

Systemic Lupus Erythematosus Dysregulates the Expression of Long Noncoding RNAs in Placentas

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Abstract

Background: Systemic lupus erythematosus (SLE) can cause placental dysfunctions, which may result in pregnancy complications. Long noncoding RNAs (lncRNAs) are actively involved in the regulation of immune responses during pregnancy. The present study aimed to determine the lncRNA expression profiles in placentas from women with SLE to gain new insights into the underlying molecular mechanisms in SLE pregnancies.

Methods: RNA sequencing (RNA-seq) analysis was performed to identify SLE-dysregulated lncRNAs and mRNAs in placentas from women with SLE and normal full-term (NT) pregnancies. Bioinformatics analysis was conducted to predict biological functions of these SLE-dysregulated lncRNAs and mRNAs. Correlation relationships between these dysregulated lncRNAs and SLE disease activity index (SLEDAI) scores were also assessed.

Results: RNA-seq analysis identified 81 dysregulated lncRNAs in SLE placentas, including 53 that were up-regulated and 28 down-regulated. Additional 221 SLE-dysregulated mRNAs were also discovered, including 209 up-regulated and 12 down-regulated. Bioinformatics analysis revealed that SLE-dysregulated genes were associated with biological functions and gene networks, such as type I interferon signaling pathway, response to hypoxia, regulation of MAPK (mitogen-activated protein kinase)/JNK (c-Jun N-terminal kinase) cascade, response to steroid hormone, heparin binding, and insulin-like growth factor binding. Correlation analysis showed that lncRNA NONHSAT246155.1 was positively correlated ($r = 0.333$, $P = 0.037$) with SLEDAI score.

Conclusions: This is the first report of the lncRNA profiles in placentas from SLE pregnancies. These results suggest that the aberrant expression and the potential regulatory function of lncRNAs in placentas may play comprehensive roles in the pathogenesis of SLE pregnancies. These lncRNAs, including NONHSAT246155.1 may potentially serve as novel therapeutic targets for SLE during pregnancy.

Background

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that predominantly affects women of reproductive age [1]. During pregnancy, the loss of immune tolerance to the fetus in SLE may cause numerous maternal and fetal complications, including lupus flare, hypertension, preeclampsia, eclampsia, spontaneous abortion, stillbirth, intrauterine growth retardation (IUGR), neonatal lupus, and neonatal deaths [2–4]. The pregnancy outcomes in mothers with SLE and the well beings of fetuses born to SLE mothers have been improved tremendously over the last five decades due to the development of disease management [5, 6]. However, the mechanism underlying pregnancy complications in SLE pregnancy remains unclear. Therefore, optimal disease control and multidisciplinary obstetrical care throughout gestation are essential to improve pregnancy outcomes in SLE.

SLE can cause placental dysfunction and insufficiencies such as decreased placental weight, ischemic hypoxic change, decidual vasculopathy and thrombi, fetal thrombi, and chronic villitis of implied

unknown etiology during pregnancy [3]. These impaired placental functions may lead to numerous maternal and fetal complications as mentioned above [7, 8].

Long noncoding RNAs (lncRNAs) are typically defined as transcripts longer than 200 nucleotides in length without recognizable protein-coding potential [9]. lncRNAs play an essential role in regulating gene expression through multiple mechanisms [9–11]. lncRNAs also actively regulate many essential biological processes, including immune cell differentiation and immune responses [12]. Meanwhile, lncRNAs have been reported to be related to autoimmune diseases such as SLE, Sjögren's syndrome, and rheumatoid arthritis [13–20].

lncRNAs are involved in the initiation and development of SLE via different signaling pathways, e.g., the nuclear factor- κ B (NF- κ B) signaling pathway [17], mitogen-activated protein kinase (MAPK) signaling pathway [18], and tumor necrosis factor (TNF) signaling pathway [21]. lncRNAs are also associated with SLE disease activity [18–20]. However, little is known about lncRNA expression profiles and functions in placentas from SLE pregnancy.

In this study, we hypothesize that SLE dysregulates lncRNA expression profiles in placentas during pregnancy, and this dysregulation is associated with the severity of SLE. Identifying SLE-dysregulated lncRNAs in placentas may aid us to identify novel therapeutic targets and disease biomarkers for SLE. RNA sequencing (RNA-seq) was performed on placentas from SLE and normal full-term (NT) pregnancies. Bioinformatics analysis was conducted to reveal the underlying biological functions of dysregulated lncRNAs.

Methods

Subjects

All procedures were conducted in accordance with the Declaration of Helsinki. Tissue collection protocols were approved by the Institutional Review Board of Qilu Hospital, Shandong University and the Scientific and Ethical Committee of Shanghai First Maternity and Infant Hospital affiliated with Tongji University. Pregnant women with SLE (n =10, with 5 female and 5 male fetuses) and NT (n =10, with 5 female and 5 male fetuses) were recruited from Qilu Hospital, Shandong University, and Shanghai First Maternity and Infant Hospital affiliated with Tongji University, respectively. Individuals excluded from the study include smokers and patients with cancer or diabetes mellitus. SLE was defined according to the American College of Rheumatology classification criteria [22]. The SLE disease activity index (SLEDAI) [23] was used to evaluate the disease activity of SLE patients. The questionnaire survey was used to collect demographic data and clinical manifestations of all patients. Laboratory data were obtained from the medical record. Clinical manifestations and laboratory records of all subjects are shown in Table 1.

Sampling and RNA isolation

Placentas were collected immediately after C-section or vaginal delivery. Six random biopsies from the fetal side of each placenta were sampled (using a 1 x 1 cm grid), snap-frozen in liquid nitrogen, and stored at -80 °C for further experiments.

Total RNA was isolated using the RNeasy mini kit (Qiagen, Germany). RNA concentration and quality were determined by the Qubit® 2.0 Fluorometer (Life Technologies, USA) and the Nanodrop One spectrophotometer (Thermo Fisher Scientific Inc., USA). The integrity of total RNA was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., USA), and samples with RNA integrity number (RIN) values above 7.0 were used for sequencing.

RNA-seq and data analysis for gene expression

RNA-seq analysis was performed on RNA samples from SLE and NT placentas (n = 8/group with 3 female and 5 male fetuses; Additional file 1: Table S1) using the VAHTS Total RNA-seq (H/M/R) Library Prep Kit (Vazyme, China) and Illumina NovaSeq 6000 platform (Illumina, USA) as described in Methods in Data Supplement. Differential expression gene (DEG) analysis for lncRNA/mRNA was performed using R package edgeR [24]. The *P*-value significance threshold in multiple tests was set by the false discovery rate (FDR) [25]. The fold-changes were estimated according to the Fragments Per Kilobase of transcript sequence per Million base pairs sequenced (FPKM) in each sample [26]. Differentially expressed RNAs with fold change > |2| and *q*-value (FDR adjusted *P*-value) < 0.05, considered as significantly modulated, were retained for further analysis. The RNA-seq data have been deposited in Gene Expression Omnibus (GEO) under accession number GSE177029.

Functional genomic analysis of dysregulated genes

To explore biological functions and involved signaling pathways of DEGs, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs were conducted using the Metascape analysis tool [27]. Restrictions: *P* < 0.05, a minimum count of 3, enrichment factor > 1.5 were considered statistically significant. The GO and KEGG terms fulfilling this condition were defined as significantly enriched GO and KEGG terms.

Gene co-expression network analysis

To study relationships between dysregulated lncRNAs and mRNAs in SLE placentas, we calculated co-expression relationships between lncRNAs and mRNAs according to the dynamic change of gene expression signal value and obtained the expression regulation relationship and direction between genes to construct the gene expression regulation network. By using the co-expression network, we can analyze the gene regulation ability and obtain the core regulatory genes. The co-expression network was constructed using Cytoscape [28]. Pearson correlation coefficients were used for the lncRNAs-mRNAs co-expression network when they were above 0.90.

***Cis*- and *trans*-target gene prediction of lncRNAs**

RNAplex was used to identify potential targets of differentially expressed lncRNAs. The lncRNA *cis*-action is predicted by searching all coding genes within the 10-kb upstream and downstream of the target lncRNA, and these neighboring genes may be regulated by lncRNAs. The lncRNA *trans*-action was predicted based on nucleic acid base pairing [29]. The GO and KEGG enrichment analyses of the lncRNA target genes were conducted.

Real-time quantitative PCR (RT-qPCR)

To validate the RNA-seq data, six lncRNAs with different expression patterns were selected for RT-qPCR [30] (n = 8/group with 4 female and 4 male fetuses; Additional file 1: Table S1) using NuHi Robustic SYBR Green Mix. Data were normalized to GAPDH. Primers are listed in Additional file 2: Table S2. The normalized data were analyzed using the $2^{-\Delta\Delta CT}$ method [30].

Correlation analysis of lncRNAs and SLEDAI scores of patients with SLE

According to RT-qPCR verification results, relative expression levels of lncRNAs (fold of NT) were used to analyze correlation with SLEDAI scores.

Statistical analyses

Data were expressed as means \pm standard deviation or medians with range. Comparison of continuous data was performed by independent Student's *t*-test. The relationships between the expression levels of genes and SLEDAI were analyzed by Pearson's correlation coefficient. Microsoft Excel (2016) and SigmaPlot (13.0) were used for statistical analyses. *P*-values < 0.05 were considered statistically significant.

Results

Patient characteristics

Demographic and clinical characteristics are shown in Table 1. All SLE patients were on maintenance medication (prednisone \leq 15 mg daily and hydroxychloroquine 200 - 400mg daily). Maternal age and body mass index (BMI) were similar between SLE and NT. The mean newborn body weight and gestation age in SLE were significantly (*P* < 0.05) lower than those in NT.

RNA-seq analysis

Deep sequencing generated the mean number of raw reads, 67,745,864 and 66,918,866, for SLE and NT groups, respectively. Removing reads with non-canonical letters or with low quality and discarding the sequences shorter than 25 nucleotides, the mean numbers of clean reads were 66,385,880 and 65,846,828 for SLE and NT groups, respectively, which were retained for analysis.

SLE-dysregulated lncRNAs in placentas

Eighty-one dysregulated lncRNAs were identified (Fig. 1a, b; Additional file 3: Table S3). Of these lncRNAs, 53 were up-regulated, and 28 were down-regulated. Among these dysregulated lncRNAs, NONHSAT152368.1 (280 fold) was the most up-regulated lncRNA, whereas ENST00000602755 (0.0023 fold) was the most down-regulated lncRNA. Two dysregulated lncRNAs were located on X-chromosome, among which NONHSAT222664.1 was up-regulated, and ENST00000602420 was down-regulated. None of these dysregulated lncRNAs was located on Y-chromosome.

SLE-dysregulated mRNAs in placentas

We also identified 221 dysregulated mRNAs (Fig. 1a, c; Additional file 4: Table S4). Of these dysregulated mRNAs, 209 were up-regulated, and 12 were down-regulated. KRT24 (246 fold) was the most up-regulated mRNA, and AC011841.1 (0.15 fold) was the most down-regulated mRNA. Six dysregulated mRNAs (ANOS1, GRIA3, HEPH, LDOC1, MAOB, and TMEM47) were located on X-chromosome and were all up-regulated. None of these dysregulated mRNAs was located on Y-chromosome.

Functional genomics analysis of dysregulated genes

We performed bioinformatics analysis with 221 SLE-dysregulated mRNAs, aiming to explore their biological functions and gene networks in SLE placentas. We found that SLE-dysregulated genes were highly enriched in signaling pathways, including type I interferon signaling pathway, response to hypoxia, regulation of MAPK cascade, regulation of JNK (c-Jun N-terminal kinase) cascade, response to steroid hormone, heparin binding, growth factor binding, and insulin-like growth factor binding (Fig. 2a). In addition, either positive or negative regulation of biological functions, such as regulation of cell adhesion, coagulation, hemostasis, and response to wounding, was also enriched (Fig. 2b). These findings suggest that a variety of placental function-associated genes/pathways were involved in the pathogenesis of SLE pregnancy.

Co-expression network of lncRNAs and mRNAs

A co-expression network was established to determine the interactions of dysregulated lncRNAs and mRNAs (Additional file 5: Fig. S1). The co-expression results in the present study strongly support a network model in which lncRNAs and mRNAs function together in placentas during SLE pregnancy. Our data showed that the co-expression network was composed of 26 lncRNAs and 111 mRNAs. The co-expression network indicated that one lncRNA could target, at most 44 mRNAs, and one mRNA could correlate with at most seven lncRNAs. ENST00000550268, NONHSAT184808.1, and NONHSAT231755.1 were the most connected lncRNAs. COL17A1 and VEGFA were the most connected mRNAs.

lncRNA target prediction analysis

The results of the “*cis*” and “*trans*” analyses are shown in Additional file 6 (Table S5) and Additional file 7 (Table S6), respectively. The prediction results indicated that 79 of 81 SLE-dysregulated lncRNAs may target 2387 genes. The two remaining lncRNAs had no potential target genes.

Bioinformatics analysis was conducted to predict the biological roles of target genes of SLE-dysregulated lncRNAs (Fig. 3). The GO project enrichment of target genes included 165 biological processes, 39 cellular components, and 7 molecular functions ($P < 0.05$), mainly involving protein catabolic process, cell cycle process, autophagy, kinase activity, and RNA metabolic process. Hence, lncRNAs may affect these biological processes, cellular components, and functions, contributing to the pathogenesis of SLE.

Confirmation of dysregulated lncRNAs

Consistent with RNA-seq data (Fig. 4a), NONHSAT159677.1, NONHSAT198272.1, NONHSAT244274.1, and NONHSAT244275.1 were up-regulated, and NONHSAT246155.1 was down-regulated (Fig. 4b). The level of NONHSAT209043.1 exhibited a similar upregulation trend as seen in the RNA-seq analysis, but this upregulation did not reach significance (Fig. 4a).

Correlations between the dysregulated lncRNAs and SLEDAI score of patients with SLE

We further analyzed correlations between SLEDAI scores and levels of NONHSAT159677.1, NONHSAT198272.1, NONHSAT244274.1, NONHSAT244275.1, and NONHSAT246155.1. Only NONHSAT246155.1 ($r = 0.333$, $P = 0.037$) was positively correlated with the SLEDAI score (Fig. 5).

Discussion

In this study, we defined, for the first time, the lncRNA expression profiles of human placentas from SLE pregnancies, though lncRNA expression profiles of human SLE have been reported in peripheral blood mononuclear cells (PBMCs) [15, 16, 18-20, 31, 32], monocyte-derived dendritic cells [33] and whole blood [34]. Our RNA-seq analysis revealed 81 dysregulated lncRNAs in SLE placentas with more up-regulated genes than down-regulated, which agrees with the pattern in monocyte-derived dendritic cells [33] but differs from the patterns reported in PBMCs and whole blood from SLE patients [15, 34]. Among these dysregulated lncRNAs, NONHSAT192272.1 and NONHSAT192274.1 have been reported in whole blood [34], while NONHSAT022132.2 has been found in PBMCs from SLE patients [32]. Interestingly, all the above 3 genes in SLE placentas show different expression patterns compared with blood samples. For instance, NONHSAT192272.1 and NONHSAT192274.1 are up-regulated in SLE placentas whereas down-regulated in SLE whole blood. NONHSAT022132.2 is down-regulated in SLE placentas but up-regulated in SLE PBMCs. This differential dysregulation of lncRNAs might be due to tissue-specific expression of lncRNAs in human tissues [35]. Specifically, the placenta is composed of different cell types, including syncytiotrophoblasts/cytotrophoblasts, mesenchymal cells, mesenchymal derived macrophages, fibroblasts, vascular smooth muscle cells, perivascular cells, endothelial cells, as well as blood cells in the intervillous space and fetal vessels [36, 37]. The diverse cell types in the placenta tissue may also contribute to the above different profiles.

Our RNA-seq analysis also identified 211 dysregulated mRNAs in SLE placentas with more up-regulated genes than the down-regulated, showing different patterns from PBMCs and whole blood from SLE patients [15, 16, 34]. The distribution of SLE-dysregulated mRNAs and lncRNAs on different

chromosomes was also analyzed. Six SLE-dysregulated mRNAs and 2 lncRNAs are located on X-chromosome. However, no SLE-dysregulated mRNA or lncRNA is located on Y-chromosome. The X chromosome contains a large number of genes that are involved in immunity and partly responsible for the hyperresponsiveness of the female immune system [38], which may contribute to the pathogenesis of SLE. To be noted, the Y-chromosome accounts for a smaller portion of the human genome compared with X-chromosome. Smaller portion may lower the possibility of locating SLE-dysregulated genes. However, we should not neglect the fact that the contribution of the Y chromosome to autoimmunity has been conducted in mouse models of SLE [39, 40].

Our bioinformatics analysis revealed intriguing biological functions of these dysregulated protein-coding genes in SLE placentas. In particular, both positive and negative regulation of biological processes, such as both positive regulation of cell adhesion and negative regulation of cell adhesion were enriched (Fig. 2b). The above two-way regulations indicate that SLE placentas are undergoing comprehensive regulations during pregnancy.

It remains to be elusive what causes these dysregulations of lncRNAs. One major factor might be tissue oxygen levels as response to hypoxia is recognized as an SLE-dysregulated network (Fig. 2a). This is supported by the current observation that higher expression of VEGFA, which is hypoxia-driven [39]. Enrichment of response to hypoxia in SLE-induced DE lncRNAs suggests that placentas from SLE pregnancies maybe undergo hypoxic compared with controls. Thus, as hypoxia (~1.5% O₂) inhibits extravillous trophoblasts outgrowth and proliferation [43], SLE-associated hypoxia may decrease placental weight, leading to IUGR [2-4]. This is supported by our current finding that the mean newborn body weight from SLE pregnancies is significantly lower than controls.

SLE-dysregulated genes in placentas were also enriched in the type I interferon signaling pathway, which plays a pivotal role in the pathogenesis of SLE [44]. Genome-wide association studies (GWAS) of SLE patients have shown that key genetic variants are involved in over-activation or regulatory deficits in the innate immune responses that are closely correlated to type I interferons (IFNs) [45]. Additionally, risk alleles that operate in IFN pathway genes have also been implicated in the pathogenesis of lupus in GWAS [46]. The placenta is a transient organ. However, its involvement with the type I interferon signaling pathway suggests that the placenta is indeed a target of SLE during pregnancy, which may cause placental dysfunctions, contributing to maternal and fetal complications commonly seen during SLE pregnancy.

Dysregulation of lncRNA has been reported to be associated with the severity of SLE. For example, lncRNA NEAT1 levels are significantly higher in PBMCs of SLE patients compared with the healthy group and are positively correlated with SLE disease activity [18]. GAS5 is down-regulated in PBMCs and plasma from SLE patients and is negatively correlated with SLE disease activity [19, 47]. linc0597 and linc0949 are both down-regulated in PBMCs from SLE patients and negatively correlated with SLE disease activity [19]. In the current study, these lncRNAs (NEAT1, GAS5, linc0597, and linc0949) were not significantly dysregulated in SLE placentas. However, our correlation analysis showed that

NONHSAT246155.1 was positively correlated with disease activity. Searching and validating biomarkers for SLE during pregnancy requires a combination of clinical manifestations and potential biomarkers. However, NONHSAT246155.1 may not serve as a biomarker for monitoring SLE during pregnancy because it is not possible to obtain the placenta tissue from patients during pregnancy. In the present study, NONHSAT159677.1, NONHSAT198272.1, NONHSAT244274.1, NONHSAT244275.1, and NONHSAT246155.1 were demonstrated to be dysregulated in SLE placentas. Expression profiles of NONHSAT198272.1 and NONHSAT246155.1 in human tissues were reported in the lncRNAs database (<http://www.noncode.org/>). NONHSAT198272.1 has been observed to be expressed in kidney and placenta tissues, whereas NONHSAT246155.1 was reported to be expressed in various human tissues, and the expression level of NONHSAT246155.1 is the second-highest among these tissues. This is the first report that NONHSAT159677.1, NONHSAT244274.1, and NONHSAT244275.1 are expressed in human placentas. Based on the above observations, we speculate that the above lncRNAs dysregulation may potentially serve as biomarkers and novel therapeutic targets for SLE. However, the functions of the above SLE-dysregulated lncRNAs are unknown.

In the current study, we defined the transcriptomic profiles in placentas from SLE pregnancies for the first time. Our findings supported the concept that lncRNAs may play comprehensive roles in the pathogenesis of SLE placentas. However, limitations of our study should be acknowledged. First, this study is limited by the small patient number that we were able to investigate, and further validation of these dysregulated mRNAs and lncRNAs in a larger cohort of patients is needed to confirm our results. Second, SLEDAI scores of SLE patients recruited in this study ranged from 0-6, and the narrow SLEDAI range strongly limited the analysis of the correlation between DEGs expression levels and SLEDAI scores. In addition, further functional studies are needed to define the role of these dysregulated lncRNAs.

Conclusions

This study demonstrates a comprehensive expression profile of lncRNAs and mRNAs in SLE placentas. The findings suggest potential regulatory functions of lncRNAs and mRNAs, which are implicated in the development and pathogenesis of SLE pregnancy. In addition, as NONHSAT246155.1 was positively correlated with the SLEDAI score, it may serve as a therapeutic target candidate for SLE.

Abbreviations

SLE: Systemic lupus erythematosus; IUGR: intrauterine growth retardation; lncRNAs: Long noncoding RNAs; NF- κ B: Nuclear factor- κ B; MAPK: mitogen-activated protein kinase; TNF: tumor necrosis factor; RNA-seq: RNA sequencing; NT: normal full-term; SLEDAI: SLE disease activity index; RIN: RNA integrity number; DEG: differential expression gene; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; FDR: false discovery rate; FPKM: Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced; RT-qPCR: real time quantitative PCR; JNK: c-Jun N-terminal kinase; PBMCs: peripheral blood mononuclear cells; GWAS: genome-wide association studies; IFNs: interferons; CPN: chronic physiological normoxia.

Declarations

Funding

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HHL collected tissues and patient information, and drafted the manuscript. LTS collected the patient information, collected tissues, and participated in manuscript preparation. YL and CF participated in manuscript preparation. KW collected tissues and edited the manuscript critically. CZ wrote and edited the manuscript critically. YJZ, JZ, and QS conceived the concept and wrote the manuscript. YJZ designed the work. All authors read and approved the final version of manuscript.

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Qilu hospital, Shandong University, and the Scientific and Ethical Committee of Shanghai First Maternity and Infant Hospital affiliated with Tongji University. Informed consent was obtained from all patients at enrollment.

Conflicts of interests

The authors declare no conflicts of interest.

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Tables

Figures

Table 1. Clinical and laboratory characteristics of the patients			
Characteristics	SLE (<i>n</i> = 10)	Control (<i>n</i> = 10)	<i>P</i>
Age (years), median (range)	29.0 (26-36)	30.5 (28-33)	> 0.05
BMI, median (range)	25.4 (23.0-32.3)	28.0 (21.8-32.7)	> 0.05
Gestation age (weeks), median (range)	38.5 (34.9-39.7)	39.1 (38.6-40.1)	< 0.05
Fetal weight (grams), median (range)	2950.0 (2150.0-3850.0)	3402.0 (2895.0-3730.0)	< 0.05
Fetal weight lower than 10 th percentile (%)	10	0	> 0.05
Disease duration (months), median (range)	40.0 (10-167)	-	-
SLEDAI score, median (range)	2.5 (0-6)	-	-
ANA > 1:320, yes/no (n)	10/0	-	-
Anti-dsDNA	2/8	-	-
Anti-phospholipid, yes/no (n)	2/8	-	-
Preeclampsia, yes/no (n)	0/8	-	-
Proteinuria, yes/no (n)	3/7	-	-
Hypocomplementemia, yes/no (n)	3/7	-	-
Steroids, yes/no (n)	10/0	-	-
Immunosuppressive drugs, yes/no (n)	0/10	-	-
Aspirin, yes/no (n)	2/8	-	-
<i>SLE</i> systemic lupus erythematosus, <i>BMI</i> body mass index, <i>SLEDAI</i> systemic lupus erythematosus disease activity index, <i>ANA</i> antinuclear antibody, <i>dsDNA</i> double stranded DNA			

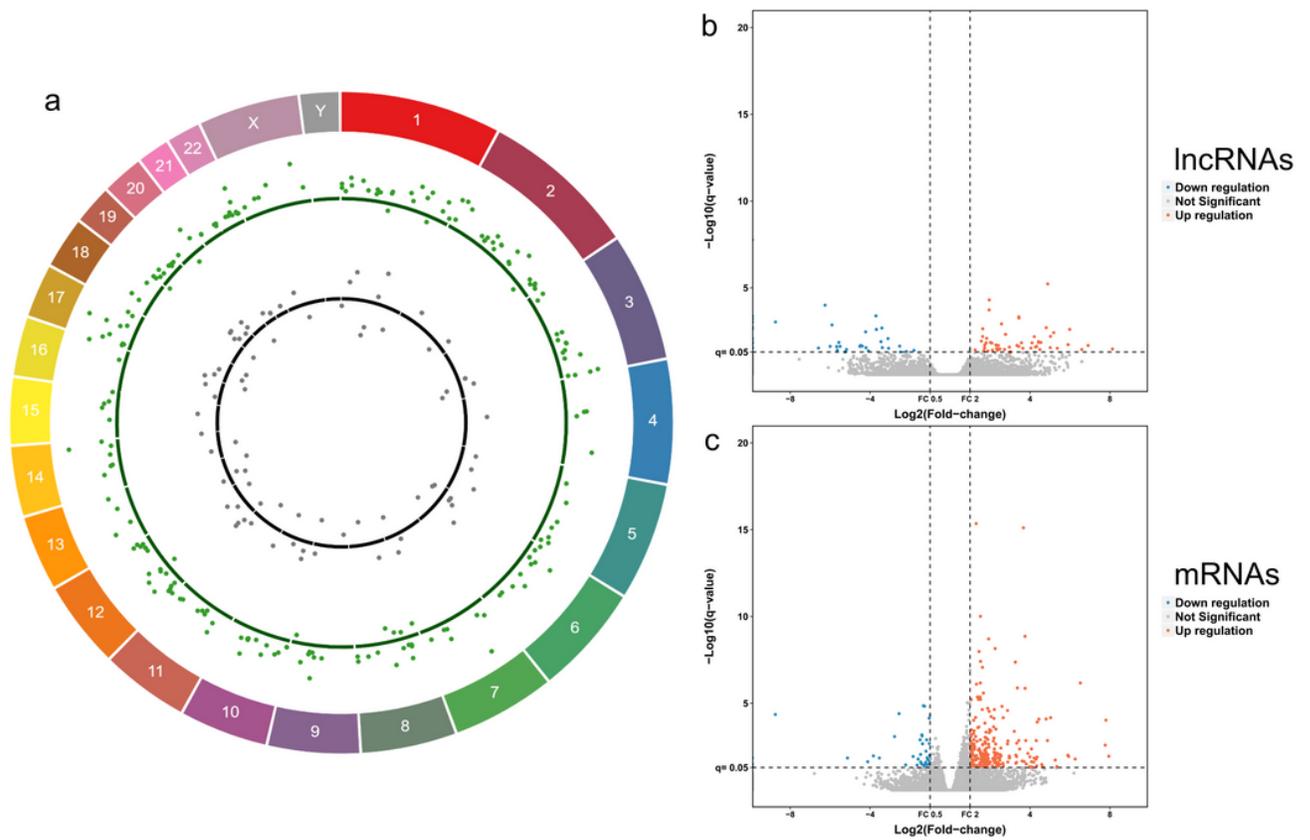


Figure 1

SLE-dysregulated transcriptomic profiles of placentas. a Circos plot illustrating the chromosomal position of dysregulated lncRNAs (gray dots) and mRNAs (green dots) between SLE vs. NT. Each dot represents one gene. The numbers and letters in the outer ring indicate the chromosomal location. For each scatter plot track, dots outside and inside of the centerline are up-regulated and down-regulated genes, respectively. b lncRNA volcano plot of SLE vs. NT. c mRNA volcano plots of SLE vs. NT. n = 8/group.

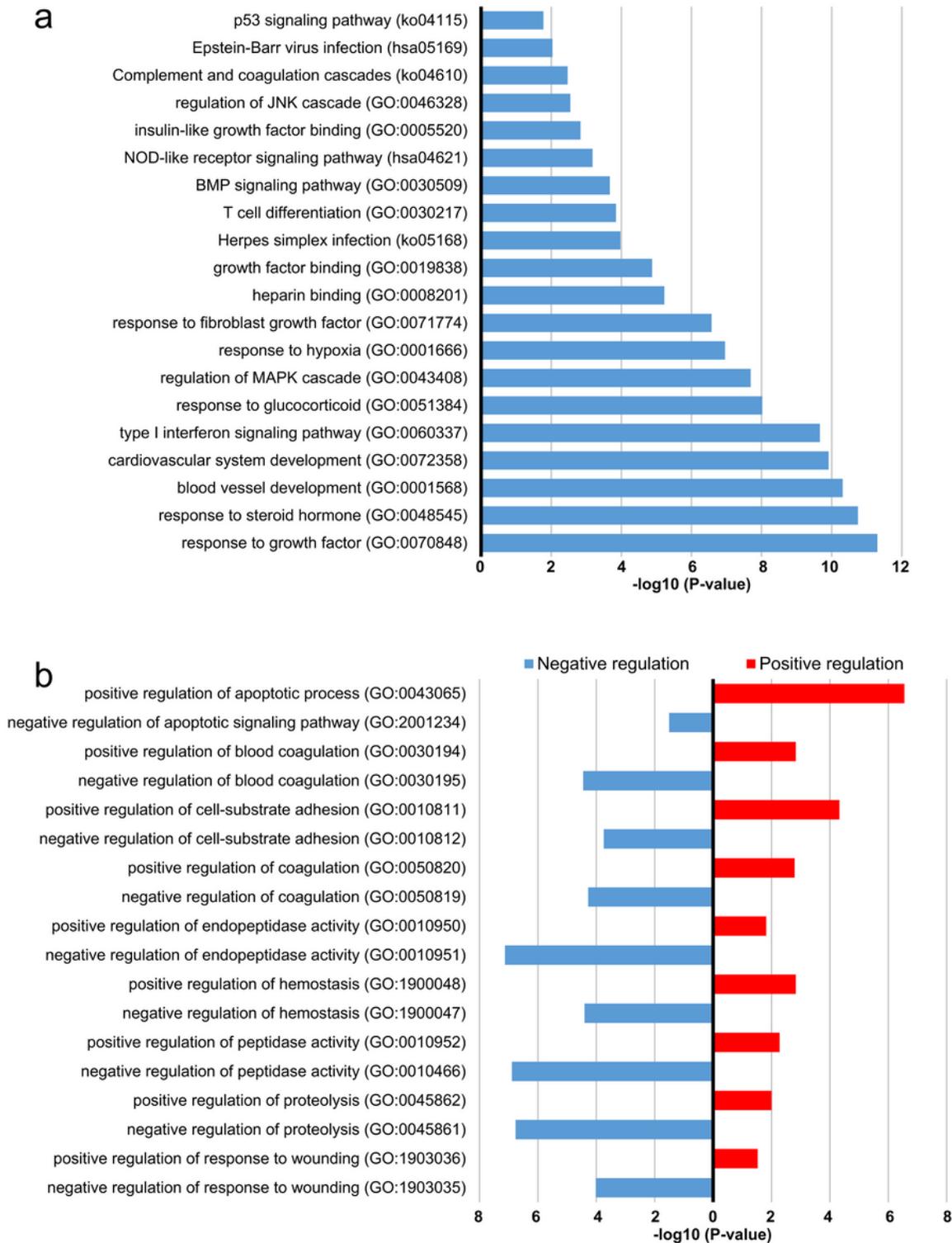


Figure 2

Enrichment analysis for SLE-dysregulated mRNAs in placentas. a Selected GOs and KEGGs in SLE. b Selected two-way enrichment.

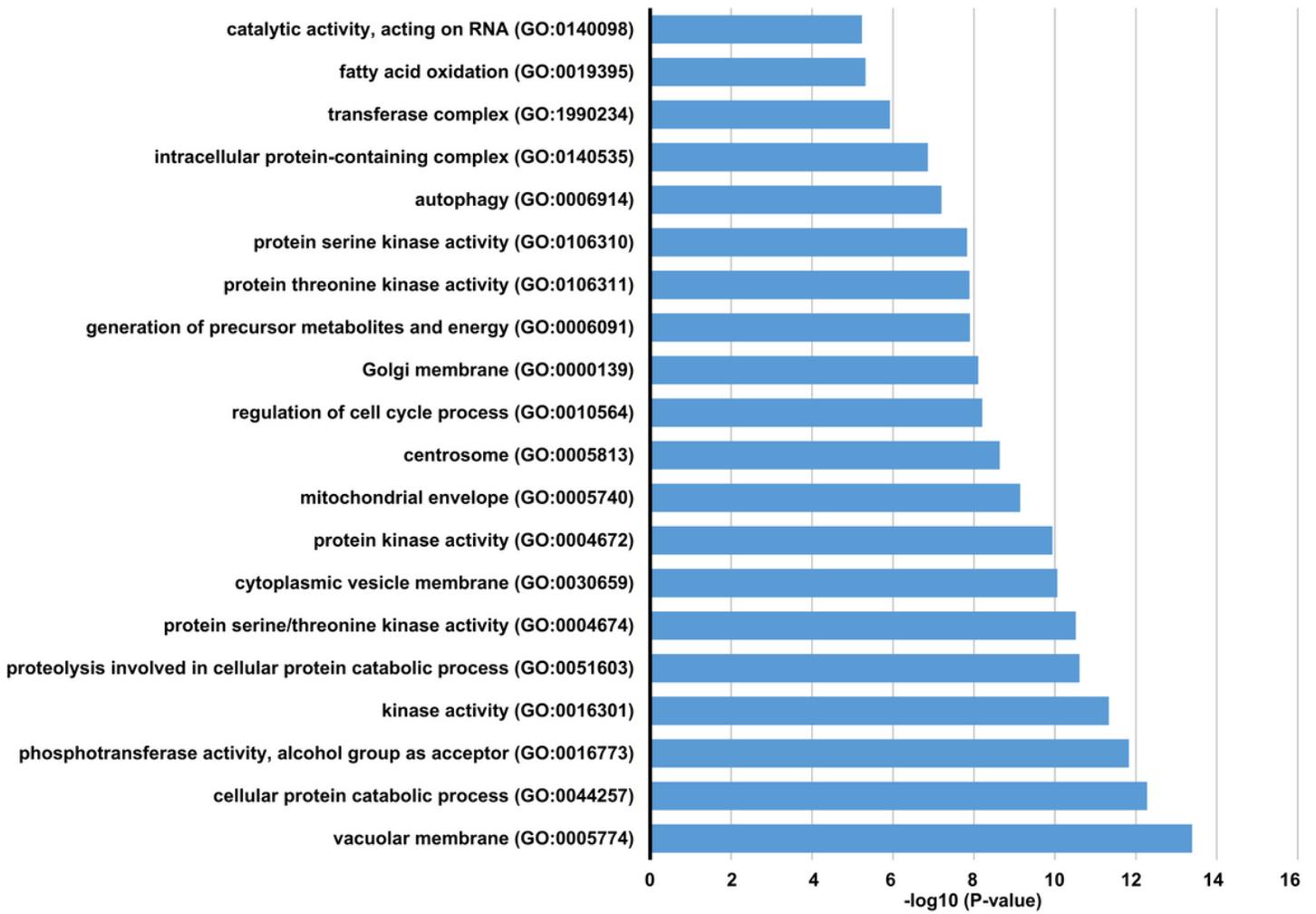


Figure 3

Enrichment analysis for lncRNA target genes. SLE-dysregulated lncRNAs may target 2387 genes including both “cis” and “trans” genes. Biological functions and involved signaling pathways of these 2387 genes were explored using Metascape online analysis tool.

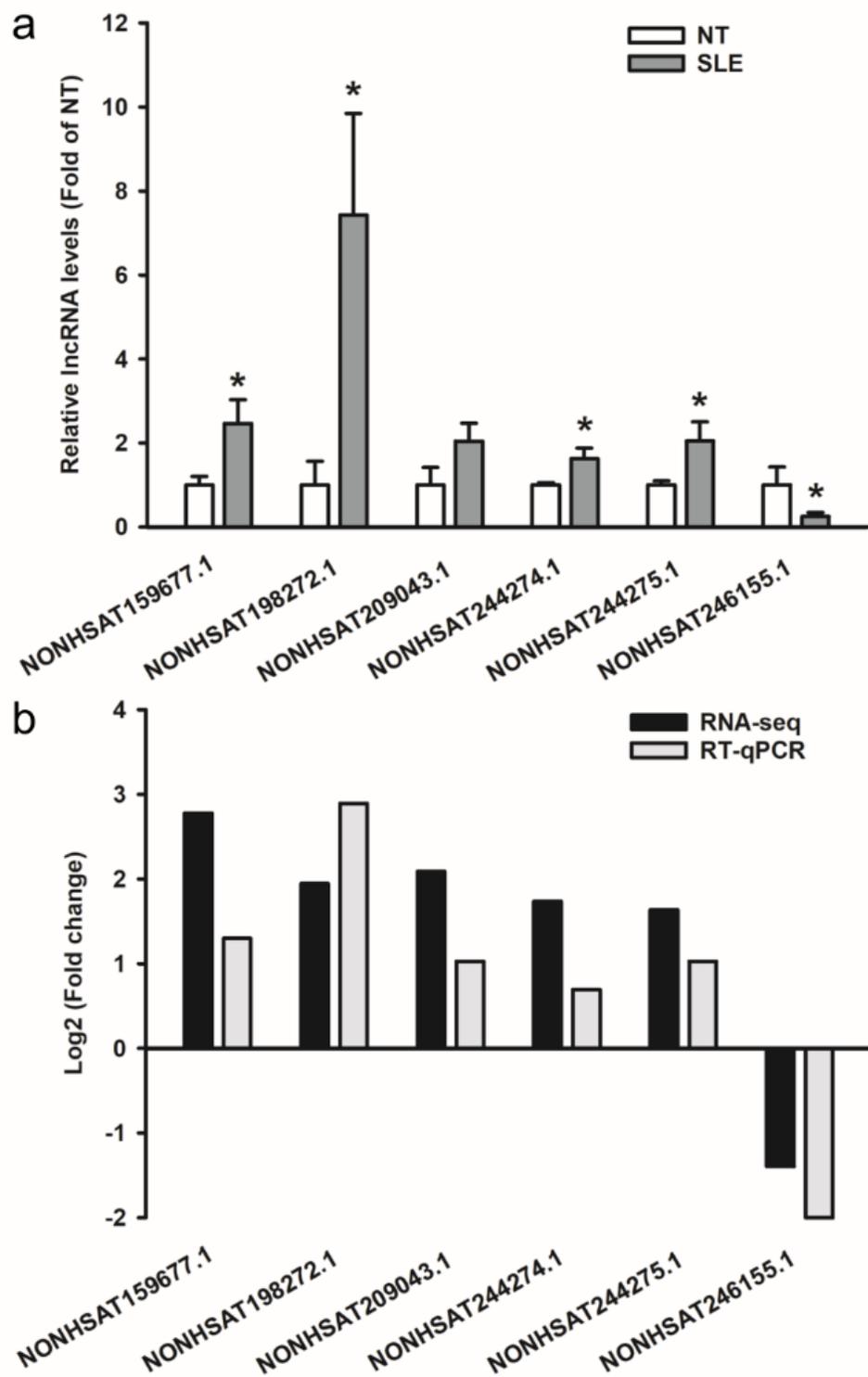


Figure 4

Validation of SLE-dysregulated lncRNAs by RT-qPCR. a Relative lncRNA levels (fold of NT control) in SLE. b The change patterns between RNA-seq and RT-qPCR. *Differ ($P < 0.05$) from NT. $n = 8$ /group.

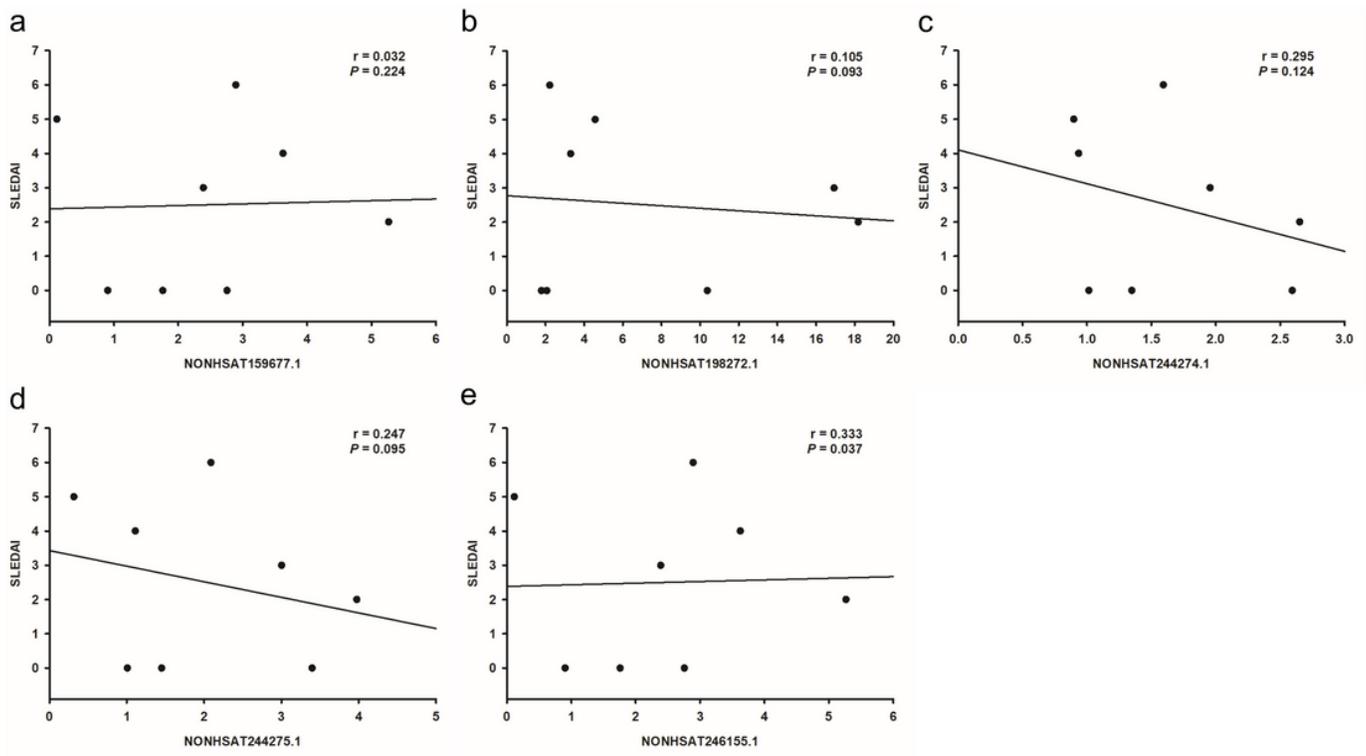


Figure 5

Correlations between SLE-dysregulated lncRNAs and SLEDAI scores. Relative expression levels of lncRNAs were obtained using RT-qPCR. Correlations between SLEDAI scores and expression levels of lncRNAs was analyzed.

Supplementary Files

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