LncRNA *XIST* alters the balance of peripheral blood immune cells in systemic lupus erythematosus by regulating the *miR-17-92, OFLM4* and *CEACAM8* axis

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Abstract

Background

X-inactive-specific transcript (*XIST*) has been shown to silence linked genes on the X chromosome that may be related to the pathogenesis of systemic lupus erythematosus (SLE) in female patients. However, the function of XIST in SLE at other levels remains unclear. The present study aimed to clarify the correlations between *XIST* expression and SLE clinical features and the contribution of *XIST* to SLE pathogenesis at the transcriptome level.

Methods

Expression of XIST in 79 SLE patients and 23 healthy controls was detected by quantitative-polymerase chain reaction. Bioinformatics methods were used to explore the function and regulatory mechanism of *XIST*.

Results

Expression of XIST was significantly upregulated in SLE patients compared with healthy controls, and had a high diagnostic value for SLE. Importantly, SLE patients with high expression of XIST tended to have elevated levels of total T cells and CD8 + T cells, but reduced levels of Treg cells and NK cells. Bioinformatics analyses suggested that XIST may regulate the expression of *OLFM4* and *CEACAM8* by acting as a spongy body for *miR-20a*, *miR-92a*, *miR-106a*, and *miR-449a*. Furthermore, *OLFM4* and *CEACAM8* are significantly upregulated in SLE patients and had significant positive correlations with expression of *XIST*.

Conclusions

We propose that *XIST* may alter the balance of peripheral blood immune cells in SLE by acting as a spongy body for the *miR-17-92* cluster and promoting the expression of *OLFM4* and *CEACAM8*, resulting in immune dysregulation and tissue damage in SLE.

Background

Systemic lupus erythematosus (SLE) is one of the most heterogeneous autoimmune diseases, but mainly occurs in women and involves multiple systems [1]. The diversity of SLE brings great challenges to its clinical diagnosis and treatment. Various pathogenic factors, including environmental, genetic, epigenetic, hormonal, and immune, lead to a variety of clinical manifestations and organ damage in SLE [2]. However, the pathogenesis of SLE remains unclear. To better understand SLE and provide effective treatments for patients, it is important to clarify the mechanism of the disease.

Epigenetic and immune factors are susceptibility factors for SLE [2, 3]. Regulation of gene expression by non-coding RNAs is one of the epigenetic processes. Long non-coding RNAs (lncRNAs) comprise a class

of non-coding RNAs that exceed 200 nt and have a variety of biological functions, including growth and development, immune regulation, and tumour microenvironment regulation. Many studies have been shown that lncRNAs were involved in disease activity, immune regulation, and tissue injury in SLE [4–6]. However, the understanding of lncRNAs in SLE remains insufficient.

As a female-dominated disease, clarification of the related genes on the X chromosome is important to achieve a deeper understanding of SLE. X-inactive-specific transcript (*XIST*) is a lncRNA that can silence gene expression on the inactive X chromosome and lead to X chromosome inactivation [7]. Recently, several investigators have reported a role of *XIST* in skewed allelic expression on the X chromosome in lymphocytes and X-linked gene dose compensation in T cells of SLE patients [8, 9]. These findings strongly suggest that *XIST* is involved in the pathogenesis of SLE. However, the studies were mainly focused on gene silencing on the X chromosome and did not investigate the function of *XIST* at other levels such as the transcriptome level. In addition, the number of samples used for verification was small (≤ 10 samples).

In the present study, we used a large sample (79 SLE patients and 23 healthy controls) to detect the expression of lncRNA *XIST* in SLE and analyze its correlations with the clinical characteristics of SLE patients. In addition, through bioinformatics methods, the target miRNAs of *XIST* were predicted, and a competitive endogenous RNA (ceRNA) network was constructed to reveal the regulatory function of *XIST* at the transcriptome level. We found that *XIST* not only has the function of gene silencing on the X chromosome in SLE, but can also play a role in altering the balance of peripheral blood immune cells in SLE patients and promoting the onset and progression of the disease by regulating miRNA and gene expression levels. Our study expands the understanding of *XIST* in the pathogenesis of SLE and may provide a basis for the development of new diagnostic and therapeutic approaches.

Methods

Patients and healthy controls

We recruited 79 SLE patients and 23 healthy controls for the study. All SLE patients met the American College of Rheumatology 1997 criteria and the Systemic Lupus International Collaborating Clinics 2012 criteria for SLE and were selected from the Department of Rheumatology, The Second Affiliated Hospital of Zhejiang University School of Medicine from August 2020 to December 2020 [10, 11]. Our study was approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University School of Medicine, China. All participants signed the written informed consent. Expression of *XIST* was determined in all 79 SLE samples to analyze the correlations with clinical features. The clinical characteristics and laboratory tests of the 79 SLE patients are shown in Table 1.

Table 1
Clinical characteristics of the 79 SLE patients

Variable	N	median (range)	Variable	N	Positive rate, n (%)
General features			**Clinical features**		
Age (years)	79	37.5 (16 to 66)	Facial erythema	79	25 (31.6%)
Gender (Female/male)	71/8	/	Oral ulcer	79	8 (10.1%)
Course (Month),	79	79.7 (0.5 to 480)	Arthralgia	79	27 (34.2%)
SLEDAI	79	9.95 (0 to 32)	Fever	79	15 (19.0%)
Laboratory test			Proteinuria	77	39 (50.7)
ESR (0-20mm/h)	78	37.73 (2 to140)	Raynaud's phenomenon	79	11 (13.9%)
CRP (mg/L)	79	10.17 (0.1 to136)	Photoallergic	79	4 (5.1%)
IgG (g/L)	78	14.11 (3.1 to 36.1)	Dropsy of serous cavity	79	36 (45.9%)
IgM (g/L)	78	0.88 (0.17 to 3.03)	Alopecia	79	16 (20.3%)
IgA (g/L)	78	2.43 (0.78 to 5.22)	Lupus nephritis	79	38 (48.1%)
IgG4 (g/L)	67	0.50 (0.01 to 2)	Neuropsychiatric lupus	79	6 (7.6%)
C3 (g/L)	78	0.55 (0.19 to 1.32)	Hypertension	78	23 (29.5)
C4 (mg/L)	43	92.81 (17 to 352)	**Autoantibodies**		
24-hour proteinuria (mg/24h)	74	1363.9 (0.22 to 8316)	ANA	79	71 (89.9%)
WBC (/L)	79	6.99 (1.7 to 18.5)	Anti-dsDNA	79	45 (57.0%)
Hb (g/L)	79	104.28 (62 to 146)	Anti-SSA	79	50 (63.3%)

Abbreviations: SLEDAI, systemic lupus erythematosus disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; C3/C4, complement 3/4; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; Treg, regulatory T cells; ANA: antinuclear antibodies; SSA, single-stranded DNA; anti-SSB, anti RNA-protein complex antibodies; RNP, ribonucleoprotein; RPP, Ribosomal P protein.

PLT (/L)	79	182.04 (10 to 838)	Anti-Ro52	79	46 (58.2%)
Lymphocyte percentage (%)	73	21.45 (3.48 to 74.58)	Anti-SSB	78	10 (12.8%)
Total T cell (%)	73	64.11 (5.13 to 92.3)	Anti-Smith	79	15 (19.0%)
CD4+/CD8 + ratio	73	1.0 (0.21 to 8.46)	Anti-RNP	79	27 (34.2%)
CD4 + T cell (%)	69	28.12 (4.5 to 54.4)	Anti-RPP	79	37 (46.8%)
CD8 + T cell (%)	69	35.18 (2.65 to 68.9)	Anti-cardiolipin	79	10 (12.7%)
Activated lymphocyte CD25+ (%)	62	17.04 (4.1 to 88.34)	Anti-nucleosome	79	37 (46.8%)
Activated helper/induced T cells (%)	62	35.18 (2.65 to 68.9)	Anti-histone	78	27 (34.6%)
NK-like T cells (%)	62	2.52 (0.1 to 12.5)			
NK cell (%)	67	7.67 (0.13 to 43.94)			
Total B/ Lymphocyte (%)	67	16.29 (1.4 to 67.13)			
Treg (%)	67	3.53 (0.45 to 18.61)			
Treg/CD4 + T cell (%)	67	6.83(0.1 to 20.9)			

Abbreviations: SLEDAI, systemic lupus erythematosus disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; C3/C4, complement 3/4; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; Treg, regulatory T cells; ANA: antinuclear antibodies; SSA, single-stranded DNA; anti-SSB, anti RNA-protein complex antibodies; RNP, ribonucleoprotein; RPP, Ribosomal P protein.

Real-time quantitative reverse transcription-polymerase chain reaction (q-RT-PCR)

Peripheral blood samples were collected from the SLE patients and healthy controls and used for extraction of peripheral blood mononuclear cells (PBMCs) by Ficoll density gradient centrifugation. Total RNA was isolated from the PBMCs using TRIzol reagent (Ambion, Thermo Fisher Scientific, Shanghai, China). Takara reverse transcription and PCR reagents (Takara, Beijing, China) were used for RT and fluorescence quantitative PCR. A 7500 Fast Real-Time PCR system (Applied Biosystems) was used for the q-PCR analyses. The $2^{-\Delta\Delta Ct}$ method was employed to determine gene expression and *GAPDH* was

evaluated as an internal reference. The primer sequences were: XIST forward, 5'-AGGGAGCAGTTTGCCCTACT-3'; XIST reverse, 5'-CACATGCAGCGTGGTATCTT-3'; OLFM4 forward, 5'-TAGGCAGCGGAGGTTCTGTGTC-3'; OLFM4 reverse, 5'-AATTCCAAGCGTTCCACTCTGTCC-3'; CEACAM8 forward, 5'-CCACCACTGCTCAGCTCACTATTG-3'; CEACAM8 reverse, 5'-AGTTGTAGCCACGAGGGTCCTG-3'; GAPDH forward, 5'-AAGGTGAAGGTCGGAGTCAA-3'; GAPDH reverse, 5'-AATGAAGGGGTCATTGATGG-3'.

Prediction of target miRNAs and genes

Four online tools, LncBase Predicted v.2 (www.microrna.gr/LncBase), lncRNome (http://genome.igib.res.in/lncRNome), miRcode (http://www.mircode.org/), and RNA22 (http://cbcsrv.watson.ibm.com/rna22.html), were used to predict the target miRNAs of *XIST*. Target genes of the miRNAs were predicted by RNA22, microT-CDS (http://www.microrna.gr/webServer), miRDB (http://mirdb.org), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/). We took the intersections as our results.

Construction of a ceRNA network

Cytoscape 3.8.0 software (https://cytoscape.org/) was used to construct and visualize the ceRNA network. The Cytohubba plug-in was used to identify hub genes [12].

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

An online tool, WebGestalt (www.webgestalt.org/), was used for Gene Ontology enrichment analysis of the target genes. ClueGO + CluePedia, a plug-in of Cytoscape, was used for KEGG enrichment analysis and the results were shown in a visual network.

Statistical analysis

IBM SPSS Statistics 25 software (IBM Inc., Chicago, IL, USA) was used to analyze the clinical data of all samples, draw receiver operator characteristic (ROC) curves, and calculate the areas under the ROC curves (AUCs). GraphPad Prism 8.0 software (GraphPad Inc., San Diego, CA, USA) was used to draw scatter diagrams and perform correlation analyses. Student's *t*-test was used to compare the differences between the two groups where applicable. $P < 0.05$ was considered statistically significant.

Results

Expression of XIST in SLE patients and its correlations with clinical traits

Initially, we detected the expression of *XIST* in the SLE patients and healthy controls by q-RT-PCR. We found that *XIST* was significantly upregulated in SLE patients compared with healthy controls (Fig. 1A, p = 0.0043). Through a ROC curve analysis, we found that *XIST* had a high diagnostic value for SLE (Fig. 1B, AUC = 0.762, 95% CI: 0.658 to 0.867, $p = 0.000136$). We further analyzed the correlations between *XIST* expression and clinical characteristics of SLE patients. Compared with male patients and older

patients, *XIST* was significantly upregulated in female patients and younger patients (Fig. 1C and 1D). Moreover, *XIST* was highly expressed in patients with arthralgia, anti-Ro-52 antibody positivity, and elevated WBC (normal range, 4–10 g/L) (Fig. 1E–1G). Interestingly, *XIST* expression was increased in patients with more T cells or fewer NK cells (Fig. 1H and 1I).

XIST may regulate the balance of peripheral blood immune cells

To better explore the relationships between *XIST* and peripheral blood immune cells, we obtained detailed immune cell test results for the patients and divided the patients into two groups based on their *XIST* expression. Consistent with our observations, total T cells (Fig. 2A, p = 0.041) were elevated in the high expression group, while NK cells were reduced (Fig. 2G, p = 0.0082). In addition, we found that CD3 + CD8 + T cells (Fig. 2B, p = 0.0301) were elevated in the high expression group, while CD4/CD8 ratio (Fig. 2D, p = 0.045), Treg cells (Fig. 2E, p = 0.0308), and activated CD25 + lymphocytes (Fig. 2F, p = 0.0389) were reduced. However, CD3 + CD4 + T cells (Fig. 2C) and total B cells (Fig. 2H) did not differ significantly between the two groups. We further found that total T cells (Fig. 3A, r = 0.2464, $p = 0.0479$) and CD3 + CD8 + T cells (Fig. 3B, r = 0.2470, $p = 0.0455$) were significantly positively correlated with *XIST* expression, while NK cells (Fig. 3C, r = − 0.4054, $p = 0.0039$) were significantly negatively correlated with *XIST* expression. Unfortunately, the correlation between total B cells and *XIST* expression was not significant (Fig. 3D, r = − 0.1424, $p = 0.2695$).

Construction of a ceRNA network and functional enrichment analysis of the target genes

A lncRNA exerts its biological functions through its target miRNAs and target genes. In the present study, we obtained 16 target miRNAs through prediction by online tools (Fig. 4A). According to the ceRNA hypothesis, an lncRNA competitively binds to an miRNA to regulate gene expression [13]. Through a literature review, we found that 8 of the 16 miRNAs were significantly downregulated in PBMCs from SLE patients, namely *miR-17*, *miR-19b*, *miR-20a*, *miR-92a*, *miR-106a*, *miR-125b*, *miR-196a*, and *miR449a* [14–18]. Next, the target genes of these downregulated miRNAs were predicted by four online tools. The intersected results for the target genes were further intersected with our previous transcriptome sequencing results (GEO accession: GSE162828; we extracted 1508 upregulated differentially expressed genes in SLE). Finally, we obtained 115 upregulated target genes and constructed a ceRNA network that included 1 lncRNA, 8 miRNAs, 115 mRNAs, and 150 edges (Fig. 4B). Hub transcripts (*XIST*, 8 miRNAs, and 6 mRNAs) were identified by the MCC algorithm of Cytohubba (Fig. 4C) [12]. The 6 hub mRNAs were *OLFM4*, *CEACAM8*, *SH3TC2*, *PRR11*, *NR4A2*, and *IRF1*. We further performed GO and KEGG enrichment analyses for the 115 upregulated target genes, and the results are shown in Fig. 4D and 4E. Most enriched GO terms were immune effector process and leukocyte-mediated immunity and immune response. Significant pathway enrichment terms were Fibronectin matrix formation, *MAP2K* and *MAPK* activation, Bladder cancer, C-type lectin receptor signalling pathway, *HIF-1* signalling pathway, and Nuclear receptor transcription pathway.

Correlations between XIST expression and hub genes

We randomly selected two of the six hub genes, *OLFM4* and *CEACAM8*, for further verification in samples from 40 SLE patients and 23 healthy controls. Consistent with the sequencing results, *OLFM4* and *CEACAM8* were significantly upregulated in SLE patients (Fig. 5A and 5B). Next, we performed correlation analyses between the expression of these two mRNAs and *XIST* in SLE patients. Interestingly, we found that expression of *XIST* was significantly positively correlated with expression of *OLFM4* (Fig. 5C, r = 0.3767, $p = 0.0166$) and *CEACAM8* (Fig. 5D, r = 0.5441, $p = 0.0003$).

These findings suggest that *XIST* may play a biological role in SLE through regulation of *OLFM4* and *CEACAM8*.

Discussion

The pathogenesis of SLE is complex and heterogeneous, rendering diagnosis and treatment of the disease very difficult. SLE also has characteristics of female dominance, immune dysregulation, multisystem involvement, and organ damage [2]. Therefore, understanding the mechanisms of the immune dysregulation and tissue damage in female SLE patients is of particular importance. LncRNAs have many functions, including involvement in the progression of various diseases such as SLE [4, 19, 20]. Previous studies demonstrated that changes in expression of the lncRNA *XIST* can silence linked genes on the X chromosome that may be related to the pathogenesis of SLE in female patients [8, 9]. However, the relationships between *XIST* expression and SLE clinical features and the contribution of *XIST* to the pathogenesis of SLE at other levels remain unclear.

In the present study, we found the lncRNA *XIST* was significantly upregulated in SLE patients and had a good diagnostic value for SLE. Furthermore, *XIST* expression was elevated in young or female patients and patients with arthralgia or anti-Ro-52 antibody positivity. It was reported that SLE mainly affects young women of reproductive age, with initial signs of physical symptoms, rash, and arthritis [1]. This suggests that *XIST* may be a potential biomarker for the diagnosis of early SLE. However, the sample size in the present study was not sufficiently large, and the results require verification in a large prospective cohort. Nevertheless, the present study lays a partial foundation for future cohort research.

To explore the effects of *XIST* on SLE at different levels, we analyzed the correlations between *XIST* expression and clinical features. Interestingly, we found that patients with elevated *XIST* expression tended to have high levels of total T cells and CD8 + T cells, but reduced levels of Treg cells, activated CD25 + lymphocytes, and NK cells. T cells contribute to the initiation and persistence of immunity in SLE and are involved in organ damage in the disease [21]. Blanco *et al.* [22] found that activated CD8 + T cells were significantly increased, had a cytotoxic effector T cell phenotype, and generated high levels of soluble nucleosomes and granzyme B in patients with active SLE. Furthermore, CD8 + T cells not only had a role in the blood, but also accumulated and infiltrated in the glomerular guard region of the kidney, leading to tissue injury and organ pathology [23]. On the contrary, in patients with active SLE, the number of CD4 + CD25 + Treg cells was reduced, and their inhibitory function was insufficient, allowing

overactivation of other T cells to cause tissue inflammation and damage [24, 25]. Like Treg cells, the number of NK cells was significantly decreased in patients with active SLE [26]. However, Suárez-Fueyo *et al.* [27] reported that NK cells in SLE patients exhibited increased cytotoxicity and pro-inflammatory phenotypes that were associated with down-regulation of *CD3ζ*. These observations are consistent with the present findings. On the one hand, *XIST* may promote the increase and overactivation of total T cells and CD8 + T cells by affecting the number and function of Treg cells, leading to immune dysregulation and tissue damage in SLE. On the other hand, *XIST* may lead to stronger cytotoxicity and inflammatory phenotypes of NK cells by reducing the number of NK cells, and ultimately reducing their protective function. Therefore, we believe that *XIST* has multiple regulatory effects on the immune system in SLE patients, regulating not only adaptive immunity, but also innate immunity. This is achieved by altering the balance of immune cells in the peripheral blood of SLE patients.

To explore the molecular mechanism of lncRNA *XIST* involvement in the pathogenesis of SLE, we constructed a ceRNA network that can reflect the regulatory mechanism of an lncRNA at the transcriptome level [13]. A lncRNA can act as a sponge body for miRNAs to regulate gene expression [20]. Here, we found that *XIST* may affect the expression of 115 genes by regulating 8 miRNAs. Enrichment analyses for these 115 genes indicated that *XIST* may be involved in leukocyte-mediated immunity and immune response through *MAP2K* and *MAPK* activation, C-type lectin receptor signalling pathway, or *HIF-1* signalling pathway. These findings are consistent with our conclusion that *XIST* can change the balance of peripheral blood immune cells in SLE. Moreover, we selected two hub genes, *OLFM4* and *CEACAM8*, to verify the accuracy and reliability of the predicted results. We found that both of these genes were significantly upregulated in SLE patients and had significant positive correlations with expression of *XIST*. These findings not only indicate that our prediction results are relatively reliable, but also suggest that *XIST* may regulate the expression of *OLFM4* and *CEACAM8* by acting as a spongy body for *miR-20a*, *miR-92a*, *miR-106a*, and *miR-449a*, thereby exerting its functions. Interestingly, *miR-20a*, *miR-92a*, *miR-106a*, and two other miRNAs, *miR-17* and *miR-19b*, belong to the *miR-17-92* cluster or its paralog [28]. This family has been shown to play important roles in the immune system, cardiovascular system, and tumours, among others. In the adaptive immune system, *miR-17-92* has a key role in the antigen response of T lymphocytes [29]. In Treg cells, *miR-17-92* is critical for the function and accumulation of Treg cells during the autoimmune-mediated stress response. Once *miR-17-92* is lost, Treg cells lose their regulatory function [30]. Similarly, both the number and function of Treg cells were partially deficient in SLE, which may be related to the low expression of *miR-17-92*. In CD8 + T cells, *miR-17-92* decreased gradually during the differentiation of CD8 + T cells, which was associated with increased proliferation potential [31, 32]. These lines of evidence also support our conclusion. However, the association between *miR-17-92* and NK cells remains unclear. Further research is needed to explore this issue in the future. It will be an interesting and meaningful topic to pursue.

Conclusions

In conclusion, we propose that the lncRNA *XIST* may alter the balance of peripheral blood immune cells in SLE by acting as a spongy body for the *miR-17-92* cluster and promoting the expression of *OLFM4* and

CEACAM8, resulting in immune dysregulation and tissue damage in SLE. However, the study still lacks some experimental data to confirm our conclusion, and thus further studies are warranted to supplement our findings in the future.

Abbreviations

X-inactive-specific transcript, XIST; systemic lupus erythematosus, SLE; Long non-coding RNAs, lncRNAs; competitive endogenous RNA, ceRNA; Real-time quantitative reverse transcription-polymerase chain reaction, q-RT-PCR; peripheral blood mononuclear cells, PBMCs; Gene Ontology, GO; Kyoto Encyclopedia of Genes and Genomes, KEGG; receiver operator characteristic, ROC; the areas under the ROC curves, AUCs.

Declarations

1. Ethics approval and consent to participate: Our study was approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (approval number: 2020-306 and 2020-445).

2. Consent for publication: Not applicable.

3. Availability of data and materials: The [GSE datasets] data that support the findings of this study are available in [GEO database] with the identifier(s) [https://www.ncbi.nlm.nih.gov/geo/, and the data Accession, GSE162828].

4. Competing interests: The authors declare no conflicts of interest regarding this work. The corresponding authors have the right to speak on behalf of all authors and do speak on behalf of all authors.

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Figures

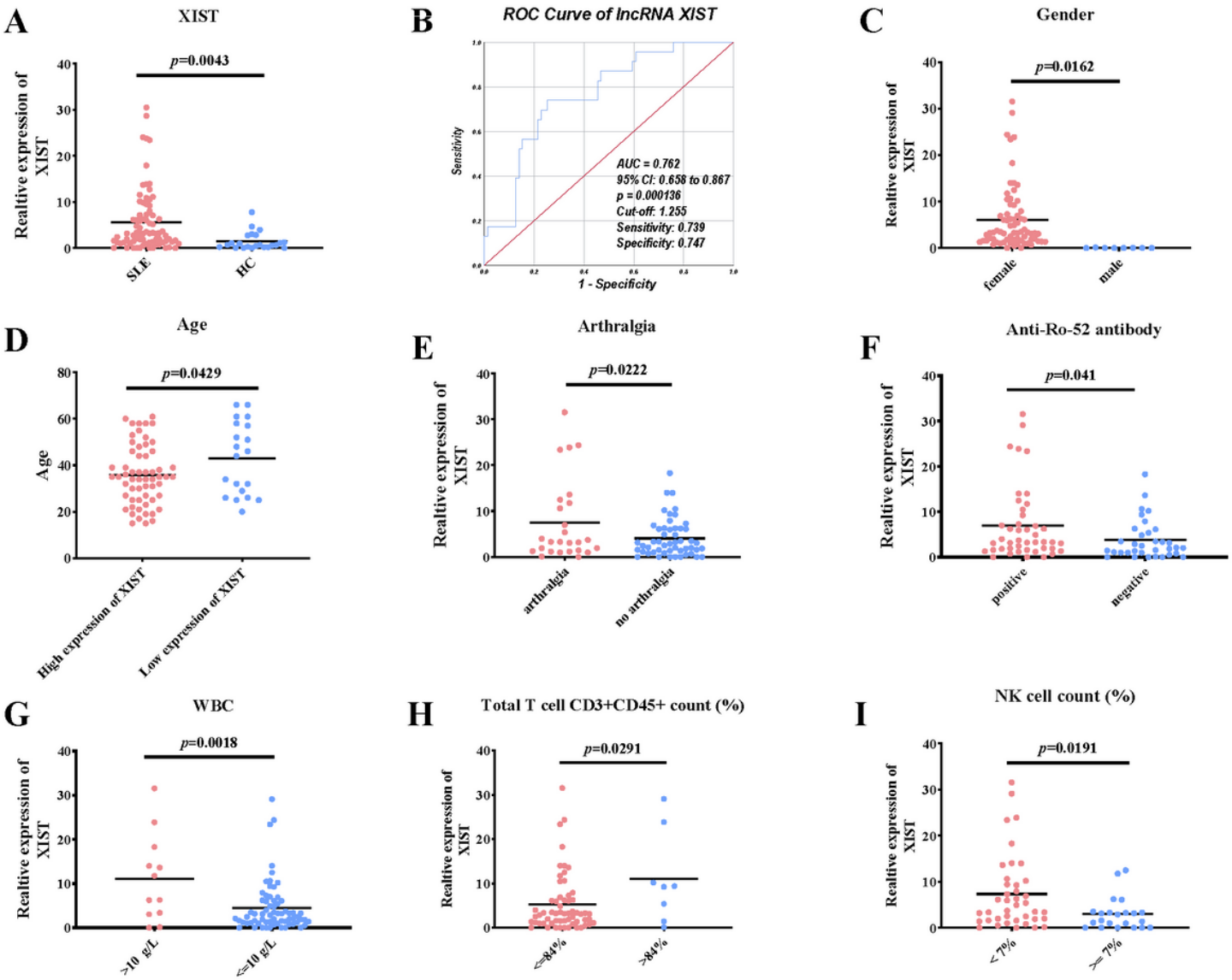

Figure 1

Expression of XIST in SLE patients and healthy controls and its correlations with clinical characteristics. A. Expression of XIST in SLE patients and healthy controls. B. ROC curves of XIST in the two groups. C and D. XIST is significantly upregulated in female (C) and young (D) patients. E–G. XIST is highly expressed in patients with arthralgia (E), anti-Ro-52 antibody positivity (F), and WBC exceeding 10 g/L (G). H and I. Expression of XIST is higher in patients with more T cells (H) or fewer NK cells (I). $P < 0.05$ was considered statistically significant. ROC, receiver operator characteristic; AUC, area under the ROC curve

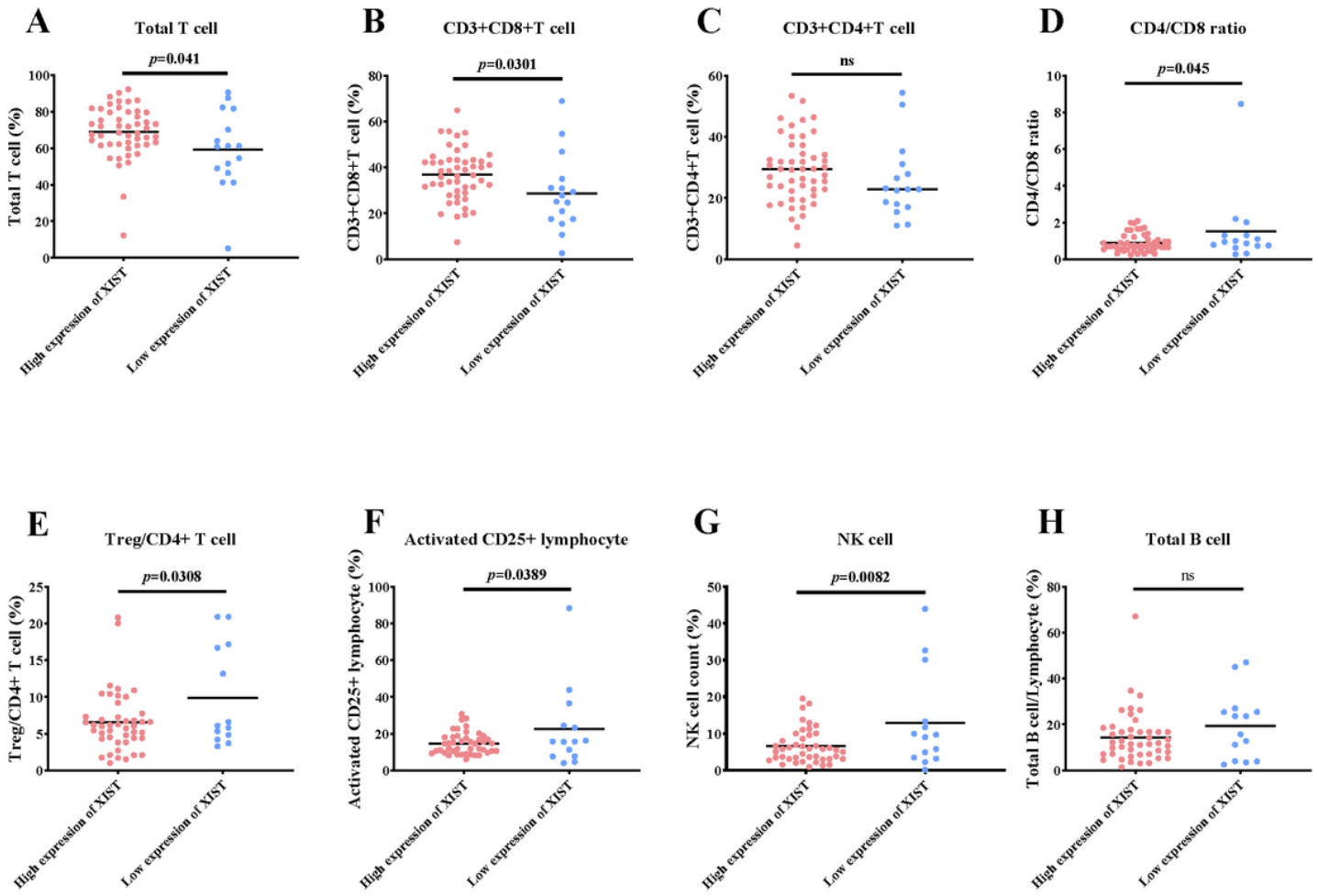

Figure 2

Levels of peripheral blood immune cells in the groups with high and low expression of XIST. The SLE patients were divided into two groups according to their XIST expression. A–F. Changes in the levels of T cells and their subsets. Compared with the low expression group, total T cells (A) and CD3+CD8+ T cells (B) were elevated in the high expression group, but CD3+CD4+ T cells (C) did not differ significantly, while CD4/CD8 ratio (D), Treg cells (E), and activated CD25+ lymphocytes (F) were reduced. G. Levels of NK cells in the two groups. H. Levels of total B cells in the two groups. $P < 0.05$ was considered statistically significant.

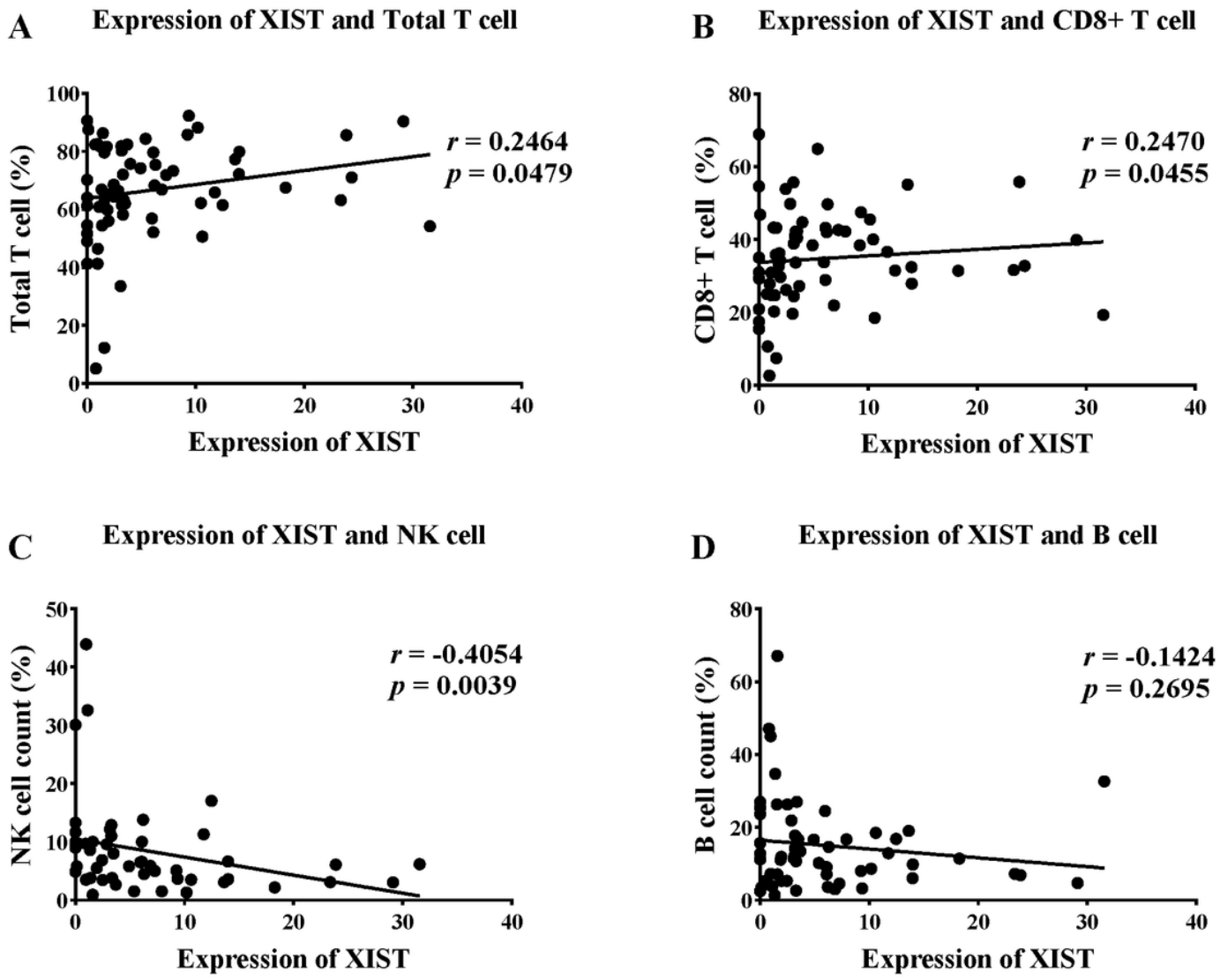

Figure 3

Correlations of XIST expression with peripheral blood immune cells. A. Correlation of XIST expression with total T cells. B. Correlation of XIST expression with CD3+CD8+ T cells. C. Correlation of XIST expression with NK cells. D. Correlation of XIST expression with total B cells. $P < 0.05$ was considered statistically significant.

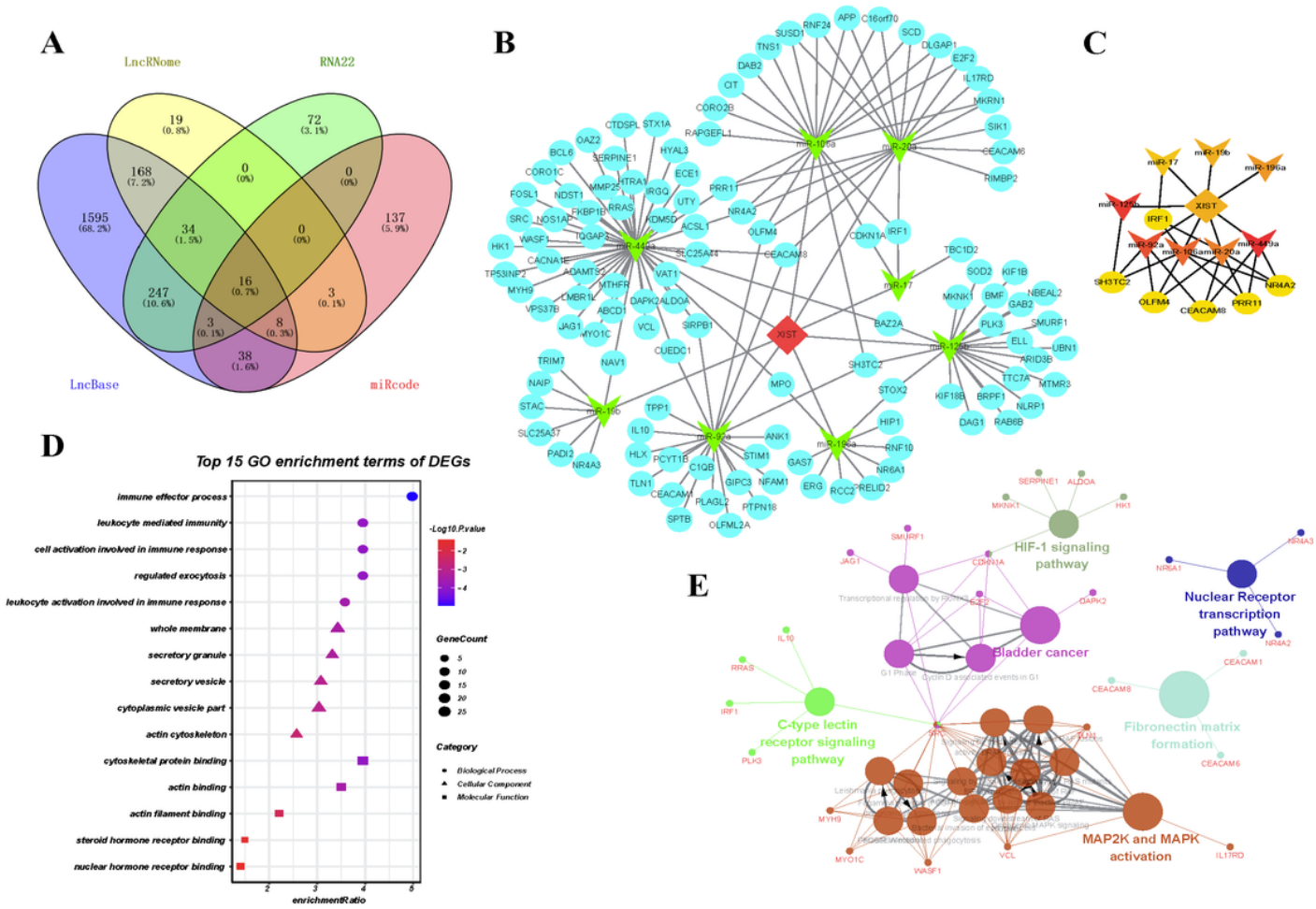

Figure 4

Function and regulatory mechanism of XIST at the transcriptome level. A. Venn diagram of the overlapping target miRNAs of XIST among four online tools. B. The ceRNA network was composed of 1 lncRNA, 8 miRNAs, 115 mRNAs, and 150 edges. Each edge represents one interaction between two transcripts. Red diamond, lncRNA; green V, miRNA; sky-blue circle, mRNA. C. Subnetwork of hub transcripts constructed by Cytoscape and its plug-in Cytohubba. D. Top 15 GO enrichment terms of the 115 target genes. E. Use of ClueGO+ CluePedia to analyze the interaction networks of enriched KEGG pathways. Multiple colour dots indicate that it was involved in multiple pathways.

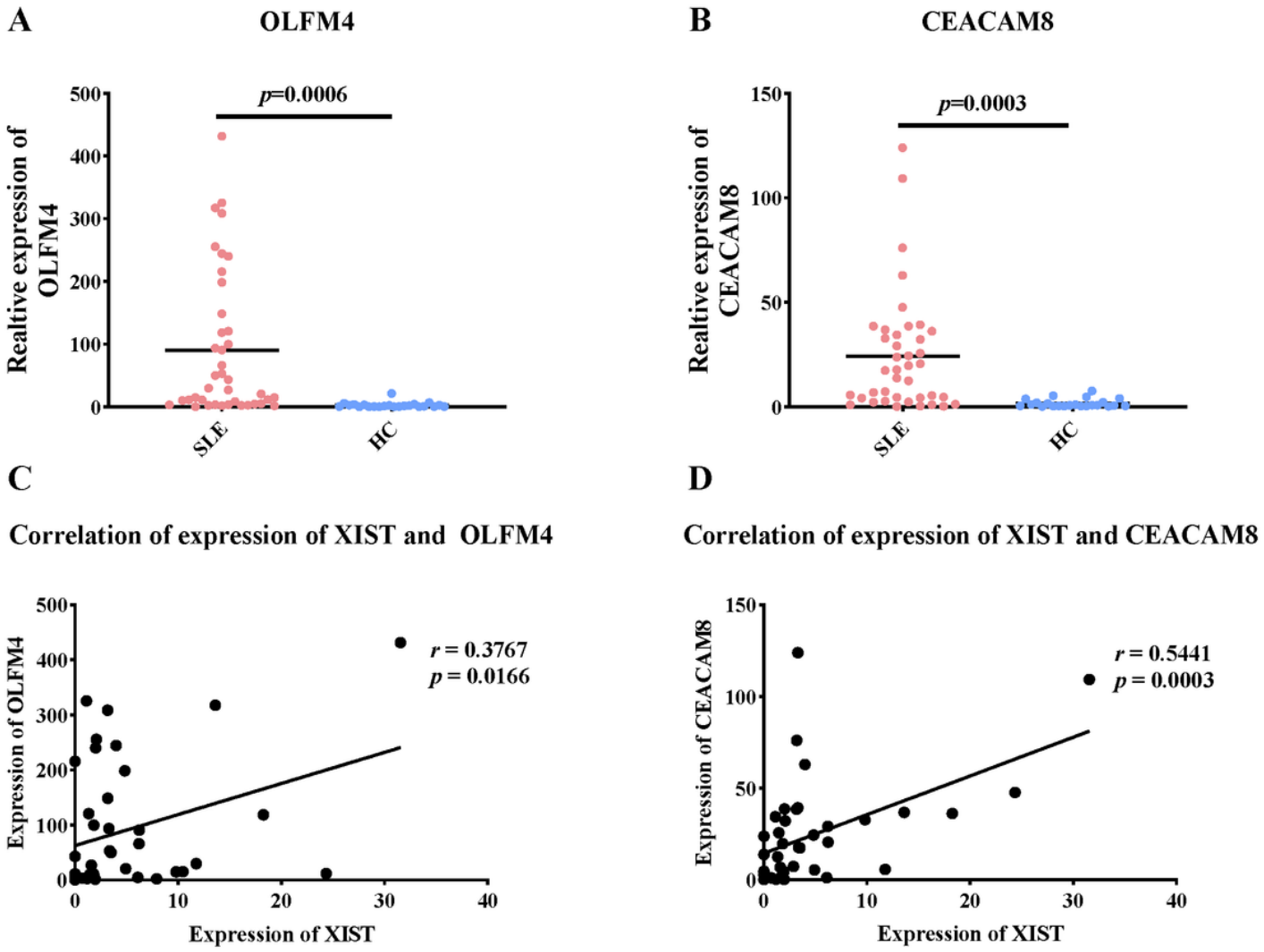

Figure 5

Expression of two selected hub genes and their correlations with XIST expression. A. Expression of OLFM4 in SLE patients and healthy controls. B. Expression of CEACAM8 in SLE patients and healthy controls. C. Correlation between expression of XIST and OLFM4. D. Correlation between expression of XIST and CEACAM8. P < 0.05 was considered statistically significant.