Interferon-alpha regulates expression of lncRNA MALAT1 and interferon-stimulated genes, as well as chemokine production, in primary Sjögren's syndrome

L.M. Amezcua-Guerra[1,2], F. Sánchez-Muñoz[1], E. Pichardo-Ontiveros[3], J. González-Ramírez[4], L.A. Martínez-Martínez[5], Y. Juárez-Vicuña[1]

[1]Department of Immunology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City; [2]Department of Health Care, Universidad Autónoma Metropolitana-Xochimilco, Mexico City; [3]Department of Nutrition Physiology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City; [4]Cell Biology Laboratory, Facultad de Enfermería, Universidad Autónoma de Baja California Campus Mexicali, Mexicali, Baja California; [5]Department of Rheumatology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico.

Abstract

Objective

This study aimed to explore the contribution of interferon-alpha (IFN-α) to MALAT1 expression in primary Sjögren's syndrome (pSS).

Methods

Peripheral blood mononuclear cells (PBMC) from pSS patients and healthy blood donors were stimulated with recombinant human IFN-α, and the expression levels of MALAT1 and several interferon-stimulated genes (ISGs) were measured by RT-PCR, while supernatant levels of interferon-regulated chemokines were measured using multiplex cytokine immunobead assay.

Results

In this work, we found that MALAT1 expression levels were increased in IFN-α-stimulated PBMC from pSS patients and healthy controls. As expected, ISG expression levels and interferon-regulated chemokine secretion levels were higher after IFN-α stimulation. However, the fold-change values for ISG15, Ly6E, OAS1, and OASL expression levels were higher in cells from pSS patients than in controls. Similarly, PBMC from pSS patients produced higher concentrations of chemokines than those from healthy controls after IFN-α stimulation.

Conclusion

Our data provide insights into the abnormal IFN-α-mediated regulation of the lncRNA MALAT1 in pSS. Based on an unusually high capacity of PBMC to express ISG and to produce interferon-responsive chemokines, it is likely that targeted therapies to block these molecules may be of benefit to patients with pSS.

Key words

long non-coding RNA (lncRNA), Sjögren's syndrome, interferon-alpha

*Luis M. Amezcua-Guerra, MD, PhD**
*Fausto Sánchez-Muñoz, PhD**
Edgar Pichardo-Ontiveros, MSc
Javier González-Ramírez, PhD
Laura Aline Martínez-Martínez, MD, PhD
Yaneli Juárez-Vicuña, PhD

**These authors contributed equally.*

Please address correspondence to:
Yaneli Juárez-Vicuña,
Department of Inmunology,
Instituto Nacional de
Cardiología Ignacio Chávez,
Juan Badiano 1, Sección XVI,
14080 Tlalpan, Mexico City, Mexico.
E-mail: yaneli2608@hotmail.com

ORCID iD: 0000-0002-6467-6638

Received on May 6, 2022; accepted in revised form on July 28, 2022.

Competing interests: none declared.

Introduction

Long non-coding RNAs (lncRNA) are transcripts longer than 200 nucleotides that are not translated into functional proteins, which play an important role in the regulation of gene expression in a variety of cell lines (1). Different studies have identified that lncRNAs are involved in physiological and pathological processes involving immune cells, either through transcriptional or post-transcriptional regulation (2-4). However, only a few studies have explored the role of lncRNAs in autoimmune diseases, and the role of almost all lncRNAs in primary Sjögren's syndrome (pSS), which is the main human autoimmune exocrinopathy, is largely unknown. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is among the most extensively studied lncRNAs, with molecular functions including alternative splicing and competitive endogenous RNA (ceRNA) (5, 6).

MALAT1 is highly expressed in different cells and can be positively or negatively regulated by transcription factors, microRNAs, hormones, and growth factors. In addition, soluble molecules such as the interferon-alpha (IFN-α) seem to regulate the expression of MALAT1 in systemic lupus erythematosus (SLE) (7-9). In pSS, IFN-α and their respective type I interferon-stimulated genes (ISG) contribute to distinct phenotypes, as several ISGs are associated with the presence of anti-SSA/Ro or anti-SSB/La antibodies (10-12). Similarly, lncRNA expression profiles in labial salivary glands and plasma are increased in pSS patients compared to healthy donors (13, 14). Consequently, it is plausible that different lncRNA-mediated molecular mechanisms may be involved in the pathogenesis of pSS. Our study aimed to evaluate the basal and IFN-α-stimulated expression of MALAT1 in peripheral blood mononuclear cells (PBMC) from patients with pSS, as well as to investigate the association of MALAT1 with ISG expression and interferon-regulated chemokine production. If a relevant role of MALAT1 expression is confirmed, this could uncover a new target to test therapeutic strategies in pSS.

Materials and methods

Study participants

This study was conducted in 14 adult patients who met the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for pSS (15), and 13 healthy blood donors with no symptoms of oral or ocular dryness were included as reference. No patient was considered to have a clinically apparent infection at the time of inclusion. Pregnant patients and those with a history of malignancy, head and neck radiation, sarcoidosis, amyloidosis, or hepatitis B or C virus or human immunodeficiency virus infection were not included. Patients underwent a clinical examination and their associated medical records and laboratory reports were evaluated. Immunological tests were obtained from medical records. The extent of disease activity was assessed using the EULAR Sjögren's syndrome disease activity index (ESSDAI) (16).

The study protocol was approved by the local ethics committee (Comité de Ética en Investigación del Instituto Nacional de Cardiología Ignacio Chávez; protocol number 21-1256) and all participants gave their consent to participate before inclusion. All procedures were in accordance with the ethical standards of our institution and the local laws, as well as with the Declaration of Helsinki and its amendments.

Cell cultures

Six ml of fasting venous blood were collected from all participants, and PBMC were isolated by density-gradient centrifugation with Histopaque-1077 (Sigma, St Louis, MO, USA) within two hours after collection. Culture medium consisted of RPMI 1640 medium (Sigma, Ronkonkoma, NY, USA) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated foetal bovine serum.

Cells were cultured in 24-well, flat-bottom plates (1×10^6 in 1 ml) for 24 hours, under one of two different conditions: PBMC alone or PBMC plus 1000 U of recombinant human IFN-α (PBL Interferon source, Biomedical Laboratories, USA). For the real-time reverse tran-

scription–polymerase chain reaction (RT-PCR) assays, cells were resuspended in TriPure Isolation Reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) for subsequent RNA isolation and supernatants were collected and frozen at -80°C until used.

Real-time reverse transcription-polymerase chain reaction

Total RNA was extracted from cultured PBMCs according to the manufacturer's instructions. The quantity and purity of the RNA was determined in a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260 and 280 nm, and only the samples with ratios from 1.8 to 2.0 were retrotranscribed using the M-MLV Reverse Transcriptase reagent (Invitrogen, UK).

RT-PCR analysis was performed on a CFX96 Bio-Rad cycler (Bio-Rad Laboratories, Hercules, California, USA) using primers for MALAT1 (NR_002819, Qiagen, Hilden, Germany). In addition, we quantified the expression of five representative type I IFN-inducible genes: ISG15 (forward primer 5′-GCG AACTCATCTTTGCCAGTA-3′, reverse primer 5′-CCAGCATCTTCACCGTCAGGTC-3′), LY6E (forward primer 5′-GCCATCCTCTCCAGAATGAA-3′, reverse primer 5′-GCAGGAGAAGCACATCAGC-3′), OAS1 (forward primer 5′-GAGAAGGCAGCTCACGAAAC-3′, reverse primer 5′-TCTTAAAGCATGGGTAATTCAGC-3′), MX1 (forward primer 5′-ACCACAGAGGCTCTCAGCAT-3′, reverse primer 5′-CAGATCAGGCTTCGTCAAGA-3′), and OASL (forward primer 5′-TTGTGCCTGCCTACAGAGC-3′, reverse primer 5′-GATCAGGCTCACATAGACCTCA-3′). Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH, forward primer 5′-AGCCACATCGCTCAGACAC-3′, reverse primer 5′- GCCCA ATACGACCAAATCC-3′) was used for relative quantification, and fold change was calculated using the $2^{-\Delta\Delta ct}$ method. Cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95° for 15 s, 60° for 1.0 min, and 65° for 0.05 s.

Table I. Clinical and laboratory data of patients.

	Primary Sjögren's syndrome (n=14)
Age, years	63 (54–71)
Female gender, n (%)	13 (93)
Disease duration, years	5 (2–12)
Comorbidities	
Antiphospholipid syndrome, n (%)	3 (21)
Diabetes, n (%)	0
Hypertension, n (%)	5 (36)
Dyslipidaemia, n (%)	2 (14)
Antirheumatic therapy	
Glucocorticoids, n (%)	4 (28)
Antimalarials, n (%)	6 (42)
Immunosuppressants, n (%)	2 (14)
Laboratory studies	
Leukocytes, total/μl	5.7 (4.6–6.5)
Haemoglobin, g/dl	13.9 (12.7–15.1)
Platelets, total/μl	194.9 (148.5–256.0)
Serum creatinine, mg/dl	1.1 (0.6–0.8)
C-reactive protein, mg/l	3.2 (1.3–4.9)
C3 complement, mg/l	108.5 (95.5–118.8)
C4 complement, mg/l	17.2 (15.0–21.2)
ESR, (mm/h)	18.8 (4.0–16.5)
Rheumatoid factor ≥20 IU/ml, + (%)	9 (64)
Antinuclear antibodies ≥1:160, + (%)	14 (100)
Anti-SSA/Ro antibodies, + (%)	8 (57)
Anti- SSB/La antibodies, + (%)	2 (14)
ESSDAI, score	1.0 (0.0-3.0)

ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index. Data are expressed as the median (interquartile range).

Bead-based multiplex assay

Supernatants samples were collected and interferon-regulated chemokine production (MCP-1, MIP-1 alpha, IP-10, and BLC) was measured using multiplexed immunobead-based assays on xMAP technology (Luminex MAGPIX System, San Francisco CA, USA) according to manufacturer's protocol.

Statistical analysis

Frequencies and percentages were used to describe categorical data, and differences were tested using the χ^2 test or the Fisher's exact tests, as appropriate. Continuous data are expressed as medians and interquartile range (IQR). Differences in MALAT1 and ISGs mRNA expression levels, as well as differences in chemokine production levels, were determined by the Wilcoxon paired t test. Correlation between groups was evaluated using the Spearman's rho coefficient. All statistical analyses were performed using GraphPad Prism v. 7.0 (GraphPad Software Inc, San Diego, CA) and a *p*-value of <0.05 was considered statistically significant.

Results

A total of 27 participants, 14 patients with pSS and 13 healthy blood donors, were included in the study. The median age of patients with pSS was 63 years (IQR, 54–71 years), while in controls it was 35 years (31–38 years; $p<0.001$). There were no differences in the distribution by gender (women, 93% *vs*. 77%; $p=0.325$). The main clinical and laboratory characteristics of pSS patients are summarised in Table I. It should be noted that all patients were positive for antinuclear antibodies, while anti-SSA/Ro antibodies were present in 8 and anti-SSB/La only in 2 patients.

We investigated whether IFN-α may contribute to MALAT1 expression levels in pSS patients and healthy blood donors. MALAT1 expression was significantly higher in IFN-α-stimulated PBMCs from patients with pSS than in unstimulated PBMC (median: 0.083, IQR 0.047–0.106 *vs*. 0.051, 0.029–0.059; $p=0.0046$) (Fig. 1), this phenomenon was replicated in PBMCs from healthy blood donors (0.075, 0.033–0.089 *vs*. 0.036, IQR 0.021–0.056; $p=0.0195$) (Fig. 1).

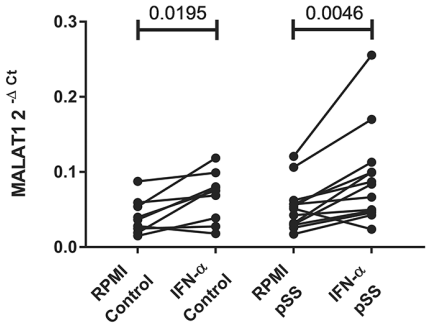

Fig. 1. MALAT1 is upregulated in IFN-α-stimulated PBMC from patients with pSS.
Peripheral blood mononuclear cells were used to study their response after stimulation with IFN-α and levels of MALAT1 expression were assessed by RT-PCR. We observed that MALAT1 was significantly upregulated after IFN-α treatment in both pSS patients and healthy blood donors. The Wilcoxon paired t-test was used to assess differences in expression levels.

We quantified ISGs expression levels in IFN-α-stimulated and unstimulated PBMCs from pSS patients as well in cells of healthy blood donors. Expression levels of all ISGs namely ISG15, Ly6E, OAS1, MX1 and OASL were significantly higher in IFN-α-stimulated than unstimulated PBMCs; this was replicated in PBMCs from healthy blood donors (Fig. 2).

We found that the mRNA levels of Ly6E (0.18, 0.07–0.41 *vs*. 4.27, 2.36–19.37; *p*=0.0001), OAS1 (0.42, 0.16–0.58 *vs*. 41.7, 32.87–56.57; *p*=0.0001), MX1 (0.64, 0.35–0.83 *vs*. 47.48, 36.04–67.00; *p*=0.0001), OASL (0.91, 0.25–1.38 *vs*. 15.22, 8.75–29.28; *p*=0.0001), and ISG15 (0.01, 0.01-0.02 *vs*. 3.00, 2.00-5.00; *p*=0.0001) were higher in IFN-α-stimulated than unstimulated PBMCs from pSS patients. Similarly, the mRNA levels of Ly6E (2.36, 1.07-4.45 *vs*. 28.93, 15.59–64.23; *p*=0.0002), OAS1 (0.53, 0.41-0.70 *vs*. 15.70, 4.34–37.52; *p*=0.0002), MX1 (1.50, 0.61–3.82 *vs*. 46.00, 36.75–74.02; *p*=0.0002), OASL (5.55, 3.18-8.34 *vs*. 46.32, 33.38–63.34; *p*=0.0002), and ISG15 (0.02, 0.01-0.06 *vs*. 2.00, 1.00-4.50; *p*=0.0002) were higher in IFN-α-stimulated than unstimulated PBMCs from heathy controls (Fig. 2). Interestingly, the fold-change values for ISG15 (100.00, 25.00–225.00 *vs*. 300.00, 91.00–500.00; *p*=0.0463), Ly6E (10.12, 3.42–34.18 *vs*. 34.38, 18.60–48.90; *p*=0.0332), OAS1 (22.11, 6.60–80.20 *vs*. 122.90, 75.64–212.4; *p*=0.0015), and OASL (11.29, 3.81–18.07 *vs*. 17.72, 12.54–53.99; *p*=0.0255) were higher in cells from patients with pSS than in those from controls, while MX1 fold-change values were similar between groups (30.67, 11.21–95.33 *vs*. 69.86, 60.12–148.40; *p*=0.0543). These results are presented in the Figure 3.

In addition, we quantified the unstim-

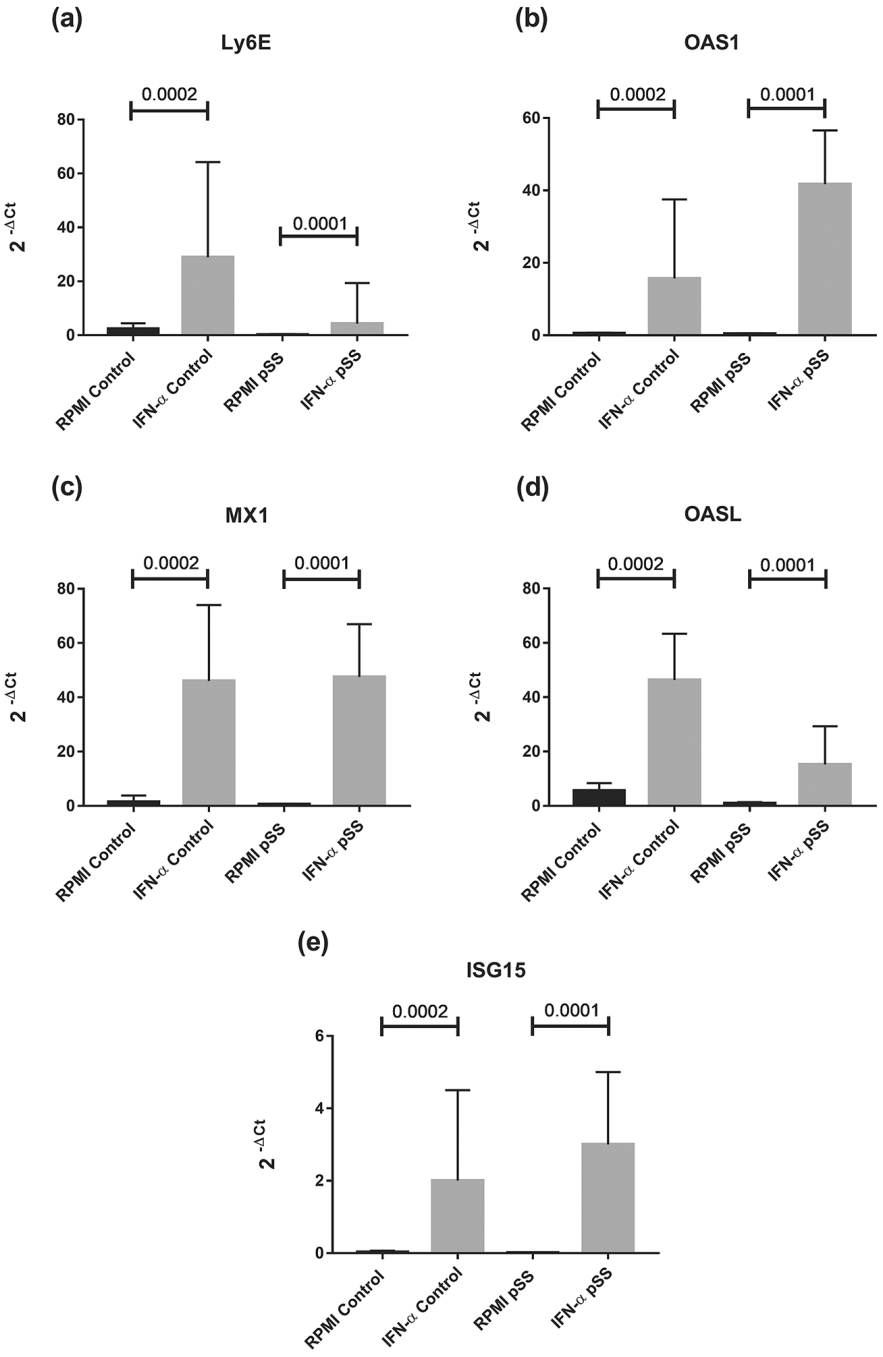

Fig. 2. Increased type I IFN-inducible gene expression in IFN-α-stimulated PBMC from patients with pSS.
Peripheral blood mononuclear cells were used to study their response after stimulation with IFN-α and levels of interferon stimulated genes (ISGs) expression were assessed by RT-PCR, GAPDH was used as a housekeeping gene. We observed that gene expression of Ly6E **(a)**, OAS1 **(b)**, MX1 **(c)**, OASL **(d)** and ISG15 **(e)** were significantly upregulated after IFN-α treatment in both pSS patients and healthy blood donors. GAPDH: glyceraldehyde-3-Phosphate dehydrogenase; LY6E: lymphocyte antigen 6 complex locus E; OAS1: 2'5'-oligoadenylate synthetase 1; MX1: myxovirus resistance 1; OASL: oligoadenylate synthase-like and ISG15: interferon-stimulated gene 15. The Wilcoxon paired *t*-test was used to assess differences in expression levels, and data are expressed as median (interquartile range).

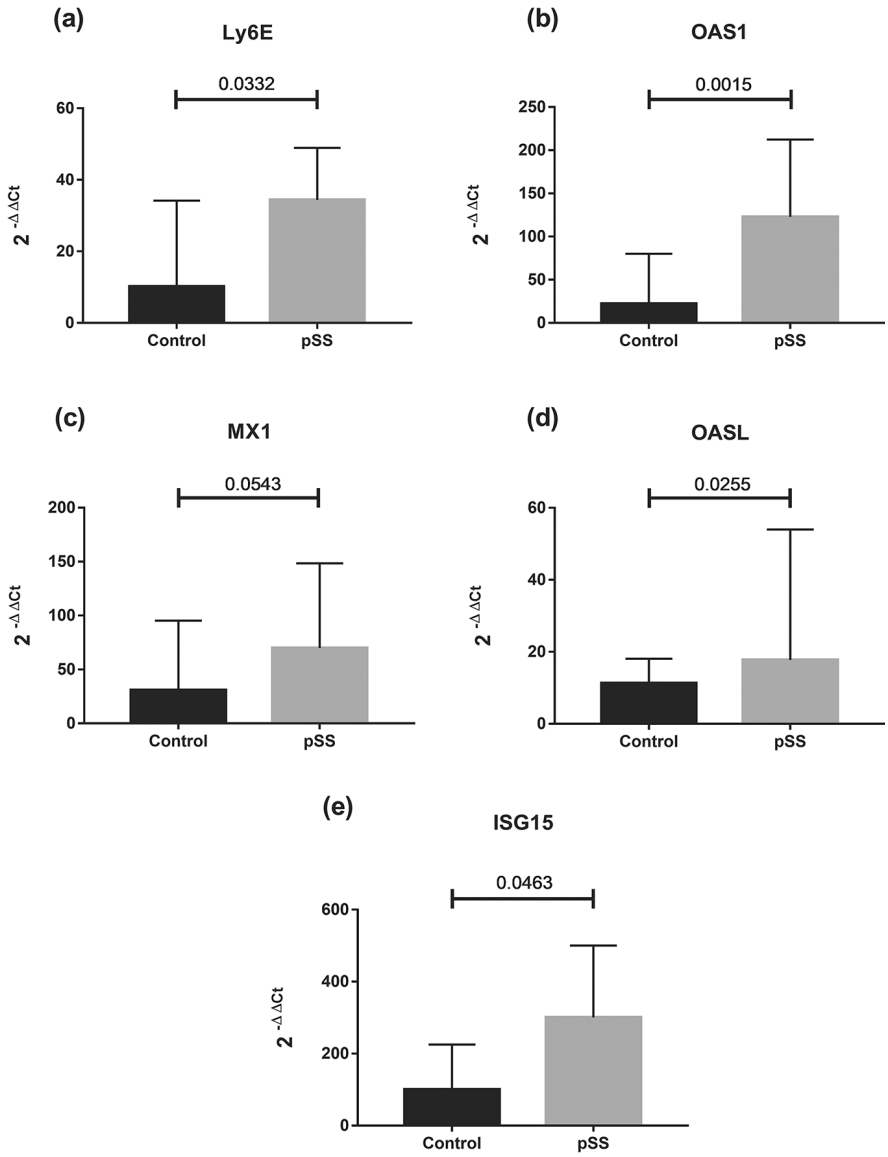

Fig. 3. Increased fold change type of I IFN-inducible gene expression in patients with pSS.
We observed that expression levels of Ly6E **(a)**, OAS1 **(b)**, OASL **(d)** and ISG15 **(e)** were significantly higher in pSS patients than those in healthy blood donors. LY6E: lymphocyte antigen 6 complex locus E; OAS1: 2'5'-oligoadenylate synthetase 1; MX1: myxovirus resistance 1; OASL: oligoadenylate synthase-like and ISG15: interferon-stimulated gene 15. The Mann-Whitney test was used to assess differences in fold change of expression levels, and data are expressed as median (interquartile range).

ulated and stimulated production of four interferon-regulated chemokines by PBMCs (Fig. 4). IFN-α stimulation increased the secretion of MCP-1 69.65, 52.93–90.89 *vs*. 228.4, 179.7–280.00; p=0.0001), MIP-1α (10.94, 3.35–34.05 *vs*. 184.10, 103.10–580.80; p=0.0001), IP-10 (0.37, 0.26–0.62 *vs*. 413.10, 238.60–514.80; p=0.0001), and BLC (1.46, 0.64–4.04 *vs*. 264.2, 124.2–503.00; p=0.0001) in PBMCs from patients with pSS. This phenomenon was replicated in PBMCs from healthy blood donors: MCP-1 (92.11, 65.12–117.7 *vs*. 186.3, 140.6–294.6; p=0.0002), MIP-1α (6.63, 4.11–9.52 *vs*. 14.81, 5.98–17.33; p=0.0215), IP-10 (0.67, 0.25–3.35 *vs*. 198.00, 80.60–705.4; p=0.0002), and BLC (3.96, 1.23–23.6 *vs*. 64.50, 24.25–112.80; p=0.0002). Notably, the fold-change values of MIP-1α (1.90, 1.20–2.80 *vs*. 21.86, 11.84–40.07; p<0.0001), IP-10 (296.00, 179.00–449.00 *vs*. 937.00, 598.00–1467.00; p=0.0040), and BLC (8.26, 6.10–21.76 *vs*. 180.90, 74.20-346.60; p<0.0001) secretion levels were higher in cells from patients with pSS than controls, while the fold-change values for MCP-1 (2.41, 1.50–4.00 *vs*. 3.06, 2.42–4.80; p=0.3255) were similar between groups. These results are presented in Figure 4.

We investigated whether MALAT1 expression levels in IFN-α-stimulated PBMC were related to serologic characteristics of patients with pSS. No significant correlation was found between MALAT1 expression levels and selected features of pSS patients (Supplementary Table S1). Finally, we also evaluated whether there was a correlation between MALAT1 expression with the expression of ISGs and with secretion levels of interferon-regulated chemokines in IFN-α-stimulated PBMC; however, there was no significant correlation of MALAT1 expression with chemokines production or ISGs expression (Suppl. Table S2 and S3).

Discussion

Attention has recently been drawn to the role of lncRNAs in pathogenesis of autoimmune diseases such as pSS. Expression of lncRNAs is regulated by several signalling molecules, including those involved in the immune response. The present study showed that IFN-α strongly regulates the expression lncRNA MALAT1, ISGs expression levels and interferon-regulated chemokines secretion levels in PBMCs from pSS patients.

The induction of MALAT1 by IFN-α has been demonstrated in $CD4^+$ T cells from SLE patients, however, the effect of IFN-α on MALAT1 expression in pSS has not been previously characterised (9). Little is known about the mechanisms that regulate MALAT1 expression, but MALAT1 has been involved in regulation of IFN inducible genes such as interferon-induced protein 44 (IFI44), since down regulation of MALAT1 results in down regulation of IFI44 (17). MALAT1 affects gene expression at the transcriptional and post-transcriptional stages of genes such as OASL, IFI44, and SPINK4 (18). IFI44 expression is upregulated in $CD14^+CD16^-$ monocytes from pSS patients (19, 20). In addition, MALAT1 contributes to the pathogenesis of autoimmune diseases by regulating silent

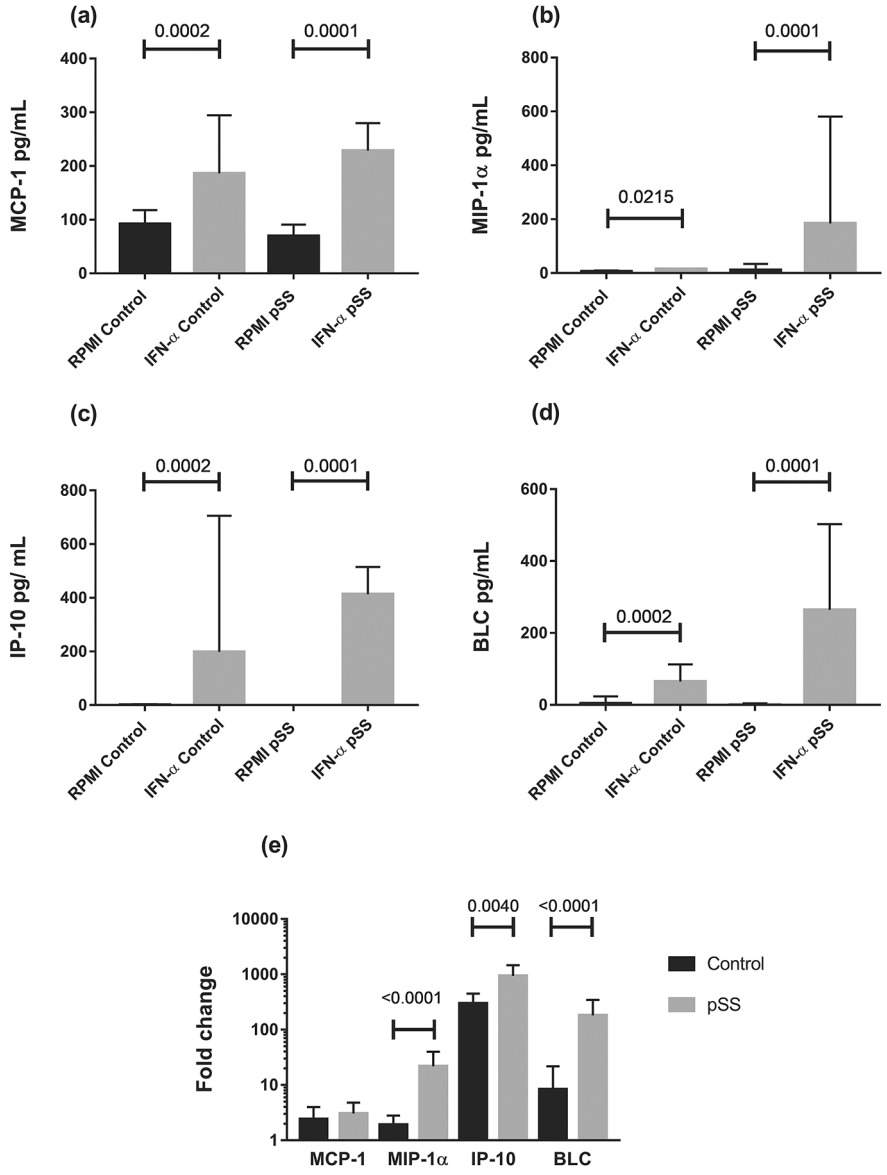

Fig. 4. Increased production levels of interferon-regulated chemokines in IFN-α-stimulated PBMC from patients with pSS.

We observed that concentrations of chemokines MCP-1 **(a),** MIP-1α **(b),** IP-10 **(c)** and BLC **(d)** in supernatants of PBMC were significantly upregulated after IFN-α treatment in both pSS patients and healthy blood donors. Furthermore, we observed increased fold change of production chemokines MIP-1α, IP-10 and BLC in patients with pSS **(e).** MCP-1: monocyte chemoattractant protein-1**;** MIP-1α: macrophage inflammatory protein-1 Alpha; IP-10: interferon-γ-inducible protein 10 and BLC: B-lymphocyte chemoattractant. The Wilcoxon paired *t*-test was used to assess differences in production levels of interferon-regulated chemokines and the Mann-Whitney test was used to assess differences in fold change of production level, data are expressed as median (interquartile range).

information regulator 1 (SIRT1) signalling and IL-21 expression in monocytes of SLE patients (21). MALAT1 expression is upregulated in patients with autoimmune diseases and has potential diagnostic value for the detection of autoimmunity. A single nucleotide polymorphisms rs4102217 in the MALAT1 gene is associated with susceptibility to SLE (22, 23).

Little is known about the potential role of lncRNAs in pSS. Expression profiles from some lncRNAs correlated with serological features of patients with pSS, and expression of lncRNA TMEVPG1 in $CD4^+$ T cells from pSS is significantly higher compared to healthy donors. In addition, the expression of TMEVPG1 corelated with circulating autoantibodies, erythrocyte sedimentation rate (ESR) and IgG levels in pSS patients (24). Interestingly, the lncRNAs expression profile in PBMCs from pSS (INC00426, TPTEP1-202, CYTOR, NRIR, and BISPR) correlated with the ESSDAI score and serum levels of IgG, C-reactive protein, and C4 complement (25). Other lncRNAs such as GABPB1-AS1 and PSMA3-AS1 are upregulated in PBMCs of pSS patients, and GABPB1-AS1 expression levels are positively correlated with IgG levels and percentage of B cells in pSS patients (26).

Recently, emerging evidence suggests that lncRNA nuclear-enriched abundant transcript 1 (NEAT1) plays crucial role in pSS. This lncRNA was overexpressed in the glands of pSS patients and there are relationships between the expression levels of NEAT1 and course of disease, rheumatoid factor and serum IgA levels (13, 27). NEAT1 regulates the expression of molecules such as IL-6 and CXCL10 and contribute to the inflammatory process mediated by TLR4 through the activation of the MAPK signalling pathway (28). In our study we found no association between serological characteristics of pSS patients and MALAT1 expression levels, which may be explained by the small sample size, although an effect dependent on ethnicity cannot be ruled out.

This study analysed the ISGs expression in IFN-α-stimulated PBMC from pSS patients; as expected ISGs levels were elevated in cells from pSS patients. Interestingly, the response to IFN-α by cells from pSS patients appear to be strongest, since a high expression of ISGs was observed in pSS patients; this may be explained by higher sensitivity to IFN-α and raised MALAT1 expression in cells from pSS patients. Other findings in the literature suggest that MALAT1 regulated ISGs expression in SLE, because silencing MALAT1 inhibits the expression of OAS2, OAS3 and OASL mRNAs in some cell from SLE patients (9). MALAT1 may function as a competing endogenous RNA (ceRNA) of some microRNAs (miR-370-3p, miR-620, miR-665, miR-1270, miR-3064-5p and miR-6504-5p) to block their effects on OAS2, OAS3, and OASL. Interestingly, the expres-

sion of miR-665 expression and other microRNA is upregulated in T cells from pSS patients (9, 29).

Excessive IFN-inducible gene expression plays role in the pathogenesis of pSS and correlates with clinical features of pSS patients, such as anti-Ro/SSA and anti-La/SSB titres (30). Furthermore, ISGs expression on CD14 monocytes from pSS patients is associated with ESSDAI and laboratory parameters (11). Similarity, transcriptomic analysis revealed a set of ISGs (IFI44, MX1 and ISG15) to be highly expressed in both minor salivary glands biopsies and peripheral blood of SS patients (31). ISGs contribute to the pathogenesis of pSS by activating some crucial biological processes of the disease, such as the induction of polyclonal B cell, resulting in increased autoantibody production (11).

In addition, we also investigated the production of interferon-regulated chemokines (MCP-1, MIP-1α, IP-10 and BLC) in IFN-α-stimulated PBMC. Cells from patients produced high levels interferon-regulated chemokines. Interestingly, we observed that the fold change production levels of MIP-1α, IP-10 and BLC were increased in pSS patients. Recently, attention has been drawn to the role of chemokines in the pathogenesis of pSS, because chronic inflammation of exocrine glands is dependent of chemokines (32). Furthermore, high levels of chemokines driven by type I IFN may disrupt normal trafficking and chemotaxis of immune cells in autoimmune diseases (33). Our results showed no association between MALAT1 expression and IFN-α-regulated chemokines in pSS patients, but in other autoimmune diseases, lncRNAs silencing reduce the expression of chemokines as well as cytokines, including IL-6 and IP-10 (28).

Some limitations of our study should be acknowledged, including the relatively small sample size, the significant age difference between patients and controls, as well as the origin of patients from a single centre.

In conclusion, our data provide an interesting insight into the abnormal regulation of the lncRNA MALAT1 in IFN-α-stimulated PBMCs from patients with pSS. Give the unusually high capacity of cells from pSS patients to express mRNAs of various ISGs and produce interferon-responsive chemokines, it is likely that targeted therapies to block these molecules may be of benefit to pSS patients.

References

1. CHEN J, AO L, YANG J: Long non-coding RNAs in diseases related to inflammation and immunity. *Ann Transl Med* 2019; 7: 494. https://doi.org/10.21037/atm.2019.08.37
2. ZOU Y, XU H: Involvement of long noncoding RNAs in the pathogenesis of autoimmune diseases. *J Transl Autoimmun* 2020; 3: 100044. https://doi.org/10.1016/j.jtauto.2020.100044
3. YANG C, ZHANG Y, YANG B: MIAT, a potent CVD-promoting lncRNA. *Cell Mol Life Sci* 2021; 79: 43. https://doi.org/10.1007/s00018-021-04046-8
4. YANG Z, LIN S-D, ZHAN F, LIU Y, ZHAN Y-W: LncRNA GAS5 alleviates rheumatoid arthritis through regulating miR-222-3p/Sirt1 signalling axis. *Autoimmunity* 2021; 54: 13-22. https://doi.org/10.1080/08916934.2020.1846183
5. SHI X, SUN M, WU Y *et al*.: Post-transcriptional regulation of long noncoding RNAs in cancer. *Tumour Biol* 2015; 36: 503-13. https://doi.org/10.1007/s13277-015-3106-y
6. HUTCHINSON JN, ENSMINGER AW, CLEMSON CM, LYNCH CR, LAWRENCE JB, CHESS A: A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMC Genomics* 2007; 8: 39. https://doi.org/10.1186/1471-2164-8-39
7. ZHANG X, HAMBLIN MH, YIN KJ: The long noncoding RNA Malat1: its physiological and pathophysiological functions. *RNA Biol* 2017; 14: 1705-14. https://doi.org/10.1080/15476286.2017.1358347
8. AMODIO N, RAIMONDI L, JULI G *et al*.: MALAT1: a druggable long non-coding RNA for targeted anti-cancer approaches. *J Hematol Oncol* 2018; 11: 63. https://doi.org/10.1186/s13045-018-0606-4
9. GAO F, TAN Y, LUO H: MALAT1 is involved in type I IFNs-mediated systemic lupus erythematosus by up-regulating OAS2, OAS3, and OASL. *Braz J Med Biol Res* 2020; 53: e9292. https://doi.org/10.1590/1414-431X20209292
10. NEZOS A, GRAVANI F, TASSISOU A *et al*.: Type I and II interferon signatures in Sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjögren's related lymphomagenesis. *J Autoimmun* 2015; 63: 47-58. https://doi.org/10.1016/j.jaut.2015.07.002
11. BRKIC Z, MARIA NI, VAN HELDEN-MEEUWSEN CG *et al*.: Prevalence of interferon type I signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. *Ann Rheum Dis* 2013; 72: 728-35. https://doi.org/10.1136/annrheumdis-2012-201381
12. ABDULAHAD WH, KROESE FGM, VISSINK A, BOOTSMA H: Immune regulation and B-cell depletion therapy in patients with primary Sjögren's syndrome. *J Autoimmun* 2012; 39: 103-11. https://doi.org/10.1016/j.jaut.2012.01.009
13. SHI H, CAO N, PU Y, XIE L, ZHENG L, YU C: Long non-coding RNA expression profile in minor salivary gland of primary Sjögren's syndrome. *Arthritis Res Ther* 2016; 18: 109. https://doi.org/10.1186/s13075-016-1005-2
14. CHEN Y, CHEN Y, ZU B *et al*.: Identification of long noncoding RNAs lnc-DC in plasma as a new biomarker for primary Sjögren's syndrome. *J Immunol Res* 2020; 2020: 9236234. https://doi.org/10.1155/2020/9236234
15. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017; 69: 35-45. https://doi.org/10.1002/art.39859
16. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9. https://doi.org/10.1136/ard.2009.110619
17. SANDHYA P, JOSHI K, SCARIA V: Long non-coding RNAs could be potential key players in the pathophysiology of Sjögren's syndrome. *Int J Rheum Dis* 2015; 18: 898-905. https://doi.org/10.1111/1756-185X.12752
18. MIYAGAWA R, TANO K, MIZUNO R *et al*.: Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles. *RNA* 2012; 18: 738-51. https://doi.org/10.1261/rna.028639.111
19. WILDENBERG ME, VAN HELDEN-MEEUWSEN, VAN DE MERWE JP, DREXHAGE HA, VERSNEL MA: Systemic increase in type I interferon activity in Sjögren's syndrome: a putative role for plasmacytoid dendritic cells. *Eur J Immunol* 2008; 38: 2024-33. https://doi.org/10.1002/eji.200738008
20. MARIA NI, BRKIC Z, WARIS M *et al*.: MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. *Ann Rheum Dis* 2014; 73: 1052-9. https://doi.org/10.1136/annrheumdis-2012-202552
21. YANG H, LIANG N, WANG M *et al*.: Long noncoding RNA MALAT-1 is a novel inflammatory regulator in human systemic lupus erythematosus. *Oncotarget* 2017; 8: 77400-6. https://doi.org/10.18632/oncotarget.20490
22. MAO YM, HE YS, WU GC *et al*.: Association of MALAT-1 gene single nucleotide polymorphisms with genetic susceptibility to systemic lupus erythematosus. *Lupus* 2021; 30: 1923-30. https://doi.org/10.1177/09612033211040366
23. FOUAD NA, SHAKER OG, MOHAMED EA *et al*.: Diagnostic potential of metastasis-associated-lung-adenocarcinoma-transcript-1 (MALAT-1) and TNF-α and hnRNPL related immunoregulatory long non-coding RNA (THRIL) in systemic lupus erythematosus patients: Relation to disease activity. *Egyptian Rheumatologist* 2019; 41(3): 197-201. https://doi.org/10.1016/j.ejr.2018.11.002

24. WANG J, PENG H, TIAN J *et al*.: Upregulation of long noncoding RNA TMEVPG1 enhances T helper type 1 cell response in patients with Sjögren syndrome. *Immunol Res* 2016; 64: 489-96. https://doi.org/10.1007/s12026-015-8715-4
25. PENG Y, LUO X, CHEN Y *et al*.: LncRNA and mRNA expression profile of peripheral blood mononuclear cells in primary Sjögren's syndrome patients. *Sci Rep* 2020; 10: 19629. https://doi.org/10.1038/s41598-020-76701-2
26. CHEN X, CHENG Q, DU Y, LIU L, WU H: Differential long non-coding RNA expression profile and function analysis in primary Sjögren's syndrome. *BMC Immunol* 2021; 22: 47. https://doi.org/10.1186/s12865-021-00439-3
27. YE L, SHI H, YU C *et al*.: LncRNA Neat1 positively regulates MAPK signaling and is involved in the pathogenesis of Sjögren's syndrome. *Int Immunopharmacol* 2020; 88: 106992. https://doi.org/10.1016/j.intimp.2020.106992
28. ZHANG F, WU L, QIAN J *et al*.: Identification of the long noncoding RNA NEAT1 as a novel inflammatory regulator acting through MAPK pathway in human lupus. *J Autoimmun* 2016; 75: 96-104. https://doi.org/10.1016/j.jaut.2016.07.012
29. WANG-RENAULT SF, BOUDAOUD S, NOCTURNE G *et al*.: Deregulation of microRNA expression in purified T and B lymphocytes from patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2018; 77: 133-40. https://doi.org/10.1136/annrheumdis-2017-211417
30. EMAMIAN ES, LEON JM, LESSARD CJ *et al*.: Peripheral blood gene expression profiling in Sjögren's syndrome. *Genes Immun* 2009; 10: 285-96. https://doi.org/10.1038/gene.2009.20
31. CINOKU II, VERROU KM, PIPERI E, VOULGARELIS M, MOUTSOPOULOS HM, MAVRAGANI CP: Interferon (IFN)-stimulated gene 15: A novel biomarker for lymphoma development in Sjögren's syndrome. *J Autoimmun* 2021; 123: 102704. https://doi.org/10.1016/j.jaut.2021.102704
32. AMFT N, BOWMAN SJ: Chemokines and cell trafficking in Sjögren's syndrome. *Scand J Immunol* 2001; 54: 62-9. https://doi.org/10.1046/j.1365-3083.2001.00970.x
33. BAUER JW, BAECHLER EC, PETRI M *et al*.: Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. *PLoS Med* 2006; 3: e491. https://doi.org/10.1371/journal.pmed.0030491