Increased expression of TNFRSF14 and LIGHT in biliary epithelial cells of patients with primary sclerosing cholangitis

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Abstract

**Background and aims:** There is a lack of biliary epithelial molecular markers for primary sclerosing cholangitis (PSC). We analyzed candidates from disease susceptibility genes identified in recent genome-wide association studies.

**Methods:** Expression was quantified using immunohistochemistry in biliary epithelia in liver biopsy samples from patients with PSC (N = 45) and controls (N = 12). Samples from patients with primary biliary cholangitis (PBC) were used as disease controls (N = 20).

**Results:** The levels of hepatic expression of ATXN2, HHEX, PRDX5, MST1, and TNFRSF14 were significantly altered in the PSC group. We focused on the immune-related receptor, TNFRSF14. Immunohistochemistry revealed that TNFRSF14 positivity was significantly higher in biliary epithelia in the PSC group (96 %) than in the control (42 %) and PBC (55 %) groups. High expression of TNFRSF14 was observed only in patients with PSC. Moreover, the expression of LIGHT, which encodes a TNFRSF14-activating ligand, was increased in PSC liver. Immunohistochemistry showed that high expression of LIGHT was more common in PSC biliary epithelia (53 %) than in the PBC (15 %) or control (0 %) groups; moreover, it was positively associated with fibrotic progression.

**Conclusions:** TNFRSF14 and LIGHT are attractive candidate markers for PSC.

Introduction

Primary sclerosing cholangitis (PSC) is an intractable idiopathic hepatobiliary disease characterized by multifocal bile duct strictures due to inflammation and fibrosis, resulting in liver failure (1). The pathogenesis of PSC remains unclear, and thus the identification of specific biomarkers is urgently needed.

Molecular markers expressed in the biliary epithelia of patients with PSC remain unidentified. Recent studies have revealed that cellular senescence and the senescence-associated secretory phenotype (SASP) are characteristics of biliary epithelial cells in PSC (2). In explanted liver samples, the expression of p16, interleukin 6 (IL-6), and interleukin 8 (IL-8) is increased in the biliary epithelia of PSC rather than in those of primary biliary cholangitis (PBC), whose pathological condition, characterized by autoimmune bile duct injury, is similar to that of PSC (2). IL-8 was also expected to be a progressive or prognostic marker for PSC (3). However, IL-8 is only expressed at low levels in biliary epithelial cells in early PSC, whereas its expression is prominent in the advanced stage (4). The identification of uniquely occurring early pathological markers in the biliary epithelia of PSC requires the analysis of biopsy samples obtained at diagnosis instead of explanted samples, which reflect the terminal conditions of the disease.

Recent genome-wide association studies (GWAS) have revealed a strong association between PSC and the human leukocyte antigen (HLA) region on chromosome 6, indicating that PSC is an autoimmune disorder (5–11). Additionally, more than 20 non-HLA disease susceptibility genes have been identified (5,
11. Despite their weaker association (odds ratios < 1.5) compared with that of HLA (odds ratio 3–5), each of them is a candidate potentially involved in the pathology of PSC through the regulation of the inflammatory immune response or bile-acid homeostasis (5, 11). A recent study showed that downregulation of *Takeda G protein-coupled receptor-5 (TGR5)*, one of the GWAS-derived risk genes regulating epithelial barrier functions, contributed to the development of cholangitis, specifically in PSC biliary epithelia (12). However, the expression profiles of other genes in patient tissues, particularly in bile ducts, remain unknown.

In this study, we aimed to identify molecular markers with altered expression in PSC biliary epithelia, focusing on GWAS risk genes. First, we analyzed gene expression in liver biopsy samples from patients with PSC, with the resected liver tissues adjacent to the tumors as controls. Next, using immunohistochemistry, we validated these expression profiles in biliary epithelial cells and hepatocytes. We further compared these results with those from control patients and patients with PBC as disease controls.

**Materials And Methods**

**Human liver tissue**

Needle liver biopsy samples were obtained from patients diagnosed with PSC and PBC at the University of Tokyo Hospital. Resected liver specimens were obtained from patients who underwent surgery at our hospital. This study was approved by the Ethical Committee for Clinical Research at our institutions (approval numbers 11902, Institute of Medical Science, Asahi Life Foundation; 2019140NI and 1302, The University of Tokyo). Informed consent was obtained from patients with PSC and liver tumors in the form of an opt-out on the website (2019140NI). Written informed consent was obtained from all patients with PBC (1302).

**RNA extraction**

Total RNA was extracted from formalin-fixed paraffin-embedded liver biopsy samples using the Recover All-TM Total Nucleic Acid Isolation kit (Invitrogen, Carlsbad, CA, USA). Total RNA was also extracted from frozen resected liver specimens using an RNeasy Mini Kit (Qiagen, Hilden, Germany).

**Real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR)**

For the synthesis of complementary DNA from RNA, ImProm-II Reverse Transcriptase (Promega, Tokyo, Japan) was used. qPCR was performed using the THUNDERBIRD SYBR qPCR Mix (Toyobo Co. Ltd., Osaka, Japan). All values were normalized to the levels of expression of β-actin mRNA. Primers are listed in Supplementary Table 1.
Immunohistochemistry

Paraffin-embedded tissue blocks were cut into 5-µm sections and mounted on glass slides. After dewaxing and rehydration, antigen retrieval was performed by heating slides in 10 mM citrate buffer (pH 6.0) at 120°C for 5 min. After blocking, sections were incubated overnight at 4°C with anti-TNFRSF14 (10138-1-AP, 1:200 dilution, Proteintech Japan, Tokyo, Japan) or anti-LIGHT (PA5-82467, 1:50 dilution, Invitrogen). Peroxidase-conjugated anti-rabbit immunoglobulin G (NICHIREI BIOSCIENCES, Tokyo, Japan) was used as secondary antibody. Peroxidase activity was visualized using Histone Simple Stain 3,3-diaminobenzidine (DAB) solution (415171, Nichirei Bioscience). We grouped TNFRSF14- or LIGHT-expressing cells by staining intensity as high- or low-positive. A sample was considered negative when the frequency of stained cells was < 1 % in each cellular fraction. Stained slides were validated by 2 gastroenterologists (SK and HF) who were blinded to patient identities and clinical information.

Statistical analysis

The Mann–Whitney U test was used to compare levels of gene expression in liver tissues and laboratory data between groups. Fisher’s exact or chi-square tests were used to compare the immunohistochemical staining data between groups. Differences were considered statistically significant at P < 0.05.

Results

Patient characteristics

The clinical characteristics of patients in all groups are shown in Table 1. The PSC group included 47 patients, of whom 19 were men and 28 were women; the median age at diagnosis was 48 years (range, 14–82). We evaluated the disease stage at the time of diagnosis using Ludwig’s classification for histological liver fibrosis. Approximately half of patients were diagnosed at an early stage (stage 1, N = 11; stage 2, N = 11; stage 3, N = 20; and stage 4, N = 5). We found that the prevalence of inflammatory bowel disease (IBD) was 36% (17/47), comparable to that in a recent study in Japan (13). The PBC group included 6 men and 14 women; the median age at diagnosis was 51 years (range, 35–67). According to Ludwig’s classification, most patients were at early stages (stage 1, N = 5; stage 2, N = 12; stage 3, N = 3; stage 4, N = 0). The control group included 7 men and 5 women who had undergone surgery for intrahepatic cholangiocarcinoma (N = 9), intraductal papillary neoplasm of the bile duct (N = 1), or liver metastasis of colon cancer (N = 2). None of them had viral hepatitis. Their resected livers were histologically evaluated using the New Inuyama classification (14); they were all classified as F0, A1, or A2. We found that serum alkaline phosphatase and gamma-glutamyl transpeptidase levels in the PSC group were significantly higher than those in the control group and equivalent to those in the PBC group. In all three groups, most patients (> 90%) were classified as Child–Pugh class A.

Table 1. Clinical characteristics of patients in PSC, PBC and control group
Values are expressed as N (%) otherwise indicated.

* Values are expressed as median (range).

UC, ulcerative colitis;

ICC, Intrahepatic cholangiocarcinoma; IPNB, Intraductal papillary neoplasm of the bile duct; LM-CRC, Liver metastasis of Colorectal adenocarcinoma

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyl transpeptidase; T-Bil, total bilirubin; Alb, albumin; PT-INR, prothrombin time-international normalized ratio; Cre, creatinine; CRP, C-reactive protein
Altered expression of risk genes identified in GWASs in the livers of patients with PSC

To screen for potential candidate genes, we performed RT-qPCR on liver biopsy samples from the PSC group and resected liver samples from the control group. We chose 9 candidate genes from a previous GWAS study for analysis (11): ATXN2, CCL20, FOXP1, HHEX, IL2RA, MST1, NFKB1, PRDX5, and TNFRSF14. We accordingly collected 10 samples from the PSC group that met the inclusion criteria: (1) confirmed expression of the housekeeping gene β-actin, and (2) quantitation of the expression of each gene using a standard curve. We found that all 12 control samples satisfied both criteria. As shown in Fig. 1, the expression of ATXN2, HHEX, PRDX5, and TNFRSF14 was significantly upregulated, whereas that of MST1 was downregulated in the PSC group compared with that of controls. However, we did not detect any significant difference in the expression of CCL20, FOXP1, NFKB1, or IL2RA between the two groups (Fig. 1).

Biliary expression of TNFRSF14 was increased in PSC but not in PBC

Among the five candidate genes identified, we focused on TNFRSF14 because it has also been reported to be a disease susceptibility gene in ulcerative colitis, the most common comorbidity of PSC (5, 15). TNFRSF14 encodes tumor necrosis factor (TNF) receptor superfamily member 14 (TNFRSF14), also known as herpesvirus entry mediator (HVEM). It is expressed in stromal, myeloid, lymphoid, and epithelial cells, and is involved in the inflammatory signal transduction through its interactions with multiple TNF-related ligands and immunoglobulin-superfamily proteins (16).

To validate the TNFRSF14 expression profiles, we performed immunohistochemical staining of liver samples from control patients and patients with PSC. We classified expression levels using staining intensity as TNFRSF14-high, -low, and -negative (Fig. 2A). We assessed a total of well-preserved 45 liver biopsy samples from patients with PSC. We also evaluated the levels of expression in hepatocytes and compared the results with those of liver biopsy samples from patients with PBC. Consistent with the results of the initial gene expression analysis, the fraction of TNFRSF14-positive (TNFRSF14-high and -low) biliary epithelial cells in patients in the PSC group (96% [43/45]) was significantly higher than that in controls (42% [5/12], P < 0.001) (Fig. 2B). Moreover, it was significantly higher than that in the PBC group (55% [11/20], P < 0.001). Notably, we observed TNFRSF14-high cells only in patients with PSC (Fig. 2B). We obtained similar results with hepatocytes; positivity was significantly higher in the PSC group than in the PBC and control groups (PSC 96% [43/45], PBC 65% [13/20], P = 0.003; control 58% [7/12], P = 0.003) (Fig. 2B). Similar to biliary epithelial cells, we detected TNFRSF14-high hepatocytes only in patients with PSC (Fig. 2B). These findings suggested that TNFRSF14 was upregulated in the biliary epithelial cells and hepatocytes of patients with PSC, whereas this upregulation did not occur in patients with PBC.
**Biliary expression of LIGHT is increased in PSC**

Recent studies have revealed that the TNF family member LIGHT (lymphotoxin-like, which exhibits inducible expression and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes), an activating ligand of TNFRSF14, is involved in fibrosis in many tissues, including the lungs, skin, and liver (17–19). The expression of both TNFRSF14 and LIGHT was increased in the synovial tissues of patients with other autoimmune disorders, such as rheumatoid arthritis (20). As the expression of LIGHT was significantly elevated in PSC (Fig. 3A), we next measured the LIGHT expression.

As in the case of TNRSF14, we performed immunohistochemistry in liver tissues from control patients, patients with PSC, and patients with PBC using antibodies against LIGHT. We classified the levels of expression of biliary epithelial cells and hepatocytes based on their staining intensity as LIGHT-high, -low, and -negative (Fig. 3B). We found that the fraction of LIGHT-positive (LIGHT-high and -low) biliary epithelial cells in patients in the PSC group was significantly higher than that in the control group (100% [45/45] vs. 83% [10/12], P = 0.041) (Fig. 3C). However, we did not detect any significant differences in staining between the PSC and PBC groups (95% [19/20], P = 0.308) (Fig. 3C). Interestingly, we observed that LIGHT-high cells were more common in PSC (53% [24/45]) than in PBC (15% [3/20], P = 0.006), whereas they were not observed in the control group (Fig. 3C). Likewise, we found that the proportion of LIGHT-positive hepatocytes was also significantly higher in PSC than in controls (PSC 100% [45/45], control 67% [8/12], P = 0.001) (Fig. 3C). We did not observe any significant differences in positivity between PSC and PBC (100% [20/20], P = 1) (Fig. 3C). We noticed that LIGHT-high hepatocytes were equally common in PSC and PBC (PSC: 64% [29/45], PBC: 55% [11/20], P = 0.655), whereas they were not found in control tissues (Fig. 3C). These observations suggested that the expression of LIGHT was increased in the biliary epithelial cells and hepatocytes of patients with PSC. We more frequently detected high expression of LIGHT in PSC than in PBC biliary tissues, although the levels of expression in hepatocytes were comparable between these groups.

**High expression of LIGHT in the bile duct is correlated with fibrotic progression of PSC**

We found that patients with PSC showed various expression patterns of TNFRSF14 and LIGHT (Supplementary Fig. 1). Therefore, we determined whether there was a relationship between increased expression of TNFRSF14/LIGHT and clinical characteristics or laboratory data in patients with PSC (Tables 2 and 3).
### Table 2
Relations between TNFRSF14 expressions and clinical characteristics and laboratory data

<table>
<thead>
<tr>
<th></th>
<th>Biliary epithelial cells</th>
<th>Hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H (N = 19)</td>
<td>L/N (N = 26)</td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (N = 20)</td>
<td>8 (42%)</td>
<td>12 (46%)</td>
</tr>
<tr>
<td>IBD (+) (N = 16)</td>
<td>6 (32%)</td>
<td>10 (38%)</td>
</tr>
<tr>
<td>Female (N = 26)</td>
<td>13 (68%)</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Ludwig stage 3 or 4 (N = 24)</td>
<td>12 (63%)</td>
<td>12 (46%)</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>3.80 (2.8–4.2)</td>
<td>3.95 (2.9–4.7)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>43 (12–274)</td>
<td>46 (11–240)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>648 (216–2002)</td>
<td>434 (157–1613)</td>
</tr>
<tr>
<td>T-Bil (mg/dL)</td>
<td>0.8 (0.5–6.5)</td>
<td>0.7 (0.3–1.9)</td>
</tr>
<tr>
<td>PT-INR</td>
<td>0.94 (0.87–1.07)</td>
<td>0.99 (0.05–1.35)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.23 (0.02–4.54)</td>
<td>0.26 (0.01–6.40)</td>
</tr>
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</table>
As shown in Table 3, we detected more LIGHT-high biliary epithelial cells (71%, \( P = 0.027 \)) and hepatocytes (69%, \( P = 0.012 \)) in later stages of the disease, whereas other clinical factors, including age, sex, and comorbid IBD, did not correlate with high expression of LIGHT in either cell type. We found that TNFRSF14 expression was not associated with disease stage (Table 2). Considering that Ludwig’s classification is based on the histological grading of liver fibrosis, these results suggest that high expression of LIGHT in biliary epithelial cells and hepatocytes is correlated with fibrotic disease progression in PSC.

Regarding the relationship with laboratory data, the following results were obtained with statistical significance. We found that patients with TNFRSF14-high hepatocytes tended to have lower levels of PT-INR and CRP.

### Table 3
Relations between LIGHT expressions and clinical characteristics and laboratory data

<table>
<thead>
<tr>
<th></th>
<th>Bile epithelial cells</th>
<th>Hepatocytes</th>
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<tbody>
<tr>
<td></td>
<td>H (N = 24)</td>
<td>L/N (N = 21)</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (N = 20)</td>
<td>12 (50%)</td>
<td>8 (38%)</td>
</tr>
<tr>
<td>IBD (+) (N = 16)</td>
<td>9 (38%)</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>Female (N = 26)</td>
<td>13 (54%)</td>
<td>13 (62%)</td>
</tr>
<tr>
<td>Ludwig stage 3 or 4 (N = 24)</td>
<td>17 (71%)</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>3.9 (2.8–4.3)</td>
<td>4.0 (3.4–4.7)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>64 (12–246)</td>
<td>35 (11–274)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>724 (243–2002)</td>
<td>388 (157–1729)</td>
</tr>
<tr>
<td>T-Bil (mg/dL)</td>
<td>0.8 (0.3–6.5)</td>
<td>0.7 (0.4–1.9)</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.01 (0.88–1.35)</td>
<td>0.97 (0.05–1.34)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.46 (0.02–4.54)</td>
<td>0.18 (0.01–6.40)</td>
</tr>
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</table>
INR (P = 0.023) (Table 2). In addition, we more often detected LIGHT-high biliary epithelial cells or hepatocytes in patients with lower levels of albumin (P = 0.042) or higher levels of total bilirubin (P = 0.008), respectively (Table 3).

Discussion

In this study, using liver biopsy samples obtained from patients at diagnosis, we found increased expression of the immune-related receptor TNFRSF14 in biliary epithelial cells of patients with PSC but not those with PBC. In addition, we observed increased expression of its activating ligand, LIGHT, in PBC biliary epithelial cells and demonstrated a positive correlation between the high LIGHT expression and fibrotic disease progression. The expression of TNFRSF14 and LIGHT was also upregulated in PSC hepatocytes. In addition to TNFRSF14, we confirmed the altered expression of several disease-susceptibility genes previously reported from GWAS: ATXN2, HHEX, MST-1, and PRDX5 in the livers of patients with PSC.

Owing to their dependency on diagnostic imaging, liver biopsies are not routinely performed for PSC diagnosis (21). Therefore, previous studies have focused on liver resection specimens obtained during transplantation. Recent reports have shown increased expression of MAdCAM-1 and VAP-1, which are involved in the recruitment of T-lymphocytes, in PSC hepatic endothelial cells (22, 23). This alteration of expression has been reported to be prominent at the end stage; however, it has not been confirmed in liver biopsy samples taken at diagnosis, which should not reflect the indirect effects of secondary liver damage (22–24). Even when using liver biopsy specimens, the altered expression of biliary epithelia could be masked by that of hepatocytes in the analysis, such as in RNA sequencing of bulk samples (25). To detect any initial changes in the biliary epithelia during the progression of this refractory disease, validation with immunohistochemical assays using liver biopsy samples is essential (Fig. 2B, 3C, Table 2, and 3).

Few reports have analyzed the expression of TNFRSF14 in hepatobiliary diseases. A previous immunohistochemical study demonstrated that the expression of TNFRSF14 was not elevated in any intrahepatic cellular fraction of patients with chronic viral hepatitis (26). Increased expression of TNFRSF14 was not observed in the biliary epithelia of patients with PBC, and its expression level was not correlated with that of serum ALP in patients with PSC (Fig. 2 and Table 2). These findings suggest that upregulation of TNFRSF14 in biliary epithelial cells is a primary feature of the pathogenesis of PSC and not a secondary change downstream of cholestasis or inflammation.

We observed not only the upregulation of TNFRSF14, but also that of LIGHT (Fig. 3B and 3C). LIGHT is a lymphokine produced by hematopoietic cells, and its serum level is elevated and correlated with disease progression in patients with inflammatory diseases such as dermatitis (27). Considering the correlation between LIGHT expression and the histological fibrotic stage of PSC (Table 3), LIGHT should be further studied as a new biomarker reflecting PSC progression, although its functional significance has not been
elucidated. In future studies, we will measure serum and bile LIGHT in patients with PSC and test for correlations with prognoses and/or comorbidities such as acute cholangitis and cholangiocarcinoma.

This study had some limitations. (1) It was a single-center retrospective study with a limited number of cases; the sample size in the PSC group was inadequate for multivariate analysis. (2) There was a small number of cases at Ludwig's stage 3/4 (only 3) in the PBC group, which might have contributed to the low rate of LIGHT-high biliary epithelial cells (Table 1 and Fig. 3C).

In conclusion, we identified 2 novel genes that were upregulated in the biliary epithelia of PSC, that is, TNFRSF14 and LIGHT. The former was not upregulated in the biliary epithelia of PBC, while the latter was correlated with the histological degree of fibrotic progression of PSC. The interactions between the two inflammatory regulators might play important roles in the pathogenesis of PSC, and their combined detection is likely to be useful as a diagnostic or prognostic biomarker for this refractory disease.

**Abbreviations**

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATXN2, ataxin2; CCL20, chemokine ligand 20; Cre, creatinine; CRP, C-reactive protein; FOXP1, forkhead box protein 1; gGTP, gamma-glutamyl transcriptase; GWAS, genome-wide association studies; HHEX, hematopoietically-expressed homeobox; HLA, human leucocyte antigen; HVEM, herpes virus entry mediator; IBD, inflammatory bowel disease; IL2RA, interleukin-2 receptor alpha chain; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes; mRNA, messenger ribonucleic acid; MAdCAM-1, mucosal adressin cell adhesion molecule-1; MST1, macrophage-stimulating 1; NF-kB, nuclear factor-kB; PBC, primary biliary cholangitis; PRDX5, peroxiredoxin-5; PSC, primary sclerosing cholangitis; PT-INR, prothrombin time-international normalized ratio; RT-qPCR, Real-time quantitative reverse transcriptase polymerase chain reaction; T-Bil, total bilirubin; TGR5, Takeda G protein-coupled receptor-5; TNFRSF14, tumor necrosis factor receptor superfamily member 14; UC, ulcerative colitis; VAP-1, vascular adhesion protein 1

**Declarations**

**Disclosures**

The authors declare that they have no conflicts of interest.

**Author Contributions**

HF, SM, TK, and KT conceived the study. SK and HF performed most experiments and wrote the manuscript. MT and TU contributed to the pathological validation of human tissue samples provided by TN, RT, YN, HI, JA, and KH. TH organized the database of clinical information. MK provided financial and material support. KK and MF supervised the study. All authors have reviewed and approved of the submitted manuscript.
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References


Figures
Figure 1

Analysis of PSC risk gene expression in liver samples from patients with PSC (PSC, N = 10) and control patients (Ctrl, N = 12). *P < 0.05; **P < 0.01. NS, not significant.
Figure 2

(A) Representative micrographs of TNFRSF14 staining of liver samples: TNFRSF14-high, -low, and -negative. (B) Number and proportion of patients with positive expression of TNFRSF14 in biliary epithelial cells and hepatocytes in each group. Scale bar, 100 mm. BEC, biliary epithelial cells; HC, hepatocytes; Ctrl, Control.
Figure 3

(A) Hepatic \textit{LIGHT} expression in samples from patients with PSC (PSC, \( N = 10 \)) and control patients (Ctrl, \( N = 12 \)). **\( P < 0.01 \). (B) Representative micrographs of \textit{LIGHT} staining of liver samples: \textit{LIGHT}-high, -low, and -negative. (C) Numbers and proportions of patients with expression of \textit{LIGHT} in biliary epithelial cells and hepatocytes per group. Scale bar, 100 mm. BEC, biliary epithelial cells; HC, hepatocytes.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.pdf
- Supplementarytable.docx