Expression profile of long noncoding RNAs in children with systemic lupus erythematosus: a microarray analysis

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Abstract

Objective

Long noncoding RNAs (lncRNAs) are reported to play crucial roles in several physiological and biological processes. However, knowledge of lncRNAs in children with systemic lupus erythematosus (cSLE) remains limited. We investigate lncRNA expression profiling of cSLE and explore the potential function of lncRNAs.

Methods

LncRNA and mRNA microarrays were performed to identify changes in lncRNA and mRNA expression between children with SLE and paired healthy children. Quantitative polymerase chain reaction (qPCR) validated these results. A Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to explore the potential lncRNA function.

Results

A comparison between children with SLE and paired healthy children revealed that 1042 lncRNAs and 1162 mRNAs were differentially expressed. By using gene co-expression network analysis, we constructed a complex lncRNA target network consisting of 817 matched lncRNA-mRNA pairs for 309 differentially expressed lncRNAs and 210 differentially expressed mRNAs. The results of further GO and KEGG pathway analyses indicated that lncRNAs were involved mainly in pathways with crucial pathobiological relevance in SLE.

Conclusion

We firstly characterised the expression profiles of lncRNA and mRNA in children with SLE and propose herein their possible roles in the pathogenesis of SLE. These results provide novel insights into the mechanisms of SLE pathogenesis and may serve as diagnostic biomarkers for SLE therapy.

Key words

lncRNA, children, systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with varous clinical manifestations affecting different tissues. It is characterised by the deposition of immune complexes accounted for by widespread loss of immune tolerance to nuclear self-antigens, as well as by excessive proinflammatory cytokine production and damage to multiple organ systems (1). To date, considerable progress in the treatment and survival of SLE has occurred. Nevertheless, current therapy remains ineffective for all SLE patients, and side-effects, including infections and renal failure caused by non-specific treatments, necessitate more efficient and specific therapies (2). Emerging biological and small-molecule therapies represent more effective and less toxic treatments, and their development hinges on a more precise understanding of SLE aetiology (3). Although genetic, environmental, epigenetic and hormonal factors are reported to participate in the initiation and progression of SLE, the exact pathogenesis of SLE remains largely unknown.

Previously, the importance of non-protein-coding RNAs has been emphasised in several biological and pathological processes. Among the 20,000 protein coding genes, only less than 2% of the total human genome sequences have been reported (4). Not surprisingly, at least 90% of the genome is actively transcribed into noncoding RNAs (ncRNAs) that have no protein coding potentiality. A heterogeneous novel class of long noncoding RNAs (lncRNAs) with a length longer than 200 nucleotides are generally characterised as nonprotein transcripts (5). Recently, several studies have claimed lncRNAs to play specific roles in different kinds of protein-coding and noncoding immune genes, confirming their role in autoimmune diseases (6-9). Furthermore, lncRNAs act as key regulators of inflammatory gene expression by a collaboration involving signal-dependent activation of transcription factors, transcriptional coregulators, and chromatin-modifying factors. lncRNAs have been revealed to play key roles in autoimmune processes and autoimmune diseases (10, 11). LncRNAs are widely recognised in the autoimmune disease paradigm and demonstrate potential roles in both the innate and adaptive immune system, both of which may be crucial players of autoimmunity (11, 12). However, it is our understanding that knowledge of SLE-related lncRNAs remains limited. This study was undertaken to explore abnormal lncRNA expression in children with SLE, and to study the potential biological functions associated with lncRNA in the pathogenesis of SLE.

Materials and methods

Patients and specimens

All samples from patients with SLE were obtained from the Department of Rheumatology of Beijing Children's Hospital (Beijing, China). All patients with SLE fulfilled at least four of the revised criteria for SLE by the American College of Rheumatology (ACR). The control group included healthy volunteers with no history of autoimmune disease or immunosuppressive therapy. Four children first diagnosed with untreated SLE and four 'control' children were used for microarray analysis. 10 patients and 10 'control' patients were used to validate the microarray results by RT-qPCR. Peripheral blood samples (4 ml) were obtained from each patient. The samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). After collection, the samples were transferred immediately into liquid nitrogen and stored in a freezer at -80°C until RNA extraction.

All participants were taken from the Han Chinese population. The study was approved by the Research Ethics Board of Beijing Children's Hospital, Capital Medical University, China. Informed consent was obtained from all study participants.

Total RNA extraction and microarray analysis

Total RNA was extracted from treated cells using Trizol Reagent (Life technologies, CA, US) and then purified with an miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany). RNA was detected using an UV-vis Spectrophotometer (Thermo, NanoDrop 2000, USA) at 260 nm absorbance, and purity

and integrity were assessed by agarose gel electrophoresis. The minimum RNA concentration for the microarray was 20 ng/μl. 250 ng total RNA was used to prepare Biotinylated cDNA according to the standard Affymetrix protocol. Following labelling, cDNA was hybridised on a GeneChip® Human Clariom™ D Assay. GeneChips were washed and stained in the Affymetrix Fluidics Station 450. GeneChips were scanned using an Affymetrix® GeneChip Command Console installed in the GeneChip® Scanner 3000 7G. Data were analysed using the Robust Multichip Analysis (RMA) algorithm with Affymetrix default analysis settings. The presented values were log2 RMA signal intensity. Differentially expressed genes between two groups were filtered with criteria $p<0.05$; fold change >1.2.

Quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted from peripheral blood using a Trizol Reagent (Life Technologies) followed by purification with an RNeasy kit (Qiagen, Valencia, CA, USA) according to manufacturer instructions. M-MLV reverse transcription (Promega) was used to synthesise cDNA. Quantitative PCR analysis and data collection were performed on the ABI 7900HT qPCR system using the primer pairs listed below. The raw quantifications were normalised to 16s values for each sample and fold changes were shown as mean ± SD in three independent experiments for each triplicate. The relative expression of each tested gene was calculated by the $2^{-\Delta\Delta}$ Ct method. Student's *t*-test was used to perform statistical analysis. $P<0.05$ was considered to indicate a statistically significant difference'

Table I. Primers used for RT-qPCR validation.

Gene name	Primers
RPSAP15_F	GCTTCACTCCTGGAACCTTCACTAAC
RPSAP15_R	CTCCGTGAGAGGCTGGTGGTC
RP11-542G1.2_F	TTAGCTTCTTCATGGAGTGGTGATGG
RP11-542G1.2_R	TGATTCTGAGTTGGCTCTGTGAGTTG
NEAT1_F	GCCAGTGTGAGTCCTAGCATTGC
NEAT1_R	AGAACTTCCTCCTCCTAAGCCTCTG
linc0949_F	TCCTGCAACCCAAGGTGGATACTT
linc0949_R	CTGCAGTGAGCAGAAATCACGCAT
ERP27_F	CCTCCACATGGTGACAGAGTACAAC
ERP27_R	GCTTGGCTGCCTTCTGGTATCTG
KLF12_F	AGTATCTTCAGCGTCATCTTCGTCAAC
KLF12_R	AGGCACCGACTGTACCACCAC
TRAF5_F	GAACACCTGGCTGTATGTCCTGAAG
TRAF5_R	GTAAGGCTGAATGCTCATGTTGCTG
CamK4_F	TGCCTATAGCTGCACGAATG
CamK4_R	CTTCTCTGTGCTCCGGTTTC

Cluster

The Hierarchical Clustering tab allows a hierarchical clustering to be performed using collected data. This is a powerful and useful method for analysing a range of large genomic datasets. Several published applications of this analysis are given in the references section at the end. Cluster currently performs four types of binary, agglomerative, and hierarchical clustering. The basic idea is to assemble a set of items (genes or arrays) into a tree, where similar items are joined by very short branches, and by increasingly longer branches as their similarity decreases. The first step in hierarchical clustering is to calculate the distance matrix between the gene expression data. Once this matrix of distances is computed, the clustering begins. Agglomerative hierarchical processing consists of repeated cycles where the two closest remaining items (those with the smallest distance) are joined by a node/branch of a tree, with the length of the branch set to the distance between the joined items. The two joined items are removed from list of items being processed and replaced by an item that represents the new branch. The distances between this new item and all other remaining items are computed, and the process is repeated until only one item remains.

GO enrichment analysis

Based on the Gene Ontology Database (http://www.geneontology.org/), the significance level of GOs of differentially expressed genes was analysed by two-side Fisher's exact test and χ^2 test. The differential expression genes were analysed independently according to up- and down-regulation of these genes. We computed *p*-values for all the differential expression genes in all GO categories, and the threshold of significance was defined as *p*-value <0.01. Each GO was also analysed by enrichment analysis using the following formula: Re = (nf/n)/(Nf/N), where nf refers to the number of differential expression genes within the particular category, n to the total number of genes within the same category, Nf to the number of differential expression genes in the entire microarray, and N to the total number of genes detected in the Microarray. R software (v. 3.4.3) was used as the Analysis platform.

Pathway enrichment analysis

Pathway analysis was used to identify significant pathways for the differential genes according to the Kyoto Encyclopedia of Genes and Genomes (KEGG), Biocarta and Reatome databases. We also used Fisher's exact and chi-square tests to select significant pathways. The threshold of significance was defined by $p<0.05$. The enrichment Re was given by: Re = (nf/n)/(Nf/N) (Re = ENRICHMENT), where nf is the number of differential genes within the particular category, n is the total number of genes within the same category, Nf is the number of differential genes in the entire microarray, and N is the total number of genes in the microarray. R software (v. 3.4.3) was used as the Analysis platform.

Co-expression network (lncRNA-mRNA)

A gene co-expression network was built according to the normalised signal intensity of specifically expressed lncRNAs and mRNAs using the Cytoscape programme (Cytoscape 3.4.0). Compute co-expression coefficient, The expression values of a gene for different samples can be represented as a

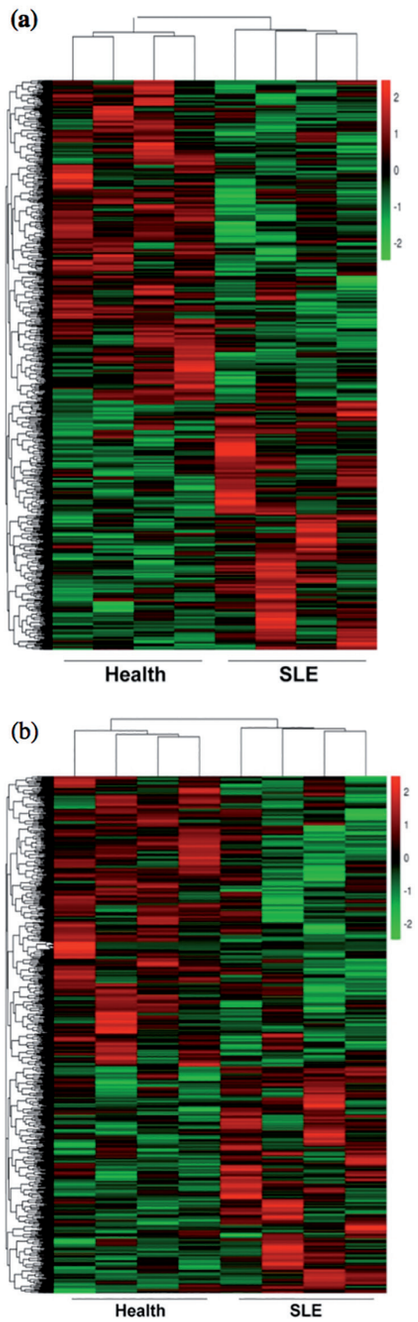


Fig. 1. Hierarchical clustering of lncRNAs **(a)** and mRNAs **(b)** in SLE and health. Red and green indicate upregulation and downregulation, respectively.

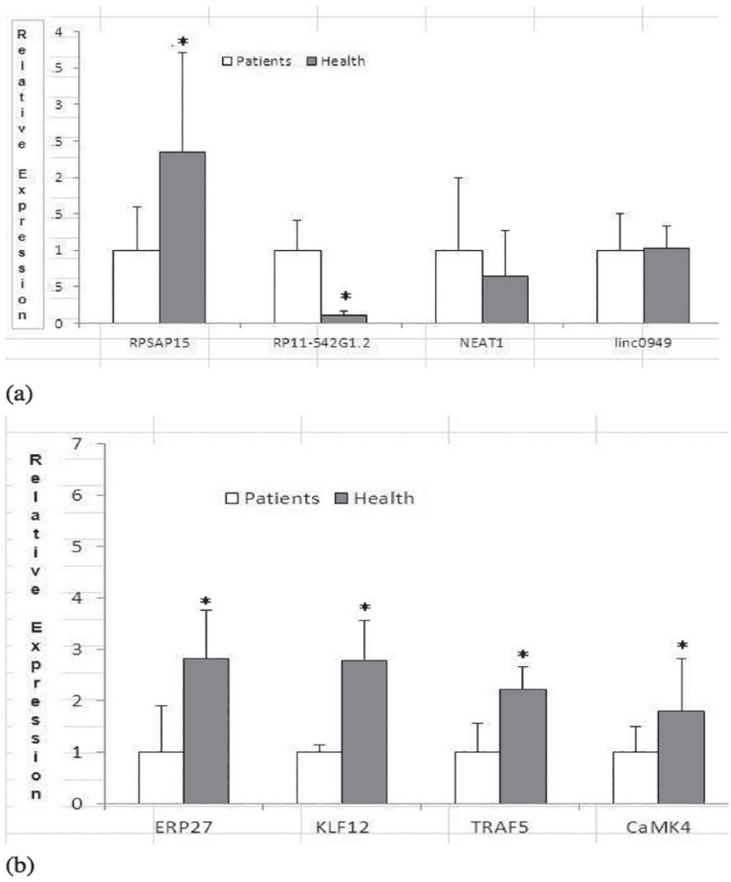


Fig. 2. RT-qPCR validation of selected lncRNAs(a) and mRNAs(b) between children with SLE and healthly children.
Significant levels are indicated by * (p<0.05)

vector, thus calculating the co-expression measure between a pair of genes is the same as calculating the selected measure for two vectors of numbers. R function cor.test (Test for Association/Correlation Between Paired Samples) was used to compute the Pearson's correlation coefficient to choose the significant correlation pairs and the correlation value cut-off was 0.99. Joint differences of lncRNA expression spectrum data and the difference of gene expression data to build a lncRNA network with the correlation coefficient of gene expression. In the hierarchy to express network clearly reflect lncRNA and the mutual relationship between genes and the overall relationship, further found that lncRNA of gene regulation relationships, and explore its possible functions. The degree was calculated to measure a gene or lncRNA centrality within a network. While considering different networks, core genes were determined by the degree differences between two group samples.

Results

Expression profile of lncRNAs and mRNAs

Hierarchical clustering revealed systematic variations between the case (n=4) and control groups (n=4) in the expression of lncRNAs (Fig. 1a) and mRNAs (Fig.1b). 1042 lncRNAs and 1162 mRNAs were identified as being significantly differentially expressed. Compared to normal children, 452 lncRNAs and 501 mRNAs were observed to be up regulated, while 590 lncRNAs and 661 mRNAs were down regulated in SLE patients.

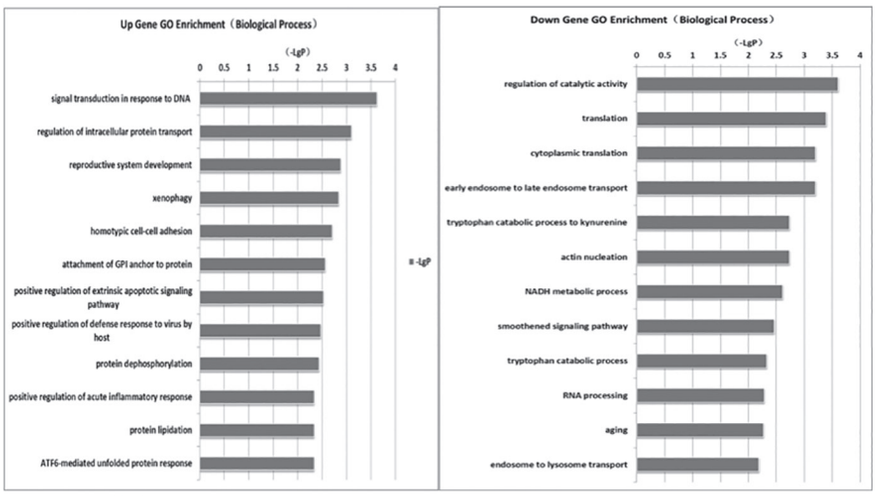
Validation of the microarray results by RT-qPCR

To verify the microarray data, four lncRNAs (RPSAP15, RP11-542G1.2, NEAT1, linc0949) and four mRNAs (ERP27, KLF12, TRAF5, CaMK4) was selected. qRT-PCR was performed to validate the expression level of these differentially expressed lncRNAs and mRNAs (Fig. 2). The expression trends of these transcripts were akin to those gathered from the microarray data.

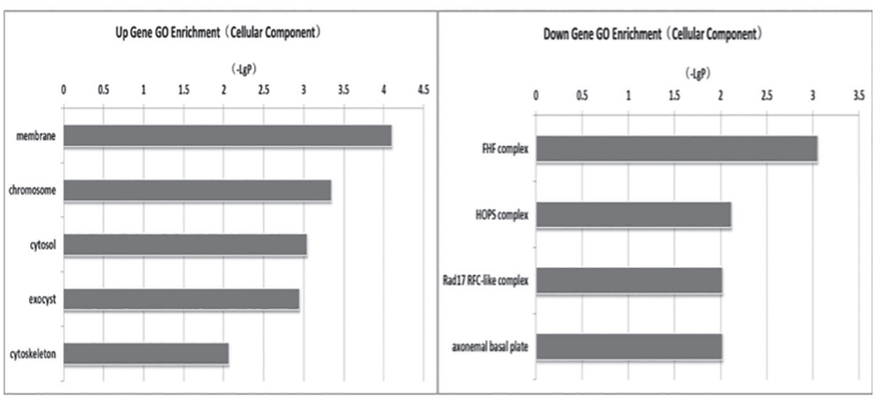
Gene ontological and pathway analysis

GO analysis provides a controlled vocabulary that can describe gene and

(a) GO biological processes



(b) GO cellular components



(C) molecular function

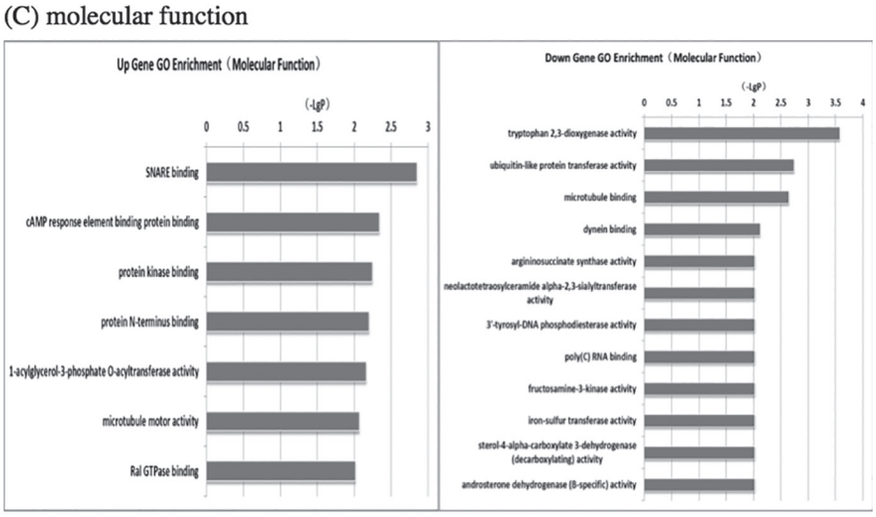


Fig. 3. Significantly changed upregulated and downregulated genes in children with SLE and healthy children chosen by GO analysis. The larger –lgP indicates a smaller *p*-value.

gene product attributes in any organism. In the present study, the ontology covered three domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Through GO analysis, it was possible to conclude that these up-regulated and downregulated transcripts of lncRNAs were associated with biological processes, cellular components, and molecular function (Fig. 3). Specifically, the top 3 upregulated biological processes were signal transduction in response to DNA damage, regulation of intracellular protein transport, and reproductive system development. Whereas, the top 3 downregulated biological processes included regulation of catalytic activity, translation, and cytoplasmic translation. The top 3 upregulated cellular components were signal transduction in response to DNA damage, regulation of intracellular protein transport, and reproductive system development. Whereas, the top 3 downregulated cellular components were regulation of catalytic activity, translation, and cytoplasmic translation.The top 3 upregulated molecular functions were SNARE binding, cAMP response element binding, and protein kinase binding. The top 3 downregulated molecular functions were tryptophan 2,3-dioxygenase activity, ubiquitin-like protein transferase activity, and microtubule binding.

KEGG pathway enrichment analysis was performed for differentially expressed genes to identify pathways represented among lncRNAs identified in the SLE gene expression signature. KEGG analysis suggested that 8 pathways were significantly correlated with upregulated gene expression, and 5 pathways were significantly correlated with downregulated gene expression (Fig. 4).

Co-expression network constructions

To uncover the possible modulating mechanisms of lncRNAs, gene co-expression network analysis was constructed. The expression network clearly reflected lncRNA and the mutual relationship between genes, and further demonstrated lncRNA gene regulation relationships by exploring their possible functions. This procedure resulted in a complex lncRNA target network consisting of 817 matched lncRNA-mRNA pairs for 309 differentially expressed lncRNAs. Among them, 161 lncRNAs were upregulated and 148 lncRNAs were downregulated. Among the 210 differentially expressed mRNAs, 106 mRNAs were upregulated, and 104 mRNAs were downregulated. Within the network, a single mRNA was potentially targeted by one to twelve lncRNAs.

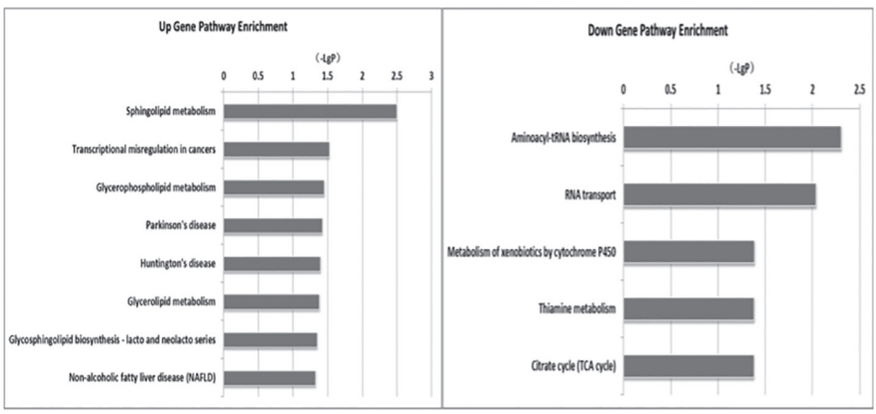


Fig. 4. Significantly changed upregulated and downregulated pathways in children with SLE and healthy children chosen by the KEGG database. Pathways with $p<0.05$ and FDR <0.05 were identified as significantly changed pathways.

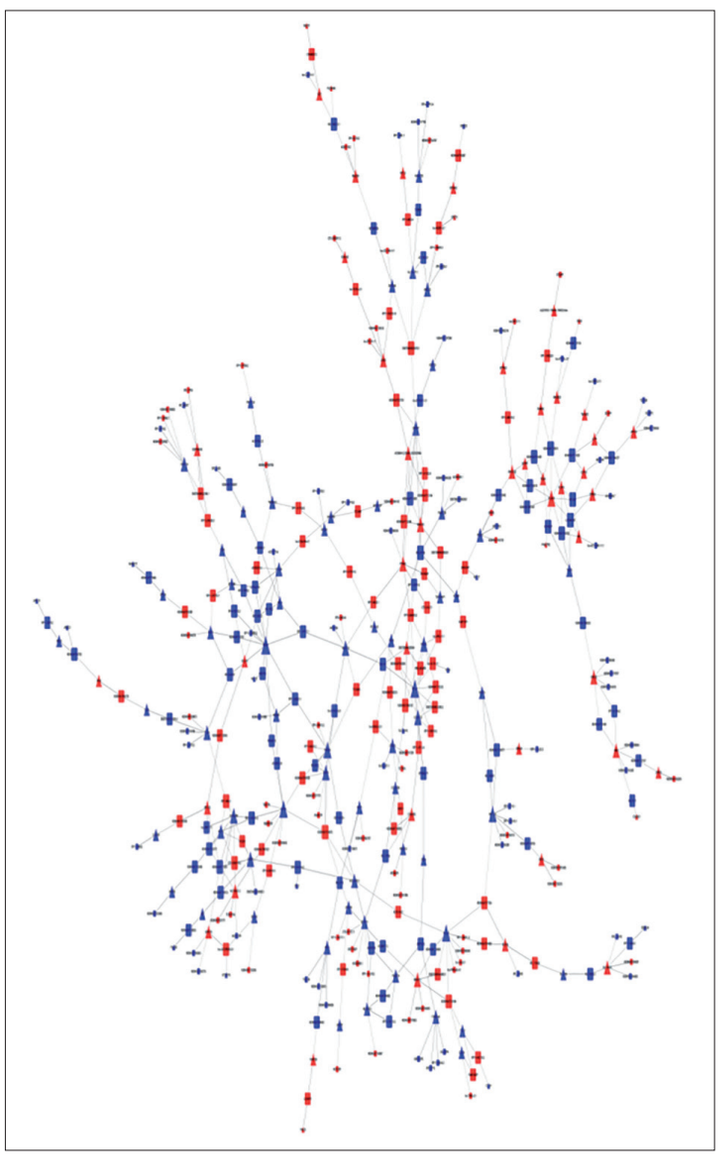


Fig. 5. lncRNA-mRNA Co-expression Network:
Blue represents down regulation and red represents up regulation. Box nodes represent lncRNA. Triangle nodes represent mRNA. Straight lines represent the possible relationship; dotted lines represent the inhibitive effect.

Discussion

Systemic lupus erythematosus (SLE) is considered a prototypic autoimmune disease. Immune cells, including T, B, and NK cells, play pivotal roles in the pathogenesis of SLE (13, 14). To date, the study of LncRNA is emerging as an important regulator of immune cell differentiation and activation (15). Accordingly, lncRNA may be important factor in the pathogenesis of SLE. In a study involving patients with SLE, linc0949 and linc0597 were significantly decreased in patients with SLE compared with patients with RA and healthy control subjects (16-18). Other preliminary data in a murine model system points to a link between lncRNA growth arrest-specific 5 (GAS5) and disease susceptibility to SLE (19). However, lncRNA expression level in children with SLE remains unclear. To understand the roles of lncRNAs in children with SLE, the present study analysed lncRNA and mRNA co-expression in children with SLE.

Although lncRNAs are an abundant class of ncRNAs encoded within the genome, their roles in SLE remain to be studied in detail. Expression profiles of lncRNAs-mRNAs in SLE indicated that the expression values of 1044 lncRNAs and 1162 mRNAs were significantly changed. qRT-PCR was used to verify the data received from the microarray assessment. The results tested by qRT-PCR confirmed the reliability of the microarray experiments. Nuclear enriched abundant transcript 1 (NEAT1), a long non-coding RNA, is encoded on chromosome 11q13.1 and expresses constitutively and widely in several tissues and cell types. Previous studies have shown that NEAT1 is essential for para-speckle structural integrity and formation by interacting with members of DBHS (Drosophila Behavior Human Splicing) family proteins (20-22). To date, emerging research has shown that NEAT1 is closely linked to SLE. NEAT1 expression was abnormally increased in SLE patients compared with normal controls, and a positive correlation was observed between NEAT1 and clinical disease activity in SLE patients (12). In the present study, however, the ex-

pression of NEAT1 was not observed to have any significant difference between children with SLE and the controls. In addition, a study by Wu demonstrated that linc0949 was significantly decreased in patients with SLE compared with patients with RA and healthy control subjects. However, no significant differences of this nature were observed in our study. A possible reason for this may be the use of different study samples. In our study, we analysed lncRNA expression in the peripheral blood of children, whereas the previous study analysed peripheral blood mononuclear cells of adults. In addition, the patients we studied were children, whereas the previous study involved adults. Therefore, more samples and further studies are needed to establish convincing results.

Most of these lncRNAs have not been functionally characterised, whereas most of the identified mRNAs are well-known. Therefore, GO and KEGG pathway analysis of expressed mRNAs was conducted to help better understand the potential role in the pathological process of SLE and speculate on the putative function of the differentially expressed lncRNAs. Pathway analyses demonstrated that differentially expressed lncRNAs were associated with biological process, cellular components and molecular function, which were associated with 13 pathways. Among the 13 pathways, seven pathways are classified as metabolism. McDonald G's study demonstrates that lipid metabolism defects contribute to SLE pathogenesis (23). Our results indicate that differentially expressed lncRNAs may play role through the metabolism pathway.

A co-expression network of lncRNA and mRNA in SLE was then constructed based on a correlation assessment between diversely expressed lncRNAs-mRNA. The network of co-expression in the experimental group consisted of 817 matched lncRNA-mRNA pairs for 309 differentially expressed lncRNAs and 210 differentially expressed mRNAs. Within the network, a single mRNA was potentially targeted by one to twelve lncRNAs. For example, Calcium/calmodulin-dependent protein kinase IV (CaMK4) is a multifunctional serine/threonine kinase that regulates gene expression by activating transcription factors. CaMK4 have shown to play important roles in immune responses including T-cell activation and T-cell development (24). Within the co-expression network, CaMK4 mRNA was potentially targeted by seven lncRNAs. Tumor necrosis factor receptor associated factor 5 (TRAF5) is an E3 ubiquitin protein ligase and signal transducer, and could regulate expression of IL-17A (25). IL-17A plays important roles in the pathogenesis of SLE by inducing the local production of chemokines and cytokines and augmenting the production of autoantibodies (26). Within the co-expression network, TRAF5 mRNA was potentially targeted by four lncRNAs. The results suggest these lncRNA may play role through interaction with CaMK4, TRAF5, etc. These data underpinned research on the mechanism of lncRNA in SLE.

However, our study has three potential limitations. First, we used a relatively small sample size. As such, our results require further validation in larger prospective patient cohorts. Second, we cannot guarantee whether the identified differentially expressed lncRNAs were the cause of SLE or whether they were secondary to the presence of SLE. Third, the functions of lncRNAs were based on bioinformatics predictions and require further experimental validation.

Conclusion

In summary, it is our understanding that this study was first to screen and analyse lncRNA expression profiles in children with SLE. This may offer new insights into pathogenesis and may be a promising modality to dissect the molecular pathogenesis of SLE. Further investigation is required to determine to what extent these lncRNAs may serve as novel therapeutic targets and diagnostic biomarkers in SLE.

References

1. PAPPAS G, GRANITO A, BIANCHI FB: Systemic lupus erythematosus. *N Engl J Med* 2008; 358: 2412; author reply 2413.
2. FANOURIAKIS A, GERGIANAKI I, SIDIROPOULOS P, BERTSIAS G, BOUMPAS DT: Evolving concepts in systemic lupus erythematosus. *Isr Med Assoc* J 2016; 18: 749-53.
3. LA PAGLIA GMC, LEONE MC, LEPRI G *et al*.: One year in review 2017: Systemic lupus erythematosus. *Clin Exp Rheumatol* 2017; 35: 551-61.
4. BONASIO R, SHIEKHATTAR R: Regulation of transcription by long noncoding RNAs. *Annu Rev Genet* 2014; 48: 433-55.
5. TANI H, MIZUTANI R, SALAM KA *et al*.: Genome-wide determination of RNA stability reveals hundreds of short-lived noncoding transcripts in mammals. *Genome Res* 2012; 22: 947-56.
6. COPE AP, FELDMANN M: Emerging approaches for the therapy of autoimmune and chronic inflammatory disease. *Curr Opin Immunol* 2004; 16: 780-86.
7. CHATENOUD L: Immune therapies of autoimmune diseases: Are we approaching a real cure? *Curr Opin Immunol* 2006; 18: 710-17.
8. BORCHERS AT, LEIBUSHOR N, NAGUWA SM, CHEEMA GS, SHOENFELD Y, GERSHWIN ME: Lupus nephritis: A critical review. *Autoimmun Rev* 2012; 12: 174-94.
9. SUAREZ-GESTAL M, CALAZA M, ENDREFFY E *et al*.; EUROPEAN CONSORTIUM OF SLEDNAC: Replication of recently identified systemic lupus erythematosus genetic associations: A case-control study. *Arthritis Res Ther* 2009; 11: R69.
10. WU GC, PAN HF, LENG RX *et al*.: Emerging role of long noncoding RNAs in autoimmune diseases. *Autoimmun Rev* 2015; 14: 798-805.
11. YAMANAKA H: TNF as a target of inflammation in rheumatoid arthritis. *Endocr Metab Immune Disord Drug Targets* 2015; 15: 129-34.
12. 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.
13. BIERMANN MH, VEISSI S, MAUERODER C *et al*.: The role of dead cell clearance in the etiology and pathogenesis of systemic lupus erythematosus: Dendritic cells as potential targets. *Expert Rev Clin Immunol* 2014; 10: 1151-64.
14. MUNOZ LE, JANKO C, SCHULZE C *et al*.: Autoimmunity and chronic inflammation - two clearance-related steps in the etiopathogenesis of SLE. *Autoimmun Rev* 2010; 10: 38-42.
15. FITZGERALD KA, CAFFREY DR: Long noncoding RNAs in innate and adaptive immunity. *Curr Opin Immunol* 2014; 26: 140-46.
16. WU Y, ZHANG F, MA J *et al*.: Association of large intergenic noncoding RNA expression with disease activity and organ damage in systemic lupus erythematosus. *Arthritis Res Ther* 2015; 17: 131.
17. LI Z, CHAO TC, CHANG KY *et al*.: The long noncoding RNA THRIL regulates TNF alpha expression through its interaction with hnRNPL. *Proc Natl Acad Sci USA* 2014; 111: 1002-7.
18. WU GC, LI J, LENG RX *et al*.: Identification of long non-coding RNAs GAS5, linc0597 and lnc-DC in plasma as novel biomarkers for

systemic lupus erythematosus. *Oncotarget* 2017; 8: 23650-63.
19. HAYWOOD ME, ROSE SJ, HORSWELL S *et al*.: Overlapping BXSB congenic intervals, in combination with microarray gene expression, reveal novel lupus candidate genes. *Genes Immun* 2006; 7: 250-63.
20. 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.
21. SUNWOO H, DINGER ME, WILUSZ JE, AMARAL PP, MATTICK JS, SPECTOR DL: Men epsilon/beta nuclear-retained non-coding rnas are up-regulated upon muscle differentiation and are essential components of paraspeckles. *Genome Res* 2009; 19: 347-59.
22. SASAKI YT, IDEUE T, SANO M, MITUYAMA T, HIROSE T: Menepsilon/beta noncoding rnas are essential for structural integrity of nuclear paraspeckles. *Proc Natl Acad Sci USA* 2009; 106: 2525-30.
23. MCDONALD G, DEEPAK S, MIGUEL L *et al*.: Normalizing glycosphingolipids restores function in $CD4^+$ T cells from lupus patients. *J Clin Invest* 2014; 124: 712-24.
24. KOGA T, KAWAKAMI A: The role of CaMK4 in immune responses. *Mod Rheumatol* 2017: 1-4.
25. WANG X, YANG J, HAN L *et al*.: TRAF5-mediated lys-63-linked polyubiquitination plays an essential role in positive regulation of RORγt in promoting IL-17A expression. *J Biol Chem* 2015; 290: 29086-94.
26. LI D, GUO B, WU H, TAN L, CHANG C, LU Q: Interleukin-17 in systemic lupus erythematosus: A comprehensive review. *Autoimmunity* 2015; 48: 353-61.