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Clinical, histopathological, and molecular features of mucosa-associated lymphoid tissue (MALT) lymphoma carrying the t(X;14)(p11;q32)/GPR34-immunoglobulin heavy chain gene

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Running title: MALT lymphoma with t(X;14)/(GPR32)-IGH

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Recurrent chromosomal translocations have been identified in MALT lymphoma, including t(11;18)(q21;q21), t(14;18)(q32;q21), t(1;14)(p22;q32), and t(3;14)(p14;q32).[1] The former three translocations lead to the generation of the BIRC3-MALT1 chimeric protein or deregulated expression of MALT1 or BCL10 under the control of the juxtaposed immunoglobulin heavy chain gene (IGH), and finally activate the nuclear-factor κB pathway.[1] t(X;14)(p11;q32) is the fifth translocation in MALT lymphoma involving the parotid gland or lung.[2, 3] The translocation results in the juxtaposition of the G protein-coupled receptor (GPCR) 34 (GPR34) gene at Xp11.4 to the IGH gene at 14q32, and t(X;14)-MALT lymphoma was found to have the highest GPR34 mRNA levels among B-cell lymphoma subtypes.[2] However, it remains unclear whether t(X;14) characterizes a subgroup of MALT lymphoma that shows a particular cytomorphology and clinical behavior. We herein describe two cases of MALT lymphoma that carried the t(X;14).

The first case (case 1) was a 66-year-old female who presented with tumors within the left parotid gland. She had been diagnosed with Sjögren syndrome and immune thrombocytopenia 6 years earlier. Sjögren antibody SS-A/Ro index was 105 (reference value, <25.0), SS-B/La was 32 (<25.0), anti-nuclear antibody was ×160 (<×40.0), and rheumatoid factor (RF) was 16.9 IU/mL. Although no residual tumor was detected after superficial lobectomy of the parotid gland, she relapsed 6.5 years after the initial presentation; the disease involved the left palatine tonsil and ileocecum with regional lymph nodes (Supplementary Figure S1A). She received 6 cycles of chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP),
leading to a complete response. The second cases (case 2) was a 66-year-old male who presented with symmetric enlargement of the bilateral parotid glands (Supplementary Figure S1B). He had been treated for rheumatoid arthritis with prednisolone and methotrexate followed by intravenous tocilizumab. His lactate dehydrogenase (LDH) level was 265 IU/L, soluble interleukin-2 receptor (IL-2R) was 2,037 U/mL, RF was 519.9 IU/mL, and matrix metalloproteinase-3 was 218.7 ng/dL (reference range, 36.9 to 121). He was treated with 6 cycles of R-CHOP, excluding prednisolone because of seropositivity for the hepatitis B virus surface antigen, and achieved a complete response. Informed consent was obtained from the patients. This study was approved by the Institutional Review Board.

Examination of histopathological specimens obtained from the parotid gland disclosed salivary gland tissues diffusely infiltrated by CD20+ lymphoma cells, and groups of these cells infiltrated into the glandular epithelium to generate the lymphoepithelial appearance (Supplementary Figure S2). The cells were medium to large in size with a moderate amount of cytoplasm, and CD79a+, BCL2+, CD5−, CD10−, and cyclin D1−. The Ki-67 proliferation index was approximately 50%. The cell surface immunoglobulins were µ-heavy and κ-light chains, and the δ-heavy chain was expressed at low levels (Supplementary Figures S3 and S4).

G-banding of metaphase spreads obtained from the specimens revealed the t(X;14)(p11;q32) in both cases, and fluorescence in situ hybridization using the IGH break-apart probe confirmed the translocation. Karyotypes according to the ISCN (2013) were 47,X,t(X;14)(p11;q32),+18,add(22)(p11)[9]/46,XX[1] in case 1
MALT lymphoma with t(X;14)(p11;q32)  

and 47,Y,t(X;14)(p11;q32),+21[8]/46,XY[2] in case 2 (Supplementary Figure S5).

To clone the t(X;14)(p11;q32) junction, we designed primers for the Cα constant gene of *IGH* and for the upstream sequence of the *GPR82* gene, which is another GPCR gene and is localized 27-kb downstream of *GPR34* (Figure 1A). Genomic DNA from the biopsies was subjected to long-distance polymerase chain reaction (LD-PCR) under the conditions for a long DNA target, resulting in the amplification of 3.6 and 6.0-kb of DNA, respectively (Figure 1B). We then cloned the LD-PCR products into the plasmid and performed Sanger sequencing (Figure 1C). Breakpoints were located at intron 1 (case 1) and the coding region of *GPR82* (case 2), and those at the *IGH* gene were within the switch region associated with *IGH Cα2*. As a result of translocation, *GPR34* and *IGH Cα2* were aligned in the same transcriptional orientation (Figure 1A).

We next performed reverse-transcriptase (RT-) PCR to investigate the mRNA levels of *GPR34* and *GPR82*, the expression of which delineates a unique molecular subset of marginal zone lymphoma.[2] As shown in Figure 1D, the level of *GPR34* mRNA in case 1 was significantly higher than those in various hematological tumor cell lines. On the other hand, the expression of *GPR82* mRNA was detected in case 1, while similar levels of RT-PCR products were generated in cell lines from B-cell precursor leukemia, follicular lymphoma, and Burkitt lymphoma. Since t(X;14) disrupted the genomic structure of *GPR82* and the primers were designed for exons 1 and 3 (Figure 1A), *GPR82* mRNA expression in case 1 theoretically represented the transcriptional activity of the non-translocated homologue at normal chromosome X.

We finally isolated the *VH-DH-JH* sequences in the current two cases in
addition to the Mayo clinic case by the inverse PCR strategy to characterize the
IGH repertoire in t(X;14)-MALT lymphoma (Supplementary Table SI). In the 3
cases, DNA fragments including the entire length of the \( V_{\text{H}}D_{\text{H}}J_{\text{H}} \) segments were
successfully amplified, and nucleotide sequencing revealed a single \( V_{\text{H}}D_{\text{H}}J_{\text{H}} \)
rearrangement in frame without stop codons. The closest germline \( V_{\text{H}}, D_{\text{H}}, \) and
\( J_{\text{H}} \) genes were identified in each case and the sequence identities of the \( V_{\text{H}} \)
genes were 95.1\%, 86.5\%, and 96.5\%, respectively, providing evidence for a
somatic hypermutation (Table I). We then calculated the excess of replacement
(R) mutations over silent (S) mutations in the complementarity determining
regions (CDRs) and the scarcity of R mutations in the framework regions
(FWRs). As shown in Table I, we found no preferential clustering of R mutations
indicative of positive selection in CDRs, while a decrease in R mutations in
FWRs was found in all cases and negative selection in the Mayo clinic case was
significant with a \( P \) value of <0.05.[4, 5] The CDR3 sequences of the 3 cases
were characterized by the GC-rich alignment, particularly in the N sequences,
and the CDR3 length was 17 or 16 at the amino acid level (Supplementary Table
SII). A database search revealed a similarity of the CDR3 sequence of cases 1
with that of an IgM RF derived from healthy immunized donors (Supplementary
Table SIII).

In line with the general concept of the development of MALT lymphoma, both
patients had chronic autoimmune disorders and showed serological evidence of
each underlying disease at presentation. By comparison with the typical MALT
lymphoma cytomorphology, lymphoma cells in the current two cases were larger
and had rounder nuclear contours, more vesicular chromatin, and more
conspicuous nucleoli, representing the features of diffuse large B-cell lymphoma (DLBCL). Nevertheless, marginal zone infiltrates of reactive follicles and lymphoepithelial lesions were in agreement with the histopathological criteria of MALT lymphoma. On the other hand, the palatine tonsil and ileocecum involved in case 1 at relapse are unusual sites of involvement of MALT lymphoma, but preferentially affected by DLBCL. In case 2, the marked enlargement of both parotid glands and elevated LDH and sIL-2R levels initially indicated aggressive lymphoma. Therefore, the current two cases appear to be characterized by intermediate features in cytomorphology and clinical behavior between low-grade MALT lymphoma and DLBCL.

**GPR34** is the first GPCR gene that is affected by chromosomal translocation in B-cell tumors. Since potent enhancer complexes are localized downstream of the two **IGH Cα** genes,[6] and since t(X;14) does not interrupt the coding region of **GPR34**, t(X;14) theoretically promotes the expression of GPR34, and we confirmed the strong expression of **GPR34** mRNA in case 1. GPCRs, including class A GPR34, are integral membrane proteins containing 7 putative transmembrane domains and mediate signals to the interior of the cell via the activation of heterotrimeric G proteins. A large number of GPCRs are overexpressed in various cancer types, and contribute to tumor cell growth when activated by circulating or locally produced ligands.[7] Lysophosphatidylserine, which is an endogenous lipid mediator generated by the hydrolysis of the membrane phospholipid phosphatidylserine, has been proposed to be the ligand of GPR34.[8] Since the current two cases developed in the setting of chronic autoimmune disorders, the potential link between the underlying inflammatory
disease and occurrence of lymphoma may be attributed to ligands being steadily generated in salivary gland tissues affected by chronic inflammation, which, in turn, stimulate GPR34-overexpressing cells resulting from t(X;14), thereby leading to the development of florid lymphoma.

We showed that $V_H$ genes were variable among the 3 cases. Of the 3 $V_H$ genes, $V_{H1-2}$ has been reported to be preferentially used in splenic marginal zone lymphoma,[9] while its usage is infrequent in MALT lymphoma that develops in the stomach, lung, and parotid gland.[10-12] In contrast, $V_{H1-69}$ is involved in not only cases of B-cell chronic lymphocytic leukemia, but also salivary gland MALT lymphoma at a frequency of 8 of 14 (57%).[13, 14] $V_{H3-7}$ appears to be preferentially used in gastric MALT lymphoma.[10-12] On the other hand, a statistical analysis of the distribution of R and S mutations within CDRs or FWRs provided no significant evidence for antigen selection, whereas negative selection for R mutations in FWRs in order to maintain the configuration of the IGH molecule appeared to have been operating. Taken together with the long CDR3 length, t(X;14)-MALT lymphoma may arise from B cells that recognize self-antigens.[15]

**Acknowledgments**

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**References**


Figure legends

Figure 1. Molecular cloning of the t(X;14)(p11;q32)/GPR34-IGH junction. (A) Schematic presentation of the anatomy of t(X;14)(p11;q32). The coding exons of GPR34 and GPR82 are intron-less (rectangles filled with black). Breakpoints in case 1 and case 2 (open triangle) as well as that in the Mayo clinic case (closed triangle) [2] are distributed within the GPR82 gene. (B) Ethidium bromide-stained gel electrophoresis of LD-PCR encompassing the t(X14)(p11;q32) junction. The positions of the primers for LD-PCR are indicated in A. (C) Nucleotide sequences of the t(X;14)(p11;q32) junction in case 1 and case 2. Vertical lines indicate nucleotide identity. (D) RT-PCR for the expression of GPR34 and GPR82 mRNA. The hematological tumor cell lines used were: ME-1, acute myeloid leukemia; CCRF-CEM, T-cell acute lymphoblastic leukemia; NALM-20 and NALM-6, B-cell precursor leukemia; SMZ-1, peripheral T-cell lymphoma; Karpas299, anaplastic large cell lymphoma; KM-H2 and L428, Hodgkin lymphoma; FL-318 and FL-218, follicular lymphoma; and Ramos and Raji, Burkitt lymphoma. RT-PCR for GAPDH is shown in the bottom to confirm that similar amounts of mRNA were loaded. The sequences of the primers for LD- and RT-PCR are described in Supplementary Table SI.
IGHA-01 primer

Cα2

Sα

Xp11-05 primer

GPR34

27 kb

IGHA-01 primer

14q32

Sα

Case 1
der(14)t(X;14)

Xp11.4

GPR34

Case 2

GPR82

Case 1

GPR34

Sα

Case 2

GPR82

Sα

Case 1

der(14)t(X;14)

GPR34

Sα

Case 2
<table>
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<td></td>
<td>Selection value ((\Sigma))</td>
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<tr>
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<td>(\text{CDR} ) R S (\text{FWR} ) R S</td>
<td>(\text{CDR} ) (\text{FWR} ) (\text{CDR} ) (\text{FWR} )</td>
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<td>0.17</td>
<td>0.06 0.57 0.21</td>
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</table>

Abbreviations: R, replacement; S, silent; CDR, complementarity determining region; FWR, framework region.

*\(\Sigma\) and \(P\) values were calculated using the Focused-Z test to detect selection.[4, 5]

**A negative sign indicates negative selection. \(P\) values < 0.05 were considered to be significant.