Ductal cells of minor salivary glands in Sjögren’s syndrome express LINE-1 ORF2p and APOBEC3B

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Background: Type I interferon activation is a hallmark event in Sjögren’s syndrome. L1 retroelements stimulate plasmacytoid dendritic cells, activating the type I interferons, and are regulated by various mechanisms, including the APOBEC3 deaminases. As L1s are potential trigger factors in autoimmunity, we aimed to investigate the immunohistochemical localization of L1 ORF2p and its inhibitor APOBEC3B protein in minor salivary glands of Sjögren’s syndrome patients.

Methods: Twenty minor salivary gland-tissue samples from 20 Sjögren’s syndrome patients, classified according to Tarpley’s histological criteria, and 10 controls were evaluated for L1 ORF2p and APOBEC3B expression via immunohistochemistry.

Results: L1 ORF2p was expressed in 17/20 SS patients and all controls. APOBEC3B expression was observed in 15/20 Sjögren’s syndrome patients, 5/5 chronic sialadenitis, and 3/5 normal minor salivary glands. Both antibodies stained the cytoplasm of the ductal epithelial cells. Negative staining was observed in the acinar cells. L1 ORF2p-positive immunostaining was significantly lower in Tarpley IV Sjögren’s syndrome patients than controls ($P = .039$), and APOBEC3B-positive staining was significantly lower in Tarpley I compared to Tarpley II Sjögren’s syndrome patients ($P = .008$) and controls ($P = .035$).

Conclusions: L1 ORF2p and APOBEC3B are expressed in the ductal epithelial cells of minor salivary glands that are among the key targets in Sjögren’s syndrome. L1 ORF2p expression may promote the L1 ability to act as an intrinsic antigen in Sjögren’s syndrome. The potential future use of L1 ORF2-reverse transcriptase inhibitors in autoimmunity supports further investigation of L1 epigenetic regulation by APOBEC3 enzymes.

Keywords
APOBEC3B, ductal salivary gland epithelial cells, L1 ORF2, long interspersed nuclear element-1, Sjögren’s syndrome

1 INTRODUCTION

Sjögren’s syndrome (SS) is a chronic autoimmune disease of unknown etiology and pathogenesis, where immunological, genetic, epigenetic, and environmental factors are thought to be involved. In SS patients, a hallmark event is activation of type I interferon (IFN), a pro-inflammatory cytokine usually stimulated by viral infections, and gene expression profile studies show overexpression of numerous IFN-inducible genes in minor salivary glands (MSGs) and peripheral blood, known as the "IFN signature." The predominant source of type I IFN are the plasmacytoid dendritic cells (pDCs), but salivary gland epithelial cells (SGECs) may, also, produce this cytokine. The exact trigger factor for type I IFN production is unknown and a model of activation of type I IFN signaling pathway through the
excessive expression of the viral-like long interspersed nuclear element-1 (LINE-1, L1) is proposed.

L1 elements are highly repetitive DNA sequences that constitute approximately 20% of the human genome. They belong to a gene superfamily termed "mobile" or "transposable elements" due to their capacity to "jump" into various genomic locations, promoting reshaping and diversity of the human genome. Human L1 contains 2 non-overlapping open reading frames, ORF1 and ORF2 that encode for L1 ORF1p and ORF2p proteins. L1 ORF2p has 3 domains supplying the enzymatic activities of endonuclease and reverse transcriptase and the RNA-binding property that are essential for L1 functional role, that is, the retrotransposition.

L1 ORF2p expression is shown in normal germ cells and somatic cells, non-neoplastic human tissues, and carcinomas. There is also an indirect evidence for L1 expression in normal salivary glands. L1 may be associated with the development of autoimmune diseases, including SS, as a potential inducer of type I IFN pathway, and recently elevated L1 mRNA levels in the MSGs of pSS patients compared to sicca controls, as well as L1 ORF1p expression in the MSG ductal epithelial cells, was reported.

L1 expression is regulated by various transcriptional and post-transcriptional mechanisms, including the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3) deaminases. APOBEC3 family consists of 7 proteins (APOBEC3A-H) that are IFN-inducible antiviral factors, acting in the innate immune system as a host-defensive mechanism against exogenous viruses and endogenous mobile elements.

The exact L1 inhibition mechanism of APOBEC3B remains elusive. APOBEC3 deaminases might affect various stages of the L1 retrotransposition pathway, either in cytoplasmic or nuclear localization. APOBEC3 enzymes might interact with L1 ribonucleoprotein particles (RNPs) in the cytoplasm and influence their intracellular transport and/or their nuclear input, while in the nucleus they could gain direct access to L1 RNA and hamper the synthesis of DNA by inhibiting the required nick generation in the first target DNA strand, thus preventing reverse transcription initiation. Stenglein and Harris demonstrated that the catalytically inactive APOBEC3B retained its L1 inhibition activity, while they did not detect via polymerase chain reaction any cDNA strand-specific C/G to T/A mutations within the retrotransposed L1s that had replicated under the impact of APOBEC3B.

The abundance of APOBEC3B in normal cells and tissues is suggestive of its role in innate immunity mechanisms. In particular, expression of APOBEC3B protein is observed in normal human salivary glands, that is, breast, prostate, gastric, and colon. Its role in salivary glands has not been studied. Due to the well-documented contribution of IFN pathway activation in autoimmunity, a link between the IFN-inducible gene APOBEC3B and autoimmune diseases also seems plausible. Crow and Wohlgemuth found that APOBEC3B gene was significantly upregulated among SLE patients. L1 retrotransposition events occur during the earliest stages of human embryonic development; thus, the APOBEC3B expression in early human embryonic tissues may be of major importance in preventing inheritable L1-induced genomic instability.

As L1 elements are among the potential trigger factors in autoimmune diseases, we hypothesized that L1 ORF2 protein and its inhibitor APOBEC3B protein are expressed in the MSGs of pSS patients and we aimed to investigate their presence via immunohistochemistry.

2 | MATERIALS AND METHODS

2.1 | Case selection

Formalin-fixed and paraffin-embedded tissues of MSGs were retrieved from the files of the Department of Oral Medicine and Pathology. The study group consisted of 20 patients with pSS (19 females and 1 male, age range 16-76 years, mean age 51.84 ± 16.49 years), diagnosed according to the revised American-European classification criteria (AECG), microscopically categorized according to standard classification criteria  as Tarpley I (n = 3), Tarpley II (n = 7), Tarpley III (n = 7), and Tarpley IV (n = 3). Biopsy focus score (lymphocytic foci/4-mm² of tissue) in all cases was ≥1.

The control group consisted of (i) MSG biopsies from 5 patients complaining for xerostomia who did not fulfill the AECG criteria, microscopically diagnosed as "Chronic Salaldenitis (CS) not consistent with SS" (sicca controls, females, age range 58-75 years, mean age 62.6 ± 6.99 years) and (ii) MSGs with none to mild chronic inflammatory infiltration (normal MGSs, NMGSs) from 5 patients, co-excised with extravasation cysts of the lower lip (non-sicca controls, 3 females and 2 males, age range 8-77 years; mean age 33.2 ± 27.42 years). Patients in both control groups were free of autoimmune diseases. Researchers were blinded as to the final diagnosis.

2.2 | Immunohistochemistry

Four-micron-thick sections were cut and stained with routine hematoxylin and eosin stain for histopathological evaluation. Immunohistochemistry was performed with a standard streptavidin–biotin–peroxidase system and the Dako Envision system (Dako, Agilent Technologies, USA). Antibodies used were polyclonal anti-L1NE-ORF2 (sc-67198, dilution 1:500; Santa Cruz Biotechnology, Inc., Heidelberg, Germany) and polyclonal anti-APOBEC3B (orb155694, dilution 1:100; Novus Biologicals, USA). Antibodies were applied at dilutions determined by titration with the relevant protein. As a secondary antibody, biotinylated goat-anti-rabbit IgG (DAKO) was used. The EnVision system (Dako) was used as the detection system. Peroxidase activity was quenched by incubating the slides in 3% hydrogen peroxide (Hydrogen Peroxide Block 30%, Merck, Darmstadt, Germany) and polyclonal anti-APOBEC3B (orb155694, dilution 1:100; Biokar, Cambridgeshire, UK). In summary, endogenous peroxidase activity was quenched by incubating the slides in 3% hydrogen peroxide (Hydrogen Peroxide Block 30%, Merck, Darmstadt, Germany) for 10 minutes; antigen retrieval was achieved by immersion into citrate buffer pH 6.1 (EnVision system) and placement into a microwave oven for 2 minutes; primary antibodies were diluted in the specific antibody buffer (EnVision FLEX Target Retrieval Solution, Dako) and placement into a microwave oven for 2 minutes; primary antibodies were diluted in the specific antibody buffer (EnVision FLEX Antibody Diluent, Dako); and slides were incubated overnight at 4°C. Following incubation with the ready-to-use secondary buffered solution containing a stabilizing protein and an antimicrobial agent (EnVision FLEX + Rabbit, LINKER, Dako) for 15 minutes at 37°C, sections were reacted with 3,3-diamino-benzidine tetrahydrochloride (DAB) solution (EnVision FLEX DAB+ Chromogen diluted into 100 ml EnVision FLEX Substrate Buffer, Dako) for 5 minutes and counterstained with Gill’s hematoxylin for...
1 minutes at 37°C. A breast carcinoma was used as positive control for both antibodies. 7,16 and substitution of primary antibodies by non-immune serum of the same specificity as negative controls.

2.3 | Immunohistochemical scoring

Immunohistochemical scoring was performed by 2 examiners independently in the whole section. Slides were scored according to the semiquantitative method of Gwak et al. 16 The final score was calculated as the product of staining extent (0 = 0-5%; 1 = 6-19%; 2 = 20-49%; and 3 = 50% of cells) and staining intensity (0 = negative; 1 = weak (Figure 1A, B); 2 = moderate (Figure 1C, D); and 3 = strong (Figure 1E, F)) and classified as negative < 4; mild positive 4-5; and strong positive > 5. The last 2 categories were finally grouped together as positive for statistical analysis.

Cases with a borderline staining intensity between 2 grades were additionally evaluated with a semi-automated computerized image analysis (CIA) system as has been previously described. 19,20

2.4 | Statistical analysis

Statistical analysis was performed with the SPSS, V22.0 Software for Windows (SPSS Inc., Chicago). The difference in immunohistochemical expression of L1 ORF2p and APOBEC3B between the four SS Tarpley subgroups, as well as between the SS patients and sicca or/and non-sicca controls, was evaluated by Fisher’s exact test at .05.

2.5 | Ethical approval statement

The study has been independently reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, National and Kapodistrian University of Athens, Greece (NKUA code number 284).

3 | RESULTS

Results are summarized in Table 1. Immunostaining for L1 ORF2p and APOBEC3B in the breast carcinoma (Figure 1G, H) was cytoplasmic in the neoplastic cells, and in the MSGs was cytoplasmic predominantly in the ductal cells (Figure 2B, C, E, F, H, I, K, L), while acinar cells (Figure 2E, F, H, I, *) and inflammatory cells were negative (Figure 2B, C, E, F, H, I, K, L), except for some positive mononuclear cells (Figure 2C, I, L, red arrows). No difference in immunostaining was seen between ducts with or without periductal lymphocytic infiltration, as well as between intralobular and interlobular ducts. Nerve bundles were L1 ORF2p negative and APOBEC3B positive, in contrast to muscle bundles that were L1 ORF2p positive and APOBEC3B negative. No L1 ORF2p or APOBEC3B immunoreactivity was seen in endothelial cells and areas of interstitial fibrosis and lipid degeneration.

3.1 | L1 ORF2p immunohistochemical expression

L1 ORF2p immunoreactivity had a slight speckled-punctuated pattern. The CIA intensity categorization was in agreement with the grading by the 2 examiners in 5/5 L1 ORF2p cases tested. Overall, 17/20 (85%) SS cases and all CS and NMSG cases were characterized as L1 ORF2p positive (Table 1). In 16/17 (94.1%) positive SS cases, 5/5 CS (100%), and 4/5 (80%) NMSG cases, the extent of staining was characterized as grade 2 or 3, and the staining intensity as grade 3, resulting in a final score of 6 or 9, classified as strongly positive. One SS and one NMSG case had a final score of 4 (mildly positive). The 3 negative SS cases showed either total absence of staining (2 cases) or mild intensity of staining (1 case).

All Tarpley I and III cases and 5/7 Tarpley II cases demonstrated strong L1 ORF2p reactivity, and 2/3 Tarpley IV cases were L1 ORF2p negative. There was no statistically significant difference in L1 ORF2p-positive cases among Tarpley subgroups (P > .05, Table 2), but a statistically significant difference was found between the SS Tarpley IV cases and the controls (P = .039, Table 2, Figure 3A).

3.2 | APOBEC3B immunohistochemical expression

APOBEC3B immunoreactivity had a fine speckled-punctuated pattern. The CIA results changed the final score and consequently the classification from positive to negative in 1/9 of the APOBEC3B tested cases. Overall, 15/20 (75%) SS, 5/5 (100%) CS, and 3/5 (60%) NMSG cases were APOBEC3B positive. In all those cases, immunostaining extended to more than 50% of ductal cells (grade 3) with moderate (grade 2) or strong intensity (grade 3), resulting in a final score 6 or 9 and a "strongly positive" classification. In all negative cases, the staining intensity was weak (grade 1) and thus, a final score <4 was defined, regardless of the staining extent.

All SS Tarpley I cases were APOBEC3B negative. A statistically significant difference was found between the SS Tarpley I and II subgroups regarding the APOBEC3B immunoreactivity (P = .008), as well as between the SS Tarpley I subgroup and the CS group (P = .018) and the combined control groups (P = .035, Table 2, Figure 3B).
3.3 | Comparison of L1 ORF2p and APOBEC3B immunohistochemical expression

Twelve of twenty (60%) SS cases, 5/5 (100%) CS, and 3/5 (60%) NMSG cases were positive for both antibodies, the difference being not statistically significant. Considering the expression of L1 ORF2p and APOBEC3B in SS cases, 0/3 (0%) Tarpley I, 6/7 (85.7%) Tarpley II, 5/7 (71.4%) Tarpley III, and 1/3 (33.3%) Tarpley IV cases were positive for both antibodies (double positive). All SS Tarpley I cases were classified as L1 ORF2p positive (Figure 2B) but APOBEC3B negative (Figure 2C); positive expression of both proteins was observed in most SS Tarpley II (Figure 2E, F) and III cases (Figure 2H, I); and 2/3 SS Tarpley IV cases were characterized as L1 ORF2p negative (Figure 2K), while 3/3 of those cases were APOBEC3B positive (Figure 2L). A statistically significant difference was detected between SS Tarpley I and II subgroups (P = .033), and between Tarpley I and CS cases (P = .018, Table 2) regarding the number of double-positive cases. It is noticed that 3/20 L1 ORF2p-negative SS cases showed strong immunopositivity for APOBEC3B, while strongly positive L1 ORF2p expression (final score of 6 or 9) was observed in all APOBEC3B-negative cases (Figure 4).

TABLE 1 L1 ORF2p and APOBEC3B immunohistochemical expression/score in SS, CS and NMSGs

<table>
<thead>
<tr>
<th>Classification/score</th>
<th>SS-I</th>
<th>SS-II</th>
<th>SS-III</th>
<th>SS-IV</th>
<th>SS-Total</th>
<th>CS</th>
<th>NMSGs</th>
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<tr>
<td>L1 ORF2</td>
<td></td>
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<tr>
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<td>0 (0)</td>
<td>2 (66.7)</td>
<td>3 (15.0)</td>
<td>0 (0)</td>
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<tr>
<td>Mildly positive/4</td>
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<td>1 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.0)</td>
<td>0 (0)</td>
<td>1 (20.0)</td>
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<tr>
<td>Strongly positive/6, 9</td>
<td>3 (100.0)</td>
<td>5 (71.4)</td>
<td>7 (100.0)</td>
<td>1 (33.3)</td>
<td>16 (80.0)</td>
<td>5 (100.0)</td>
<td>4 (80.0)</td>
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<td>Total (%)</td>
<td>3 (100.0)</td>
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<td>7 (100.0)</td>
<td>3 (100.0)</td>
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<td>APOBEC3B</td>
<td></td>
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<tr>
<td>Strongly positive/6, 9</td>
<td>0 (0)</td>
<td>7 (100.0)</td>
<td>5 (71.4)</td>
<td>3 (100.0)</td>
<td>15 (75.0)</td>
<td>5 (100.0)</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>3 (100.0)</td>
<td>7 (100.0)</td>
<td>7 (100.0)</td>
<td>3 (100.0)</td>
<td>20 (100.0)</td>
<td>5 (100.0)</td>
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</table>

SS, Sjögren’s syndrome; I, Tarpley I; II, Tarpley II; III, Tarpley III; IV, Tarpley IV; CS, Chronic Sialadenitis; NMSGs, normal minor salivary glands.

FIGURE 2  Histological images of the four Tarpley subgroups (A, D, G, J) and representative images of L1 ORF2p (B, E, H, K) and APOBEC3B (C, F, I, L) cytoplasmic immunostaining in the ductal epithelium of MSGs. All SS Tarpley I cases were L1 ORF2p positive (B) but APOBEC3B negative (C). Double-positive immunostaining was observed in most SS cases of Tarpley II (E, F) and III (H, I). Most cases in SS Tarpley IV subgroup were L1 ORF2p negative (K) and APOBEC3B positive (L) (A, D, G, J) hematoxylin and eosin stain, (B, E, H, K) L1 ORF2p immunohistochemical stain, (C, F, I, L) APOBEC3B immunohistochemical stain; original magnifications (A, D, G, J) × 100, (B, C, E, F, H, I, K, L) × 400

4 | DISCUSSION

This is the first study to show that L1 ORF2 and APOBEC3B proteins are co-expressed in the ductal cells of MSGs of pSS patients and non-autoimmune controls. We performed CIA in order to provide a more accurate evaluation of the staining intensity and overcome the subjectivity of the manual visual semiquantitative evaluation.19 The full agreement between CIA and semiquantitative evaluation that we found is in accordance with previous studies.20 L1 ORF2p detection was hindered until recently, due to the high percentage of L1 adenosine content (up to 40%) that may cause a defective transcription elongation, or the unconventional translation mechanism of L1 RNA that results in low levels of L1 ORF2p (~one ORF2p molecule per L1 mRNA).21 The antibody applied in the present study recognizes the C-terminal region of ORF2 that remains in the cytoplasm after truncation of the C-terminal fragment, a process that is necessary for the nuclear entrance of L1 ORF2p.22 The fine punctuated staining pattern seen in our cases is described in...
An alternative explanation is the presence of L1 ORF2p in stress granules or P-bodies, in association with the Ro 60/TROVEC2 protein, and consequently both proteins with the Ago2-GFP fusion protein. It is noted that Ro60/TROVEC2 protein is a major SS autoantigen and stress granules have been recently recognized as potential autoantibody targets in systemic sclerosis.

No significant difference in L1 ORF2p expression was observed between SS patients and controls in the present study. In another study, increased levels of full-length L1 mRNA and ORF1p were found in MSGs of SS patients in comparison to non-autoimmune sicca controls. Divergent results may be explained by posttranscriptional events that influence the amount of L1 mRNA eventually translated into L1 ORF2p. The detection of L1 ORF2p in ductal cell of MSGs may indicate the presence of an active L1 element in those cells that may trigger pDCs or SGECs to produce type I IFN and promote the immune response in SGECs via Toll-like receptor (TLR)-dependent or independent mechanisms. Alternatively, apoptotic death of SGECs’ or exosomes’ formation may expose L1 RNA or its encoded ORF1p and ORF2p to the immune system and trigger the generation of autoantibodies. As a result, DNA (L1 and endogenous nucleic acids) and autoantibody-containing immune complexes may stimulate pDCs to produce type I IFNs. In addition, as L1 ORF2p plays a key role in the successful L1 retrotransposition, L1 retrotransposition-competent elements integrating into new genomic sites may affect mRNA splicing or exons, resulting in the production of a protein that is recognized as foreign by the immune system.

### Table 2

<table>
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<tr>
<th>Study group</th>
<th>Findings</th>
<th>L1 ORF2p</th>
<th>APOBEC3B</th>
<th>Both proteins</th>
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<tbody>
<tr>
<td>CS (n = 5)</td>
<td>n (%) positive</td>
<td>5 (100.0)</td>
<td>5 (100.0)</td>
<td>5 (100.0)</td>
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<tr>
<td>NMSGs (n = 5)</td>
<td>n (%) positive</td>
<td>5 (100.0)</td>
<td>3 (60.0)</td>
<td>3 (60.0)</td>
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<td>CS and NMSGs (n = 10)</td>
<td>n (%) positive</td>
<td>10 (100.0)</td>
<td>8 (80.0)</td>
<td>8 (80.0)</td>
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<tr>
<td>SS Tarpley I (n = 3)</td>
<td>n (%) positive</td>
<td>3 (100.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>SS Tarpley II (n = 7)</td>
<td>n (%) positive</td>
<td>6 (85.7)</td>
<td>7 (100.0)</td>
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<tr>
<td>SS Tarpley III (n = 7)</td>
<td>n (%) positive</td>
<td>7 (100.0)</td>
<td>5 (71.4)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>SS Tarpley IV (n = 3)</td>
<td>n (%) positive</td>
<td>1 (33.3)</td>
<td>3 (100.0)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>P value (VS CS and NMSGs)</td>
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<td>0.4118</td>
<td>0.485</td>
<td>1.000</td>
</tr>
<tr>
<td>P value (VS SS Tarpley II)</td>
<td></td>
<td>1.000</td>
<td>0.018</td>
<td>0.018</td>
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<td>Bold values correspond to significant results (P &lt; .05) of Fisher’s exact test.</td>
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</table>

SS, Sjögren’s syndrome; CS, Chronic Sialadenitis; NMSGs, normal minor salivary glands
An intriguing finding in the present study is that 2/3 SS Tarpley IV cases were negative for L1 ORF2p, in contrast to most SS Tarpley I-III subjects and all controls that were positive. These cases were histologically characterized by high lymphocytic focus score and/or germinal center formation. A more intense immunoeexpression of ORF1p is observed in high-grade B-cell lymphomas compared to low grade. Our data demonstrated a weaker L1 ORF2p in SS patients with adverse histopathological factors for lymphoma development among SS patients, indicative of a role of L1 element in lymphoproliferative processes in SS that should be further investigated.

The anti-APOBEC3B antibody applied in the present study specifically targets the N-terminal amino acid residues 20-60 of the human APOBEC3B protein, overcoming the problem of the high degree of homology between the APOBEC3 genes. As the IFN pathway activation is a hallmark event in SS, a link between the IFN-inducible gene APOBEC3B and this autoimmune disease seems plausible. APOBEC3B gene was upregulated among systemic lupus erythematosus patients, whereas it was found among the top 10 downregulated genes in rheumatoid arthritis synovial RNA compared to RNA from healthy controls.

The colocalization of L1 ORF2p and APOBEC3B in the cytoplasm of ductal cells of MSGs in our study is in accordance with a coordinated expression of "intrinsic enemies." APOBEC3 proteins are among the inhibitory factors of the endogenous L1 retroelements and are found in conjunction with L1 elements. An interesting finding of our study was that Tarpley I MSGs was negative for APOBEC3B but strongly positive for L1 ORF2p, indicating a possible defect in the APOBEC3B inhibitory mechanism in this subgroup. Although APOBEC3B enzymes are not the only restricting factor of L1 retroelements, this dysfunction could allow the inadequately controlled L1 elements to trigger autoimmunity in SS patients.

L1 ORF2p and APOBEC3B proteins were mostly absent in the inflammatory infiltrate of MSGs. Low to absent APOBEC3B mRNA expression was found in freshly isolated monocytes and lymphocytes, and L1 ORF1p was detected in MSGs inflammatory cells.

L1 ORF2p expression in skeletal muscles and APOBEC3B in the nerve bundles found in the present study is in accordance with previous findings at mRNA level. Capillary endothelial cells in our cases were negative for L1 ORF2p and APOBEC3B expression, but in other studies were described as positive or negative for L1 ORF2p, while APOBEC3B protein expression in the endothelial cells has not been previously described.

In conclusion, our study showed that L1 ORF2p and APOBEC3B are expressed in the MSGs that are among the major target organs in SS. The localization of L1 ORF2p in the ductal epithelial cells of MSGs that have a key role in SS pathogenesis may promote the ability of L1 to act as an intrinsic antigen in SS. The potential future use of L1 ORF2-reverse transcriptase inhibitors in autoimmunity supports further investigation of L1 element epigenetic regulation by APOBEC3 deaminases.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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REFERENCES


