*IGH-BCL2* in FL and t(11;14)/*CCND1-IGH* in MCL and consist of short copies of at least 5 nucleotides of the adjacent flanking sequences inserted in the breakpoints.[9,10] Therefore, we investigated our cases with de novo nucleotide additions of at least 5 nucleotides at the breakpoints (cases 1-5; Tables 2-3) and examined their sequences for the presence of T-nucleotides. Interestingly, we identified T-nucleotides with highly significant homologies to the join coding segments involved in the translocation in all 5 cases (Table 4).

In case 1, for example, T-nucleotides were found both in the direct and reciprocal fusion products. The J_H4 segment involved in the *IGH-MALT1* fusion served as template. In the direct junction, the homology sequence consisted of a fragment 21 basepairs (bp) in length with 3 mismatches. Eight of these 21 bp with 1 mismatch were also present in the reciprocal junction (Table 4). Taken together, in our cases and the published case, T-nucleotides were observed in 5 of 6 cases.

Table 3. Sequences at the reciprocal breakpoint junctions of the *DH-MALT1* fusion products

Cases	D_H	Processing of ends*	D_H sequence (14q32)	De novo nucleotide additions†	*MALT1* sequence (18q21)‡	Processing of ends*
Germline	*D2-15*		AGGATATTGTAGTGGTGGTAGCTGCTACTCC		[27556]GCAACCTCTCGTGTGCATACGA–GTTAAATG[27585]	
Germline	*D2-21*		AGCATATTGTGGTGGTGACTGCTATTCC			
Case 1	*D2-15*	−29 bp	CCAGTAGGGACAGGAGGATTTTGTGGGGGC–TCGTGTCACTGTGAG	CAGAACCCGT	GTGTGCATACGAGTTAAATG[27585]	+8 bp
Case 2	*D2-21*	−13 bp	AGCATATTGTGGTGG	AAACT	CATACGAGTTAAATG[27585]	−4 bp

*Status of coding ends at *IGH* locus or *MALT1* end processing (−bp indicates deletion; +bp, addition).
†These sequences are deposited as GenBank Accession nos. GQ406057 and GQ406059.
‡Numbers in superscript in the *MALT1* sequence indicate the position of the first nucleotide in BAC clone RP11-126O1 according to GenBank sequence AC104971.

Characteristics of the breakpoints at the *MALT1* gene

At the *MALT1* locus, the analysis of the direct *MALT1-J_H* sequences showed that all 5 breaks were located in the 5′ noncoding region of *MALT1* on chromosome 18 (Table 2). The same was found for the published case.[7] In all cases, the breakpoints were clustered in a 87-bp region between nucleotides 27.489 and 27.573, according to GenBank sequence AC104971 (BAC clone RP11-126O1, which contains the 5′ end of *MALT1*).

This is the first report that describes the presence of a putative major breakpoint region (MBR) for the t(14;18)/*IGH-MALT1* in the 5′ noncoding region of the *MALT1* gene.

The analysis of the reciprocal *D_H-MALT1* junctions could be performed in 2 of our cases. One case (case 2) showed a deletion of 4 nucleotides of *MALT1*. The other case (case 1) showed a duplication of 8 *MALT1* nucleotides with the sequence GTGTGCAT found in the direct and the reciprocal *IGH-MALT1* fusion (Table 3; Figure 1).

We identified one cryptic RSS sequence in the putative *MALT1* MBR. This *MALT1* RSS-like sequence fulfilled almost all requisites for its recognition by the recombination activating gene-1 (RAG-1)/RAG-2. It consisted of a heptamer with the essential trinucleotide CAC at the first 3 positions and an A residue at the fourth position, a 23-bp spacer, and a nonamer with a core of 3 consecutive A residues in the positions 4, 5, and 6 flanked by 2 residues other than A (Figure 1).[15] However, this cryptic RSS was present in the 3′ to 5′ opposite orientation.

J_H and D_H utilization and nucleotide mutations at the breakpoints of the t(14;18)/*IGH-MALT1*

We have further investigated whether MALT lymphomas show a preferential use of determined J_H and D_H segments and/or the presence of somatic mutations as it has been described in FL and MCL.[9,10] We observed that exclusively the J_H4 and J_H5 gene segments were used in the t(14;18)/*IGH-MALT1* (Table 2). The rearranged D_H genes were determined in 2 cases and showed a biased usage of the D2 family of the D_H segments, *D2-15* and *D2-21* (Table 3).

We found mismatches to the germline close to the breakpoints in the sequences of the *IGH* and *MALT1* genes in 4 patients (Table 2).

We detected *IGH* mutations in 2 cases with a C-to-T transition and an insertion of one G residue into the J_H5 segment (case 6) and a T-to-A transversion into the J_H5 segment (case 5) (Table 2).

MALT1 mutations were seen in 2 further cases with an A-to-T transversion (cases 2 and 4). In case 2, the origin of the sequence CTTTCG found at the direct breakpoint is ambiguous and can either be assigned to the *MALT1* sequence CTCTCG with a C-to-T transition or to the *IGH* sequence CTTTG with a C insertion (Figure 1).

Discussion

The t(14;18)(q32;q21) involving the *IGH* and the *MALT1* genes is a recurrent translocation in MALT lymphomas. However, since its
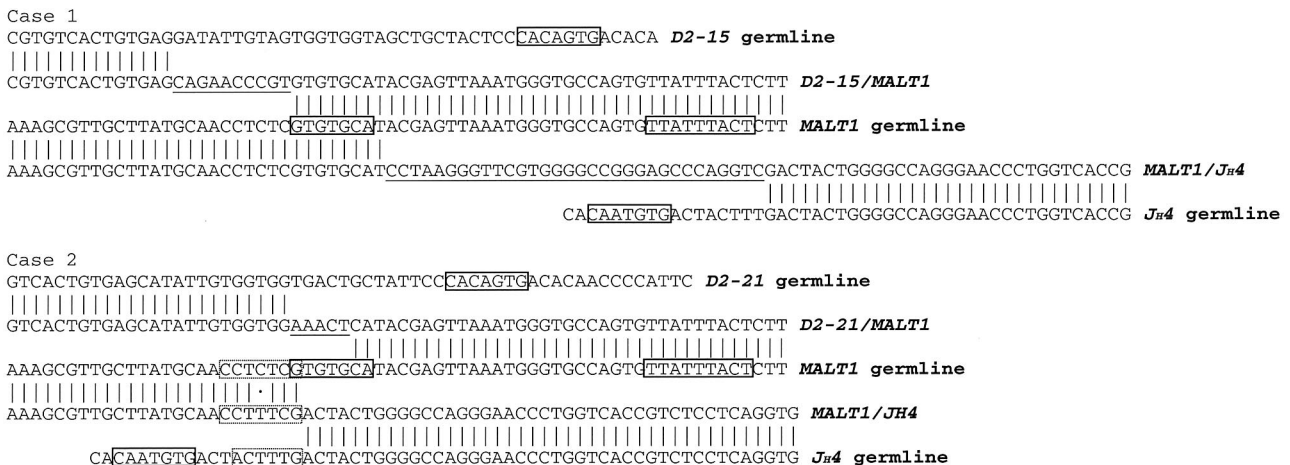

Figure 1. Comparison of the DNA sequences at the direct *MALT1-J_H* and reciprocal *D_H-MALT1* breakpoint junctions and the *IGH* and *MALT1* germline. The *IGH-MALT1* fusion products of the cases 1 and 2 are aligned with germline sequences of *IGH* (GenBank accession no. X97051.1) and *MALT1* (GenBank accession no. AC104971). Nucleotide homologies to the germline are represented by vertical lines and mismatches by dots. De novo nucleotide additions are underlined. Solid boxes indicate RSS heptamers in the J_H and D_H segment germlines and a cryptic RSS heptamer and nonamer in the *MALT1* germline. Dashed line boxes in case 2 indicate the 6-bp microhomologous sequence flanking the translocation breakpoint.

Table 4. Templated nucleotide additions identified at the breakpoint junctions of the t(14;18)/*IGH-MALT1*

Case no.	De novo nucleotide additions*	Breakpoint	Template	P
1	TGGGGCC$_a$GGGA$_g$CCC$_a$GGTC	MALT1-J_H	J_H4	
	GAACCC^{g}T	D_H-MALT1		< .001
2	AAACT	D_H-MALT1	J_H4	.041
3	GCCAG$_a$G$_{aa}$CC^{g}C$_t$C$_{cg}$C	MALT1-J_H	J_H4	< .001
4	A$_c$TGGTT$_t$^{t}G$_g$C	MALT1-J_H	J_H5	.047
5	CGACCC$_{ct}$GG	MALT1-J_H	J_H5	< .001

*Mismatches to the germline are in lowercase letters. Point mutations are underlined, nucleotide insertions are specified above the line, and deletions are specified below the line. The sequence shown is the one of the copy.

identification 6 years ago, the molecular characteristics of this translocation have not been elucidated. This may be due to the relatively low incidence of this translocation and to the difficulties to obtain a sufficient amount of tumor tissue from these rare extranodal lymphomas.[1,3,8]

In this study, we performed a sequence analysis of the *IGH-MALT1* junctions in 5 MALT lymphomas harboring the t(14;18)/*IGH-MALT1*. The results of our investigation strongly indicate that a failure in the V(D)J-mediated recombination process is responsible for the generation of breaks at the *IGH* locus in the t(14;18)/*IGH-MALT1*: the breakpoints in *IGH* were located at a RSS, and coding end processing, such as nucleotide deletions and addition of N-nucleotides, was detected in all cases.[14,16] Similar findings have been published for other translocations associated with V(D)J recombination, such as the t(14;18)/*IGH-BCL2* in FL and the t(11;14)/*CCND1-IGH* in MCL.[9,10,17]

On chromosome 18, all the breakpoints clustered in a 87-bp region in the 5′ noncoding region of *MALT1*. This clustering of breakpoints on chromosome 18 suggests the presence of a MBR in the *MALT1* gene, as it occurs for *BCL2* in t(14;18)/*IGH-BCL2* and for *CCND1* in the t(11;14)/*CCND1-IGH*.[18,19]

Interestingly, we observed one cryptic RSS sequence in the putative *MALT1* MBR. However, several observations indicate that a RSS-guided V(D)J recombination is not responsible for the breaks at the *MALT1* locus. First, the essential trinucleotide 5′CAC3′ of the heptamer and the nonamer sequence of this cryptic RSS were present in the 3′ to 5′ opposite orientation.[15] Second, one of the breakpoints in *MALT1* is located 75-bp downstream of this pseudo signal. Third, the *IGH-MALT1* fusion sequence in one case showed a duplication of 8 nucleotides of *MALT1*, which were present both at the direct and reciprocal fusion products.

The presence of duplications is not consistent with the V(D)J recombination mechanisms. Duplications of sequences are supposed to be generated by a staggered double-stranded DNA break mechanism that is independent of the V(D)J recombination. Similar duplications have also been reported in the *BCL2* and *CCND1* genes in the *IGH-BCL2* and *CCND1-IGH* translocations.[9-11,20]

Therefore, in the absence of any convincing recombination signals in the *MALT1* locus, the definitive mechanisms of DNA recognition and cleavage leading to breaks on chromosome 18 remain to be elucidated.

In FL, most breaks on chromosome 18 cluster in a region of *BCL2* known as MBR without a functional RSS sequence.[13,17,18,21] Recent reports have shown that the RAGs recognize a specific "non-B DNA" conformation of the *BCL2*-MBR even in the absence of an RSS sequence.[11,21,22] Therefore, the RAGs are supposed to be responsible for both cleavage at the *IGH* and the *BCL2* loci by recognition of 2 different motifs, a sequence-dependent RSS signal at *IGH* and a non-B DNA structure at

BCL2.[22] It can be speculated if similar mechanisms play a role in the generation of breaks in *MALT1* in the t(14;18)/*IGH-MALT1*.

Another novel finding of our study is the presence of T-nucleotides at the *IGH-MALT1* junctions, which we have detected in all of our 5 analyzed cases. T-nucleotides have so far been reported in the translocations t(14;18)/*IGH-BCL2* and t(11;14)/*CCND1-IGH* and consist of insertions of short copies of the surrounding J_H, D_H, and *BCL2*, or *CCDN1* sequences at the breakpoints.[9,10]

The ascertainment of T-nucleotides has provided new insights into the mechanisms of *IGH*-associated translocations, in which processes other than illegitimate V(D)J-mediated recombination might be involved.[23] For instance, T-nucleotides exhibit such a high homology to their template sequences that their synthesis by the template-independent terminal deoxynucleotidyl transferase of the V(D)J machinery is unlikely. Accordingly, T-nucleotides have not been described in normal V(D)J recombination products and RSS-guided V(D)J-mediated translocations.[20,23] Moreover, the template sequences do not always border on the breakpoints, and mismatches with the germline have been observed, indicating that T-nucleotides are generated by an error-prone and template-dependent DNA polymerase.

T-nucleotides are not exclusively present in chromosomal translocations, but they have also been identified at the junctions of end-joining events in *Drosophila*. However the molecular mechanism that create T-nucleotides remains to be determined.[24] On the basis of studies from flies and other organisms, McVey and Lee[25] speculate that T-nucleotides might be generated by the action of an error-prone polymerase in an attempt to create microhomologies when flanking microhomologous sequences are inadequate. Furthermore, the investigators suggest that a nonhomologous end joining (NHEJ)–independent mechanism named microhomology-mediated end joining might be responsible for repairing of these DNA double-strand breaks in human neoplasias.[25] In line with this hypothesis, all *IGH-MALT1* junctions but one analyzed in this study displayed nucleotides insertions. The only *IGH-MALT1* junction that did not show this feature contained a 6-bp imperfect microhomology sequence at the translocation breakpoint of *MALT1-IGH*.

The cell of origin of MALT lymphomas is still debated and probably belongs to more than one stage of B-cell differentiation.[26] Interestingly, 4 MALT lymphomas analyzed here showed a total of 5 somatic mutations in the rearranged *MALT1* and *IGH* genes; however, all mutations but one were found within the 20 bp of the *IGH-MALT1* breakpoint junctions. The only mutation located distal from the breakpoint, namely a C-to-T transition described in the case published by Sánchez-Izquierdo et al,[7] did not show the target motifs for a somatic hypermutation.[27] Therefore, the mutations detected in our study could result from error-prone repair by end-joining events rather than from somatic hypermutation.[28]

In conclusion, our observations indicate that the pathomechanism underlying the t(14;18)/*IGH-MALT1* in MALT lymphomas is probably based on an illegitimate V(D)J-mediated recombination at the *IGH* locus on chromosome 14 and a staggered double-stranded DNA break at the *MALT1* locus on chromosome 18. Furthermore, we could identify a putative MBR proximal to the 5′ noncoding region of *MALT1*. The molecular characteristics of the t(14;18)/*IGH-MALT1* resemble those found in the t(14;18)/*IGH-BCL2* in FL and t(11;14)/*CCND1-IGH* in MCL, suggesting that these translocations could be generated by a common pathomechanism involving new synthesis of T-nucleotides and NHEJ or alternative NHEJ repair pathways.

Authorship

Contribution: E.M.M.P. designed the research, performed FISH experiments and molecular genetic analyses, analyzed the data, and wrote the manuscript; E.C.-B., S.G., F.B., and G. Salles performed cytogenetic analysis and contributed patients' data; H.Y. and M.-Q.D. performed FISH experiments and contributed patients' data; G. Schilling and N.A.-K. performed FISH experiments and molecular genetic analyses; E.V. performed statistical analyses; I.W. contributed patients' data; C.B. supervised research and wrote the manuscript; and J.D. designed and supervised the research, and wrote the manuscript. All authors have read and reviewed the manuscript.

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