Rare occurrence of *IgVH* gene translocations and restricted IgVH gene repertoire in ocular MALT-type lymphoma

FISH studies on 37 ocular MALT-type lymphomas yielded chromosomal translocations affecting *MALT1* and *BCL10* in 1 case each, no evidence for a break in the *FOXP1* locus, and trisomy 3 in 14 out of 34 cases (41%). Three out of 8 cases analyzed used the highly mutated VH3-23 gene and showed ongoing somatic hypermutations.

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Extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue type (eMZBCL) arise, amongst other organs, in the ocular adnexa.[1] Interestingly, frequency and distribution of genetic alterations, use and specifity of immunoglobulin heavy chain variable region (IgVH) genes, and the association with chronic inflammatory processes vary remarkably between affected organs and geographic regions.[2] In ocular adnexal eMZBCL, some data suggest an association with chronic *Chlamydia psittaci* infection.[3]

We studied 37 biopsies of ocular adnexal eMZBCL diagnosed between 1999 and 2004 at the Reference Centre for Lymph Node Pathology in Würzburg, Germany. Cases were classified according to WHO criteria.[1] For fluorescence *in situ* hybridization (FISH) studies, representative tissue cores from each case were assembled into a tissue microarray (TMA). FISH analyses for the detection of chromosomal breaks in *BCL10* (from DakoCytomation, Germany), IgH and MALT1 loci and for trisomy 3 (all Abbott, Germany) as well as for *FOXP1*[4] were performed. PCR was successfully used to amplify rearranged *IgVH* genes in 8 cases. After subcloning, the mutational status of *IgVH* gene was determined using a 2% somatic mutation cut-off and compared with the corresponding germline sequences. The study was approved by the local ethics committee.

Results are summarized in Table 1. FISH studies provided evidence of chromosomal breaks affecting the *IGH* locus in 3 cases only. In 1 case each, *MALT1* and *BCL10* were the putative translocation partners. In the third case, no candidate translocation partner was indicated by the FISH results. However, in this case, the *BCL10*

Table 1. Results of the FISH-based molecular genetic and immunohistochemical analyses of the 37 cases of ocular MALT type extranodal marginal zone B-cell lymphoma.

	FOXP1 BAP	*FOXP1 numeric*	*FOXP1 immuno strong nuclear (%)*	*MALT1 BAP*	*IgH BAP*	*Centromere 3*	*BCL10 BAP*	*VH family*	*Germ strain homology*
1	–	X2	5	-;X2	–	X2 in 100%	-;X2		
2	–	X3 in 40%	30	-; X3 in 40%	–	X3 in 40%	-;X2		
3	–	X2	80	-; X2	–	X3 in 90%	-;X2		
4	–	X2	30	-; X2	–	some X3	-;X2		
5	–		60	-;X2	–	X3,4 in 30	-;X2		
6	–		NA	NA	–	NA	NA		
7	–	X2	20	-; X2	–	10% X3	-;X2		
8	–	X3 in 10%	40	-; 3 in 10%	–	X3 in 60%	-;X2		
9	–	X3 in 70%	60	–	–	X3 in 50%	-;X2		
10	–	X2	10	-;X2	–	X2 in 100%	-;X2		
11	–	X2	10	-: X 3 in 20%	-, someX3	X3in 40%	-;X2		
12	–	X2	80	–	–	X2 in 97%	-;X2		
13	–	X2	30	-;X3 in 30%	–	X2 in 100%	-;X2		
14	–	X3 in 10%	NA	-	–	X2 in 90%	NA		
15		X2	10	NA	–	NA	-;X2	VH3-23	92% (ongoing)
16	–	X2	5	-;X2	–	X2 in 95%	NA	VH3-23	94% (ongoing)
17	–	X3 in 10%	40	–	–	X2	-;X2		
18	–	X2	10	–	–	X3 in 10%	-;X2	VH4-39	94%
19	–	X3 in 10%	NA	–	–	X2 in 100%	-;X2		
20	–	X2	10	–	–	X3 in 10%	-;X2		
21	–	X3 in 70%	40	-; X3 in 50%	–	X3 in 20%	-;X2	VH3-23	88% (ongoing) (9bp insertion)
22	–	X2	NA	NA	–	X2	NA		
23	–	X2	60	–	–	X3	NA		
24	–	X3 in 60%	NA	–	–	NA	NA		
25	NA	NA	60	-;X3 in 10%	–	X2	-;X2		
26	–	X3 in 10%	10	-; X2	–	X2	-;X2	VH3-15	93%
27	–	X3 in 10%	80	-;X2	–	X2	-;X2	VH3-72	92%
28	–	X3 in 70%	50	-; X2	–	X3 in 30%	-;X2		
29	–	X2	40	-; X2	–	X3 in 70%	-;X2		
30	–	NA	NA	-;X3 in 10%	–	X3 in 15%	NA		
31	–	X2	5	-;X2	–	X2			
32	–	X3 in 40%	40	-; X3 in 10%	–	X3in 30%	-;X2		
33	–	X3 in 50%	30	+	+	X3 in 70%	-;X2		
34	–	X2	80	-; X3 in 10%	–	X2	-;X2		
35	–	X2	5	-; X2	+	X2	NA	VH7-81	92%
36	–	X2	5	-; X2	–	X2 in 100%	-;X2		
37	–	X3 in 30%	30	-; X2	+	X3in 60%	+	VH3-30	93%

BAP: FISH-based break-apart assay. X2: disomy. X3: trisomy. IgH BAP: FISH-based break-apart assay of immunoglobulin heavy chain gene locus.

locus analysis failed for technical reasons, and potential *BCL10* rearrangement could not, therefore, be excluded. Trisomy 3 was detectable in 14 out of 34 cases (41%). None of the cases showed evidence of a breakpoint in the *FOXP1* locus.

Sequencing results revealed somatically mutated *IgVH* genes in all 8 cases with a mutation frequency ranging between 88-94% (Table 1). The VH3 family was used in 6 cases. Remarkably, *VH3-23* was used in 3 cases and all of these showed evidence of intraclonal heterogeneity (ongoing mutations), whereas the remaining 5 cases carried a high load of somatic mutations without detectable intraclonal heterogeneity. Neither of the 2 cases with a detectable IgVH translocation (cases 35 and 37) showed ongoing mutations.

These results shed further light on the varying genetic and immunological features in ocular MALT-type lymphomas. In contrast to Streubel *et al*.[2] who reported the presence of the t(14;18) involving *MALT1* in 24% of ocular MALT-type lymphomas, we could only detect a single case (1 out of 34 cases, 3%) carrying this genetic alteration, whereas the frequency of trisomy 3 in our series was almost identical to the published data.[2] In addition, the revelation of a preferential usage of *VH3-23* among the VH3 family members is a unique finding in our series and has not been previously reported.[5,6] Although the antigen specificity of the VH3-23 segment is not known in ocular adnexal MALT-type lymphomas, *VH3-23* is frequently used by autoreactive B-cells in rheumatoid arthritis[7] and Wegener's granulomatosis.[8] The fact that all 3 *VH3-23* gene positive lymphomas in our series showed evidence of ongoing somatic hypermutations, in contrast to the remaining cases, may indicate a continuous stimulation of the neoplastic B-cells by an as yet unknown (auto)antigen leading to a sustained affinity maturation process during clonal expansion. In contrast, the somatically mutated eMZBCL without intraclonal heterogeneity may have acquired self-sufficient growth properties, e.g. by their underlying genetic alterations, and may not depend on continuous stimulation by (auto)antigen. Interestingly, *VH3-23* was also among the most frequently used Ig genes in two other lymphoma subsets. In particular, thymic eMZBCLs which are common among the Asian population and have a strong association with Sjogren's syndrome, show overusage of this VH gene. However, in contrast to ocular adnexal MALT-type lymphomas, two of three thymic eMZBCLs carried an unmutated or only minimally mutated VH3-23 gene and no intraclonal heterogeneity was observed.[9] It is, therefore, likely that the pathogenetically relevant (auto)antigens may differ between ocular adnexal and thymic MALT-type lymphomas. Primary intraocular lymphoma (PIOL), an aggressive primary CNS lymphoma has also recently been reported to use the *VH3-23* gene in a subset of cases.[10] The primary intraocular lymphoma cases using *VH3-23* were also characterized by a high load of somatic mutations, but no information was provided about the presence of ongoing mutations.[10]

To summarize, translocations involving the *MALT1* gene were a rare event in ocular eMZBCL in our study and 3 out of 8 cases used the highly mutated *VH3-23* gene with evidence of ongoing somatic hypermutations. This implies a specific, pathogenetically relevant (auto)antigen in a subset of cases.

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