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Source / Izvornik: **Pathology International, 2007, 57, 47 - 51**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/doi: 10.1111/j.1440-1827.2007.02056.x

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:125763

MALT1, BCL10 and FOXP1 in salivary gland MALT lymphomas

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Running Title: Salivary gland MALT lymphomas

Abstract

In view of the certain anatomic site dependent frequency of chromosomal translocations involved in extranodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) pathogenesis, 17 salivary gland MALT lymphoma cases were analyzed for *MALT1* and *FOXP1* translocations.

BCL10 and FOXP1 protein expression were studied by immunohistochemistry and translocations identified using fluorescence *in situ* hybridization (FISH)-specific probes *FOXP1*, t(11;18)(q21;q21)/*API2-MALT1* and t(14;18)(q32;q21)/*IgH-MALT1*.

None of the 11 analyzed cases showed *FOXP1* rearrangement or amplification. The t(11;18) was present in five of 13 cases and the t(14;18) in three of 13 cases. *MALT1* translocations were mostly mutually exclusive except in a single case. FOXP1 protein expression showed differences in the proportion of tumor cells with nuclear expression but not in their intensity, with the exception of one case where very intense nuclear staining was noted. BCL10 nuclear expression was present in four of 17 cases, two of which lacked the t(11;18).

Our results suggest that *MALT1* specific translocations and *FOXP1* rearrangements are not commonly involved in pathogenesis. A case with strong FOXP1 protein expression indicates the possibility that the up-regulation of FOXP1 expression is significant in a small subset of salivary gland MALT lymphomas. Also a single case where both *MALT1* translocations were present indicates that these are not always mutually exclusive.

Key words

BCL10, FOXP1, MALT1, MALT lymphomas

Introduction

Extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma) is recognized as a distinct clinicopathological entity in the World Health Organization (WHO) classification of malignant lymphomas and constitutes 8% of all Non Hodgkin lymphomas (NHL).[1] The disease is generally indolent and localized but can transform into a more aggressive diffuse large B cell lymphoma (DLBCL). MALT lymphomas most often occur in the stomach (70%) or less commonly in other extranodal sites such as the salivary glands, lungs, ocular adnexa and thyroid among others.[2-4]

Primary NHL constitute 2-5% of salivary glands malignant lesions and represent an uncommon tumor.[5] Morphological characteristics are the basis of classification of lymphoproliferative lesions in the salivary glands.[5-7] Immunophenotypic analysis of aberrant phenotype and flow cytometric or molecular genetic analysis of immunoglobulin light chain restriction are useful methods to support a diagnosis of malignant lymphoma.[5,8,9] MALT lymphomas occur most often in organs where organized lymphatic tissue is normally absent and usually arise from MALT that has been acquired as a result of a chronic inflammatory disorder.[2,3] In the salivary gland, MALT lymphomas are usually preceded by lymphoepithelial sialadenitis, often associated with Sjögren's syndrome.[5]

Three well characterized chromosomal translocations t(11;18)(q21;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21) are implicated in the pathogenesis of MALT lymphoma**.** Also, the specific translocations occur at variable frequencies in MALT lymphomas from different anatomical sites, t(11;18)(q21;q21) and t(1;14)(p22;q32) more often in gastrointestinal and pulmonary lesions and t(14;18)(q32;q21) in salivary glands, skin, ocular adnexa and liver lesions.[10-13]

The t(11;18)(q21;q21) translocation is most common, occurring in 15-40% of cases, and results in a fusion of the *API2* and *MALT1* genes. The t(1;14)(p22;q32) translocation, detected in 1-2% of cases, results in overexpression of the *BCL10* gene. Moreover, this translocation alters the subcellular localization of BCL10 and cases with either the t(1;14) or the t(11;18) show strong nuclear BCL10 expression, rather than the cytoplasmic expression seen in normal germinal centre B cells.[14] The t(14;18)(q32;q21) translocation, described in 20% of cases, juxtaposes the *MALT1* gene and the *IgH* locus and thus deregulates its expression.[15]

FOXP1 is located within a human tumor suppressor locus at 3p14.1 and its expression is deregulated in a number of tumours.[16] The recently described t(3;14)(p14;q32) identified the *FOXP1 forkhead* transcription factor as recurrent translocation partner involving the enhancer region of the *IgH* locus to deregulate gene expression in MALT lymphoma.[17] This translocation was detected in MALT lymphomas arising at certain anatomic sites: skin, thyroid and ocular adnexa.[17] In addition, Wlodarska and colleagues also described the t(3;14)(p14;q32) in gastric MALT lymphoma and initial data suggest that the *FOXP1* translocation occurs mutually exclusively with the three specific MALT lymphoma associated translocations.[17,18]

The pathogenetic relevance of t(3;14)(p14;q32), trisomy of chromosome 3 and FOXP1 protein expression is still unclear but recent data indicate FOXP1 expression predicts poor prognosis and transformation to DLBCL in MALT lymphomas.[18,19] In view of the certain anatomic site dependent frequency of chromosomal translocations involved in MALT lymphoma pathogenesis we have analyzed the frequency of *FOXP1* gene rearrangement and its relationship with patterns of FOXP1 protein expression in salivary gland MALT lymphoma. In parallel we have also examined cases for the most common MALT1

translocations t(11;18)(q21;q21) and t(14;18)(q32;q21), and for BCL10 protein expression in an attempt to elucidate the molecular mechanisms involved in salivary gland MALT lymphoma pathogenesis.

Methods and materials

Surgically resected parotid or submandibular salivary glands, diagnosed with MALT lymphoma (n=17), presenting at Merkur University Hospital between 1992-2004 were used with ethical approval and patient consent. Cases were processed routinely with H&E, Giemsa and analyzed according to the WHO classification diagnostic criteria by a qualified pathologist (MD).[1] All cases were immunohistochemicaly CD20 positive and CD3, CD5 and CD10 negative with the exception of five cases which were CD5 cytoplasmic positive (data not shown).[4] The B cell monoclonality in all cases was confirmed by polymerase chain reaction according to the BIOMED 2 protocol (data not shown).[20] Deparaffinized tissue sections (4 μm thick) were immunostained for BCL10 (Zymed Laboratories Corporation, San Francisco, CA, USA) and FOXP1 (JC12 monoclonal antibody provided by AHB, Nuffield Department of Clinical Laboratory Sciences, University of Oxford, Oxford, UK).[16] Heat induced antigen retrieval by pressure cooking treatment (Pascal, Dako, Glostrup, Denmark) was performed, for BCL10 in 0.01M sodium citrate buffer pH6, at 125ºC, 30 seconds and for JC12 antibody in 1mM EDTA solution pH8, at 125ºC, 30 seconds. The universal labeled streptavidin biotin kit LSAB (Dako) was used to visualize BCL10 expression and the HRP+ (Dako) for JC12 labeling. Sections were stained in an Autostainer (Dako) and counterstained with hematoxylin.

Tissues were also analyzed using dual fusion fluorescence *in situ* hybridization (FISH) assays: t(14;18)(q32;q21)/*IgH-MALT1*, t(11;18)(q21;q21)/*API2-MALT1* (Abott Diagnostics, Abbott Park, IL, USA) and a *FOXP1* probe as previously described.[21,22] The gastric MALT lymphoma cases with t(14;18) and t(11;18) translocations and primary nodal DLBCL cases with *FOXP1* rearrangements were used as positive controls in our laboratory. In some cases FISH analysis or immunostaining were not performed due to poor tissue processing or insufficient quantities of material.

Results

Results of the FISH analysis of chromosomal translocations t(11;18)(q21;q21)/*API2-MALT1*, t(14;18)(q32;q21)/*IgH-MALT1*, *FOXP1* rearrangements and immunostaining for BCL10 and FOXP1 protein expression in salivary gland MALT lymphomas are described in Table 1.

The BCL10 immunolabeling demonstrated cytoplasmic BCL10 protein expression in all 17 cases and nuclear positivity within the tumor cells was also present in four of these cases. Immunohistochemical staining with JC12 in 11 of 17 cases showed that two cases lacked FOXP1 protein expression in the tumor cells and five cases showed nuclear positivity in 10-30% of the tumor cells (score 1). In the remaining four cases FOXP1 protein was observed in nuclei in more than 30% of tumor cells (score 2). The intensity of JC12 staining was generally weak; however, one case (no.12) did exhibit very intense nuclear FOXP1 protein expression.

Eleven cases were suitable for analysis by FISH using the *FOXP1* specific breakapart probe and all were negative for *FOXP1* rearrangements. None of the cases showed *FOXP1* amplifications or trisomy 3. Primary nodal DLBCL cases from Merkur University Hospital

have been found to contain *FOXP1* translocations, confirming the ability of this probe to detect these rearrangements in our laboratory. Analysis for the *MALT1* translocations was successful in 13 cases. The *API2-MALT1* rearrangement, t(11;18)(q21;q21), was identified in five of 13 (38.5%) cases, and the t(14;18)(q32;q21)/*IgH-MALT1* in three of 13 (23.1%) cases. The results of compared FISH analysis and immunostaining for BCL10 and FOXP1 protein expression (Figure 1) showed the two of four cases with nuclear BCL10 harbored the t(11;18)(q21;q21) and the remaining two BCL10 nuclear cases lacked the *API2-MALT1* rearrangement. The t(11;18)(q21;q21) and t(14;18)(q32;q21) translocations or BCL10 nuclear staining were absent in three of the four cases with nuclear FOXP1 expression in more than 30% of tumor cells. This was consistent with the finding that four of the five cases with the *MALT1* translocations or BCL10 nuclear expression had FOXP1 nuclear expression in 10-30% of tumor cells.

Discussion

Although, Streubel and colleagues suggested clustering of t(3;14)(p14;q32) at certain anatomical sites and reported that MALT lymphomas arising in the stomach, salivary gland and lung were negative. Wlodarska and colleagues have shown a gastric MALT lymphoma case with this translocation.[17,18] Also Streubel and colleagues selected primary MALT lymphoma cases without known translocations to look for FOXP1 translocation and studied fewer cases with the known translocations to determine their mutual exclusivity.[17]

The FISH analysis for translocations involving *MALT1* show a lower frequency of *IgH-MALT1* rearrangements (23.1% of cases) compared to the more common *API2-MALT1* fusion (38.5% of cases) in analyzed MALT lymphomas presenting in salivary glands.

According to the literature, the t(14;18)(q32;q21) is found in 20% of salivary gland MALT lymphomas and the t(11;18)(q21;q21) in approximately 40% of gastric and lung MALT lymphomas, but this frequency is lower in salivary gland and orbital MALT lymphomas.[11-13,23-25] Although the t(11;18)(q21;q21) is more commonly present in gastric and pulmonary MALT lymphomas, the frequency of 1-6% in salivary gland MALT lymphomas is described.[11,12] The high frequency of *API2-MALT1* fusion observed in the present study of salivary gland MALT lymphomas could be due to the small number of cases.

According to the literature four recurrent translocations t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p14;q32) occur mutually exclusively in MALT lymphomas.[13,17,26] The two analyzed *MALT1* translocations in the present study were mutually exclusive except in a single case (no. 16) where both translocations were present. To the best of our knowledge this is the first report showing the presence of both translocations in the same case. Clinically this women (57 years old) presented with unilateral enlargement of the parotid gland. Pathohistological examination of surgically resected parotid gland resulted in a diagnosis of MALT lymphoma without peripheral blood and bone marrow infiltration. Also chronic *Helicobacter pylori* positive gastritis and autoimmune leucopenia were diagnosed. After surgical resection the patient is still well without any disease symptoms in the follow up period of 36 months.

Compering the clinical course of patient no.16 with both *MALT1* translocations and three of six patients (no. 4,11,17) with t(11;18) or t(14;18) and available clinical data no differences in clinical course were observed. Also no differences in clinical course were observed in four of eight patients (no. 4,11,15,17) with *MALT1* translocations and/or BCL10 nuclear expression and the one patient (no. 13) lacking *MALT1* translocations and BCL10 nuclear expression. In

the follow up period between 12-50 months the patients presented with local disease and any additional therapy after surgical resection was applied.

BCL10 is strongly expressed in the nuclei of MALT lymphoma cells with t(1;14)(p22;q32) and t(11;18)(q21;q21), and in the cytoplasm of normal germinal centre B cells and in MALT lymphoma cells with t(14;18)(q32;q21).[3] In line with observations that aberrant nuclear expression may play a role in the development of MALT lymphoma, in two cases with evident BCL10 nuclear expression and absent *API2-MALT1* rearrangement the presence of t(1;14)(p22;q32) could be inferred.[3,5,27] However, there is currently no commercial FISH probe available to enable us to confirm this.

Our results demonstrate the absence of *FOXP1* rearrangements in salivary gland MALT lymphomas. The absence of this translocation in cases harboring *MALT1* translocations confirms previous findings.[17] In cases that lacked FOXP1 expression in more than 30% of the tumor cells we commonly observed *MALT1* translocations and/or BCL10 nuclear expression. This may suggest that in cases with activation of the BCL10/MALT1 pathway FOXP1 has a limited role in disease development. Through analysis of FOXP1 protein expression in MALT lymphoma, we observed differences in the proportion of tumor cells with nuclear expression but not in the intensity of nuclear positivity with the exception of a single case with strong nuclear FOXP1 staining. This case did not exhibit a rearrangement of the *FOXP1* gene or display trisomy 3 / amplification of the *FOXP1* locus. Thus alternative mechanisms are implicated in up regulating FOXP1 expression in a subset of MALT lymphomas. Because only two of four cases showing higher proportion of tumor cells with FOXP1 protein expression were able to be examined with *FOXP1* FISH probe, the relationship between *FOXP1* rearrangements and protein expression in these cases is unclear.

Salivary gland MALT lymphomas lack *FOXP1* rearrangements suggesting that these are not commonly involved in the pathogenesis of MALT lymphomas at this anatomical site that lack *MALT1* specific translocations. The finding of a case with strong intensity of FOXP1 protein expression indicates the possibility that up regulation of FOXP1 expression by alternative mechanisms may yet play a role in a small subset of salivary gland MALT lymphomas. Also a single case where both *MALT1* translocations were present indicates that these are not always mutually exclusively presented in MALT lymphomas.

Acknowledgments

This work was funded by the Leukaemia Research Fund (AHB + RV).

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Table 1 FISH analysis and immunostaining in salivary gland MALT lymphomas

	FISH *analysis*			*Immunostaining*	
Patient	t(11;18)(q21;q21) / *API2-MALT1*	t(14;18)(q32;q21) / *IgH-MALT1*	*FOXP1*	BCL10	JC12
1	-	+	-	c +	0
2	ND	ND	ND	c +	2 +
3	ND	ND	ND	c +	2 +
4	+	-	-	c + n +	1 +
5	-	+	-	c +	ND
6	-	-	ND	c +	ND
7	-	-	-	c +	2 +
8	ND	-	ND	c +	ND
9	ND	ND	ND	c +	0
10	-	-	ND	c + n +	ND
11	+	-	-	c +	1 +
12	+	-	-	c + n +	2 ++
13	-	-	-	c +	1 +
14	-	-	-	c +	ND
15	-	-	-	c + n +	1 +
16	+	+	-	c +	1 +
17	+	ND	-	c +	ND

API2, BIRC 3 baculoviral IAP repeat-containing 3; BCL10, B cell CLL/lymphoma 10; FOXP1, forkhead box P1; MALT, mucosa-associated lymphoid tissue; ND, not done due to poor tissue processing or insufficient quantities of material;

+, rearrangement present; -, rearrangement absent,

c +, cytoplasmic positive; n +, nuclear positive;

0, negative JC12 staining; 1, nuclear JC12 staining in 10-30% of tumor cells; 2, nuclear JC12 staining in >30% of tumor cells; +, weak intensity of nuclear FOXP1 protein expression; ++, strong intensity of nuclear FOXP1 protein expression.

Figure legends

Figure 1

(a) FISH, salivary gland MALT lymphoma, case 11, probe for detection t(11;18)(q21;q21)/*API2- MALT1*. Fused signals represent translocations, red signal, the *MALT1* gene and green signal, the *API2* gene.

(b) MALT lymphoma of the salivary gland, case 12, nuclear expression of BCL 10.

(c) MALT lymphoma of the salivary gland, case 13, weak intensity of nuclear FOXP1 protein expression.

(d) MALT lymphoma of the salivary gland, case 12, strong intensity of nuclear FOXP1 protein expression.