Identification of the key genes and long non-coding RNAs in ankylosing spondylitis using RNA sequencing

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Abstract. Ankylosing spondylitis (AS) is an insidious and debilitating form of arthritis that involves the axial skeleton, and its etiology and pathogenesis remain unclear. In the present study, three patients with AS and three normal controls from our hospital were enrolled. RNA sequencing and bioinformatics analysis were performed in order to identify the differentially expressed (DE) mRNAs (DEmRNAs) and DE long non-coding RNAs (DElncRNAs) between the patients with AS and normal controls. Construction of an AS-specific protein-protein interaction network, a weighted DElncRNA-DEmRNA co-expression network and functional annotation of the DEmRNAs co-expressed with DElncRNAs was performed. Nearby cis-targeted DEmRNAs or DElncRNAs were identified by searching for DEmRNAs that were transcribed within 100-kb up- or downstream of DElncRNAs. Based on the Gene Expression Omnibus datasets GSE25101 and GSE73754, the expression of selected DEmRNAs and DElncRNAs were verified using published RNA sequencing data from blood samples, and receiver operating characteristic analysis of selected DEmRNAs was performed. Compared with the normal controls, 1,072 DEmRNAs and 372 DElncRNAs in the patients with AS were identified. Caspase recruitment domain family member 11 and DNA methyltransferase 1 have great diagnostic value for AS. MSTRG.8559 and LINC00987 were also identified as two hub DElncRNAs. The T-cell receptor signaling pathway was a significantly enriched pathway of the DEmRNAs co-expressed with DElncRNAs in patients with AS. In conclusion, the present study identified the key DEmRNAs and DElncRNAs in AS, which provides novel information for understanding the pathogenesis of AS and developing potential biomarkers for AS.

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Abbreviations: AS, ankylosing spondylitis; DEmRNAs, differentially expressed mRNAs; DElncRNAs, differentially expressed long non-coding RNAs; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction

Key words: ankylosing spondylitis, RNA sequencing, lncRNAs

Introduction

Ankylosing spondylitis (AS) is the prototypic and debilitating type of spondyloarthritis, which is an autoimmune disorder (1). AS often affects the axial joints, including the sacroiliac joint and spine, and induces new bone formation and ultimately ankylosis (2,3).

AS is associated with the interplay of genetic risks and environmental triggers, and its etiology and pathogenesis remain largely unknown. A number of studies have reported a strong association between the major histocompatibility complex class I allele human leukocyte antigen B27 (HLA-B27) and AS (4-6). However, the mechanism by which HLA-B27 causes a predisposition to AS remains unclear. T cells and a number of immune pathways, including the interleukin (IL)-17/IL-23 pathway and control of nuclear factor-κB (NF-κB) activation, have been reported to be closely associated with AS (5,6). Currently, it is difficult to diagnose AS during the early stages due to the lack of accurate diagnostic biomarkers.

Long non-coding RNA (lncRNA) is a type of non-protein-coding transcript with a length of >200 nucleotides; they are emerging as key regulators in various biological processes (7). Recent studies have indicated that lncRNAs, including lncRNA-AK001085, lnc-zinc finger protein 354A (ZNF354A)-1, lnc-Lin-54 DREAM MuvB core complex component (LIN54)-1, lnc-Facioscapulohumeral muscular dystrophy region gene 2 family member C (FRG2C)-3 and lnc-ubiquitin specific peptidase 50 (USP50)-2, serve roles in AS (8,9). Some researchers have also revealed that mRNAs, such as programmed cell death 1 (PDCD1) (1), caspase recruitment domain-containing protein 11 (CARD11) (10), phospholipase Cγ1 (PLCG1) (11,12) and DNA methyltransferase 1 (DNMT1) (13), may be involved in the pathogenesis of AS.

In the present study, the key differentially expressed (DE) mRNAs (DEmRNAs) and DElncRNAs in AS were identified using RNA sequencing and bioinformatics analysis. DElncRNA-DEmRNA co-expression network construction, identification of nearby target DEmRNAs of DElncRNAs and functional annotation of DEmRNAs were performed in order

to understand the biological functions of the key DEmRNAs and DElncRNAs in AS.

Materials and methods

Patients and samples. Three patients with AS and three normal controls were enrolled in the present study from 2nd Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China). The patients with AS were aged 37, 36 and 40 years old, and all were HLA-B27$^+$ and male. These patients were diagnosed with AS based on kyphotic deformity, bilateral damage of the sacroiliac joint observed in the computed tomography results, and spinal fusion and sacroiliac arthrodesis observed in the X-ray results. All of the patients had not received treatment with non-steroidal anti-inflammatory drugs or biologics and they had not exhibited complications. The normal controls were aged 35, 36 and 37 years old and were male. None of the six participants had any other type of autoimmune disease. Blood samples were obtained from all six participants. All of the participants submitted written informed consent and the present study was approved by the Ethics Committee of 2nd Affiliated Hospital, School of Medicine, Zhejiang University.

Library preparation and high-throughput sequencing. Total RNA was isolated from the blood samples with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. A NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Inc.) was used to check the concentration and purity of the RNA. The integrity of the RNA was confirmed using 2% agarose gel and the RNA integrity number (RIN) was obtained using an Agilent 2100 Bioanalyzer instrument (Agilent Technologies, Inc., Santa Clara, CA, USA). The thresholds of total RNA for cDNA library construction were as follows: i) Amount of RNA, >5 μg; ii) concentration of RNA; ≥200 ng/ml; iii) 1.8<optical density $(OD)_{260/280}$<2.2; iv) 2.0<$OD_{260/280}$; and v) RIN value, >8.0.

Firstly, the ribosomal RNA was removed from the total RNA using a Ribo-Zero Magnetic kit (Epicentre; Illumina, Inc., San Diego, CA, USA). In addition, 'Soap' software (version 1.03; soap.genomics.org.cn/SOAPdenovo-Trans.html#intro2) was used to compare the reads of the rRNA, and then to write a perl script to remove it from the FastQ file. Subsequently, the cDNA library for RNA sequencing was contrasted using the TruSeq RNA Sample Prep kit (Illumina, Inc.). Briefly, the retrieved RNA was fragmented by adding First Strand Master Mix and then first-strand cDNA was generated using the First Strand Master mix and Super Script II reverse transcription (Invitrogen; Thermo Fisher Scientific, Inc.) with the following reaction conditions: 25˚C for 10 min, 42˚C for 50 min and 70˚C for 15 min. Following purification of the product with Agencourt RNA Clean XP beads (Beckman Coulter, Inc., Brea, CA, USA), Second Strand Master mix and dATP, dGTP, dCTP and dUTP mix (Beckman Coulter, Inc.) were added to synthesize the second-strand cDNA (16˚C for 1 h). Subsequently, the purified cDNA was combined with End Repair Mix (Thermo Fisher Scientific, Inc.) and incubated at 30˚C for 30 min. Following purification with beads (Qiagen, Inc., Valencia, CA, USA), A-Tailing mix (Qiagen, Inc.) was added into the reaction system, which was incubated at 37˚C for 30 min. Adenylate 3' Ends DNA, Index Adapter and Ligation mix (Qiagen, Inc.) were combined and incubated at 30˚C for 10 min. Subsequently, the Uracil-N-Glycosylase enzyme was added into the purified ligation product and incubated at 37˚C for 10 min. A total of 15 rounds of polymerase chain reaction (PCR) amplification were conducted with PCR Primer Cocktail (Illumina, Inc.) and PCR Master Mix (Illumina, Inc.) to enrich the cDNA fragments. The PCR products were then purified with AMPure XP beads (Qiagen, Inc.). The qualified libraries were amplified on cBot (Illumina, Inc.) to generate a cluster on the flow cell (TruSeq PE Cluster kit v3-cBot-HS; Illumina, Inc.). The amplified flow cell was sequenced on an Illumina HiSeq X Ten platform (Illumina, Inc.).

Quality control of raw sequence. Quality control of the raw reads derived from the RNA sequencing was performed to obtain clean reads of high quality. Briefly, quality control involved trimming low-quality reads, including adaptor sequences, sequences with a quality score <20 and sequences with an N-base rate of raw reads >10%, using SeqPrep (version 1.2; github.com/jstjohn/SeqPrep) and Sickle (version V3.4.0; github.com/najoshi/sickle).

Clean reads mapping. Sequences were aligned to the human reference genome GRCh38.p7 (Ensembl v84; www.ensembl.org/index.html) using hierarchical indexing for the spliced alignment of the transcripts programme HISAT2 (version 2.1.0; ccb.jhu.edu/software/hisat2/index.shtml). Then, StringTie (version v1.3.4; ccb.jhu.edu/software/stringtie/) was used to assemble and quantify the transcripts in each sample. Ultimately, differential gene expression analysis was performed with Ballgown (version 3.7; www.bioconductor.org/packages/release/bioc/html/ballgown.html) in R environment.

Identification of DEmRNAs and DElncRNAs. Using Ballgown, the DEmRNAs and DElncRNAs between the patients with AS and normal controls were identified with P<0.05. The false discovering rate (FDR)-adjusted P-value of the test statistic was used. Hierarchical clustering of the DElncRNAs and DEmRNAs expression profile was performed using hcluster in R language (version 3.3.3; stat.ethz.ch/R-manual/R-devel/library/stats/html/hclust.html).

AS-specific protein-protein interaction (PPI) network construction. PPI networks of the top 30 up- and down-regulated DEmRNAs, respectively, were constructed using BioGRID (version 3.5; thebiogrid.org).

AS-specific weighted DEmRNA-DElncRNA co-expression network analyses. Weighted Gene Co-expression Network Analysis (WGCNA_1.64-1; horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/) (14) is an R package for weighted correlation network analysis, which is also known as weighted gene co-expression network analysis. Using WGCNA, AS-specific weighted DEmRNA-DElncRNA co-expression network analysis was performed. The pairwise Pearson's correlation coefficients (PCCs) between the DElncRNAs and DEmRNAs in patients with AS were calcu-

Table I. Top 10 up- and downregulated DEmRNAs between ankylosing spondylitis and normal controls.

DEmRNAs	log2.Fold change	P-value	SD_fpkm_C	SD_fpkm_T	Regulation
IGHG1	-2.021979095	0.047255959	8.80361236	0.515381703	Down
IGHG4	-1.859252007	0.025210597	3.368882506	0.227307135	Down
TRAJ18	-1.643179443	0.035866398	10.07677638	4.09159482	Down
TRAJ11	-1.524711275	0.014505975	8.654159887	4.620202967	Down
TRAJ5	-1.49467071	0.000440647	6.737567008	2.426348758	Down
TRAJ44	-1.46271809	0.001143618	4.42364856	1.848651282	Down
ETS1	-1.335519855	0.031765089	32.5763664	9.312805914	Down
LAIR2	-1.302776176	0.044790098	0.834353066	1.050550336	Down
ESYT1	-1.259200819	0.002673524	15.14634661	6.658023234	Down
NCR3	-1.247053856	0.033379705	5.641796936	2.719067002	Down
CLC	1.194865831	0.043003696	21.60725157	83.0330712	Up
S100A12	0.98788469	0.00518168	40.98384277	110.0364144	Up
CMPK2	0.904925259	0.01474919	0.622597776	2.021786682	Up
ZFP36	0.842014426	0.012071156	6.481954564	7.910723637	Up
MME	0.746984037	0.031312145	9.013396028	20.24882244	Up
LIN7A	0.711395439	0.049387957	3.456739121	4.043733587	Up
CYP4F3	0.707298362	0.025967428	4.648357447	8.950444777	Up
CXCL8	0.651965182	0.048148598	6.882829746	11.04384805	Up
CLEC4D	0.636486225	0.047074356	2.579585737	3.692774365	Up
FAM118A	0.634156346	0.010715671	0.992441488	2.574184644	Up

DEmRNAs, differentially expressed mRNAs; fpkm, fragments per kilobase million; C, control group; T, case group.

Table II. Top 10 up- and downregulated DElncRNAs between the patients with ankylosing spondylitis and the normal controls.

DElncRNAs	log2.Fold change	P-value	SD_fpkm_C	SD_fpkm_T	Regulation
MSTRG.9221	-2.900321700	0.018550654	4.278074312	0.276811741	Down
MSTRG.1368	-2.619486415	0.003067593	1.673345961	0	Down
AL928768.3	-2.320417178	0.018613919	1.432426837	0.766958784	Down
WDR86-AS1	-1.749482818	0.045879379	5.820719606	0.430167793	Down
GS1-393G12.12	-1.595574045	0.025413087	2.892968281	0.573066828	Down
RP11-75C10.7	-1.578454562	0.028918409	1.681378986	0.27755832	Down
MSTRG.22984	-1.515487489	0.028151217	6.307372767	6.856887292	Down
RP1-29C18.8	-1.491219357	0.007182308	1.813751882	0.780203396	Down
RP11-20I20.4	-1.489870084	0.045895116	1.882568915	0.702213888	Down
CTD-2531D15.5	-1.452569255	0.044459184	1.198212257	0	Down
AC010084.1	1.812545185	0.005654266	0	0.808113485	Up
PSMD5-AS1	1.788651473	0.011076928	8.927660428	20.42027077	Up
RP11-535M15.1	1.639437785	0.00820557	0	1.477784502	Up
LLPH-AS1	1.498657545	0.005471274	0.230318651	2.298519226	Up
RP4-811H24.9	1.452389476	0.004968106	0.053490925	0.499762058	Up
MSTRG.30231	1.342076952	0.040559246	2.874551067	6.364192991	Up
TMEM92-AS1	1.339293051	0.014889801	0	1.352052251	Up
WI2-87327B8.2	1.289651543	0.017494757	1.241932483	1.714464098	Up
AC099668.5	1.25464443	0.023121561	0	1.059053016	Up
LINC01151	1.231390756	0.00790838	0.384761558	0.385097694	Up

DElncRNA, differentially expressed long non-coding RNA; fpkm, fragments per kilobase million; C, control group; T, case group.

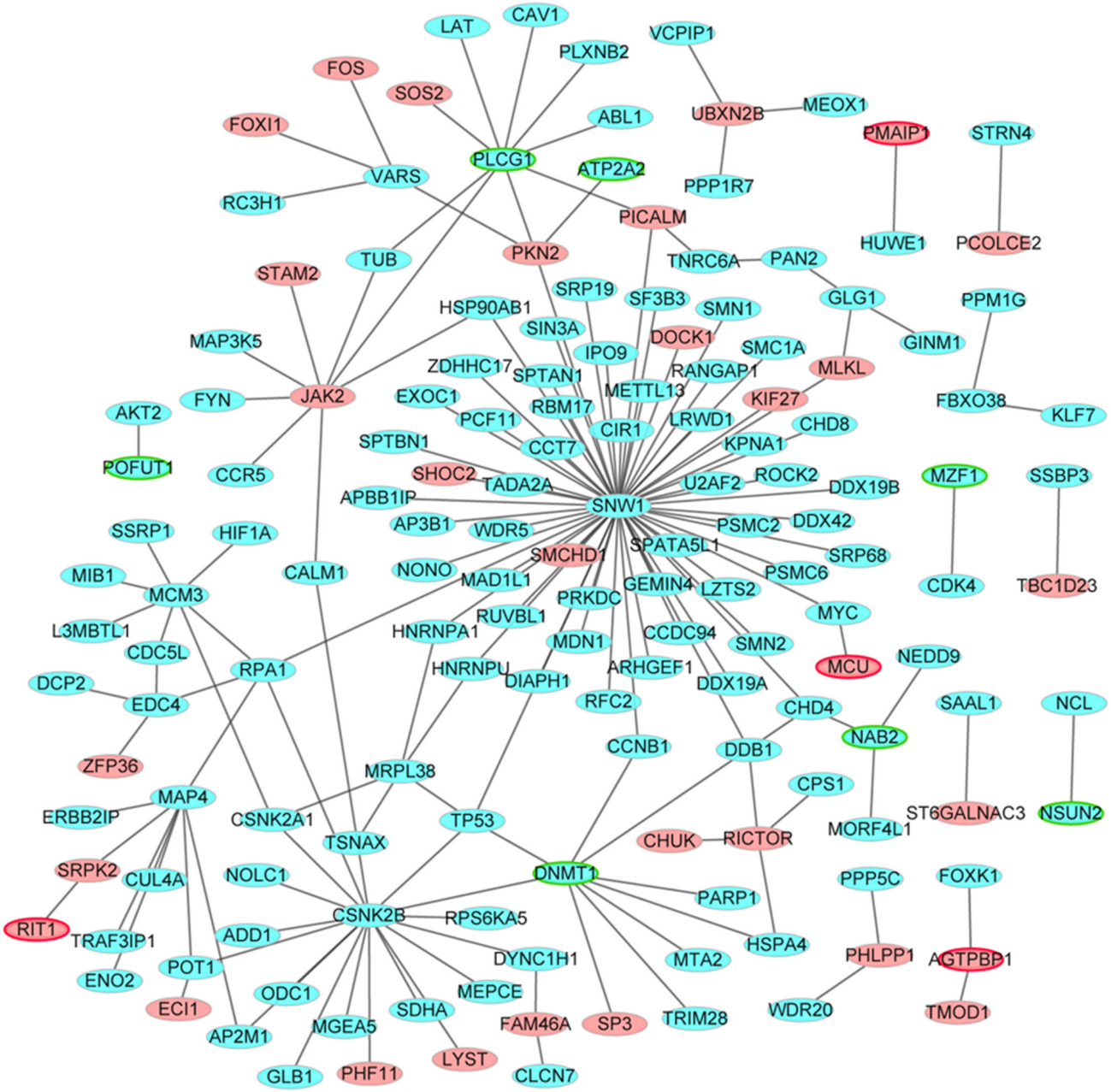

Figure 1. AS-specific PPI network. Blue and red shapes represent proteins encoded by down- and upregulated DEmRNAs, respectively, when comparing patients with AS and normal controls. Among these, blue shapes with green outlines represent proteins encoded by the top 10 downregulated DEmRNAs; red shapes with red outlines represent proteins encoded by the top 10 upregulated DEmRNAs. AS, ankylosing spondylitis; DEmRNA, differentially expressed mRNA.

lated. DElncRNA-DEmRNA pairs with |PCC value ≥0.90| and P<0.001 were used to construct an AS-specific weighted DEmRNA-DElncRNA co-expression network, which was deciphered using the online-based software GeneCodis3 (genecodis.cnb.csic.es/analysis).

Functional analyses of DEmRNAs co-expressed with DElncRNAs in AS. The DEmRNAs of these DElncRNA-DEmRNA pairs with |PCC value ≥0.90| and P<0.001 were used to conduct Gene Ontology (GO; www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/) molecular pathway enrichment analysis using GeneCoDis3.

DEmRNAs close to DElncRNAs with cis-regulatory effects. Previous studies have reported that lncRNAs can regulate genes that are transcribed near to them, consistent with activity in cis-regulatory elements (15,16). Therefore, in the present study DEmRNAs that were close cis targets of DElncRNAs were identified by searching for DEmRNAs that were transcribed within a 100-kb window up- or downstream of DElncRNAs between the patients with AS and the normal controls (17).

Validation of the expression of DEmRNAs and DElncRNAs using the GSE73754 and GSE25101 datasets. The mRNA expression profile of 52 patients with AS and 20 normal controls (Canadian cohort) in the GSE73754 dataset (GPL10558 Illumina

Table III. The top 100 co-expressed differentially expressed mRNA-differentially expressed long non-coding RNA interaction network.

mRNAsig	lncRNAsig	corsig	pvalsig
OR5B2	RP11-524O1.4	0.999999784	$7.02x10^{-14}$
ADAMTS14	RP5-906A24.2	0.999998855	$1.97x10^{-12}$
ADAMTS14	KCNMB2-AS1	0.99999619	$2.18x10^{-11}$
DMRTC2	RP11-756H6.1	0.9999959	$2.52x10^{-11}$
PRY	RP11-355N15.3	0.999995538	$2.99x10^{-11}$
CAMSAP3	LINC01337	0.999993952	$5.49x10^{-11}$
GPR62	LINC01337	0.999993931	$5.52x10^{-11}$
ACTN3	AC004471.9	0.999985033	$3.36x10^{-10}$
COL21A1	LINC01337	0.999984742	$3.49x10^{-10}$
OR5B2	RP11-91I20.2	0.9999843	$3.70x10^{-10}$
SFTA2	AC004471.9	0.999975676	$8.88x10^{-10}$
ADAMTS14	TSPEAR-AS1	0.999948189	$4.03x10^{-9}$
DMRTC2	TSPEAR-AS1	0.999936548	$6.04x10^{-9}$
FOXI1	RP5-906A24.2	0.999888814	$1.85x10^{-8}$
FOXI1	AC022431.3	0.999855298	$3.14x10^{-8}$
ERICH6	RP1-72A23.4	0.999853976	$3.20x10^{-8}$
DMRTC2	KCNMB2-AS1	0.99982545	$4.57x10^{-8}$
FOXI1	KCNMB2-AS1	0.999815958	$5.08x10^{-8}$
OR2C3	RP11-355N15.3	0.999806865	$5.59x10^{-8}$
FOXI1	RP11-320N21.1	0.999759427	$8.68x10^{-8}$
PAK6	PSMD5-AS1	0.999743004	$9.91x10^{-8}$
DMRTC2	RP5-906A24.2	0.999736476	$1.04x10^{-7}$
CAMSAP3	RP11-254I22.2	0.999726506	$1.12x10^{-7}$
GPR62	RP11-254I22.2	0.99972637	$1.12x10^{-7}$
SFTA2	RP11-320N21.1	0.99970509	$1.30x10^{-7}$
ADAMTS14	RP11-756H6.1	0.999704563	$1.31x10^{-7}$
ERICH6	RP11-254I22.2	0.999685951	$1.48x10^{-7}$
COL21A1	RP11-254I22.2	0.999676565	$1.57x10^{-7}$
OR2C3	RP11-524O1.4	0.999662773	$1.71x10^{-7}$
FOXI1	TSPEAR-AS1	0.999646107	$1.88x10^{-7}$
SFTA2	AC022431.3	0.999573412	$2.73x10^{-7}$
PRY	RP11-203E8.1	0.999549564	$3.04x10^{-7}$
PRY	RP11-104N10.1	0.999534887	$3.24x10^{-7}$
OR2C3	RP11-91I20.2	0.999522111	$3.43x10^{-7}$
PRY	RP4-736L20.3	0.999509557	$3.61x10^{-7}$
FCRL4	CTD-2534I21.8	0.999489932	$3.90x10^{-7}$
ADAMTS14	AC022431.3	0.999441016	$4.69x10^{-7}$
PRY	AC008781.7	0.999421699	$5.02x10^{-7}$
ACTN3	RP11-320N21.1	0.999325423	$6.82x10^{-7}$
FZD9	AC008781.7	0.999315145	$7.03x10^{-7}$
RP11-505K9.4	RP11-756H6.1	0.999273631	$7.91x10^{-7}$
ADAMTS14	RP11-320N21.1	0.999264308	$8.12x10^{-7}$
FZD9	RP4-736L20.3	0.999211985	$9.31x10^{-7}$
FZD9	RP11-104N10.1	0.99917912	$1.01x10^{-6}$
FOXI1	RP11-756H6.1	0.999170485	$1.03x10^{-6}$
FZD9	RP11-203E8.1	0.99915935	$1.06x10^{-6}$
PDZD4	RP11-159N11.4	0.999141006	$1.11x10^{-6}$
ACTN3	AC022431.3	0.999132502	$1.13x10^{-6}$
RP11-219A15.1	RP11-457M11.7	0.999106184	$1.20x10^{-6}$
CAMSAP3	RP1-72A23.4	0.999004264	$1.49x10^{-6}$
GPR62	RP1-72A23.4	0.999004022	$1.49x10^{-6}$
OR5B2	RP11-355N15.3	0.998989141	$1.53x10^{-6}$
ERICH6	LINC01337	0.998988895	$1.53x10^{-6}$
CXXC5	LINC01588	0.998981114	$1.56x10^{-6}$
FZD9	RP1-72A23.4	0.998951532	$1.65x10^{-6}$
COL21A1	RP1-72A23.4	0.99891675	$1.76x10^{-6}$
NDUFC2-KCTD14	RP11-45A17.2	-0.998877807	$1.89x10^{-6}$
MAP4	MSTRG.8559	0.998862021	$1.94x10^{-6}$
PRY	RP11-524O1.4	0.998818651	$2.09x10^{-6}$
SRGN	CTD-2562G15.3	0.998661182	$2.69x10^{-6}$
RP11-505K9.4	TSPEAR-AS1	0.998635484	$2.79x10^{-6}$
OR2C3	RP11-203E8.1	0.998613936	$2.88x10^{-6}$
OR2C3	RP11-104N10.1	0.998588286	$2.99x10^{-6}$
FOXI1	AC004471.9	0.998585363	$3.00x10^{-6}$
PRY	RP11-91I20.2	0.998566098	$3.08x10^{-6}$
ZNF804A	TCEAL3-AS1	0.99856452	$3.09x10^{-6}$
FAM111A	MSTRG.6714	0.998552192	$3.14x10^{-6}$
OR2C3	RP4-736L20.3	0.998544416	$3.18x10^{-6}$
DMRTC2	AC022431.3	0.998494339	$3.40x10^{-6}$
OR2C3	AC008781.7	0.99839571	$3.86x10^{-6}$
HHAT	AC012074.2	0.998323875	$4.21x10^{-6}$
IL18BP	MSTRG.8559	0.998273402	$4.47x10^{-6}$
RP5-862P8.2	RP11-1H8.5	0.998269298	$4.49x10^{-6}$
SMAD6	RP5-1092A3.5	0.998257122	$4.55x10^{-6}$
CFAP20	MSTRG.8559	0.998252424	$4.58x10^{-6}$
RP11-505K9.4	KCNMB2-AS1	0.998220553	$4.75x10^{-6}$
DMRTC2	RP11-320N21.1	0.998212166	$4.79x10^{-6}$
PCOLCE2	RP11-105C19.2	-0.998176846	$4.98x10^{-6}$
SFTA2	RP5-906A24.2	0.998131862	$5.23x10^{-6}$
FZD9	RP11-254I22.2	0.998116114	$5.32x10^{-6}$
ARL11	DNAJC3-AS1	0.998052713	$5.68x10^{-6}$
MYOF	LINC01151	-0.998035735	$5.78x10^{-6}$
NT5C2	RP11-139H15.6	0.998022562	$5.86x10^{-6}$
MME	RP11-295I5.4	0.998001494	$5.99x10^{-6}$
RP11-505K9.4	RP5-906A24.2	0.99795661	$6.26x10^{-6}$
IL18BP	MYCBP2-AS2	0.997935664	$6.39x10^{-6}$
HSPA1L	LINC01588	0.997924492	$6.46x10^{-6}$
SAMHD1	MYCBP2-AS2	0.997902735	$6.59x10^{-6}$
SFTA2	KCNMB2-AS1	0.997861652	$6.85x10^{-6}$
CSNK2B	RP11-214K3.23	0.997833473	$7.04x10^{-6}$
NOLC1	MSTRG.8559	0.997817608	$7.14x10^{-6}$
TNXB	RP11-441F2.2	0.997791211	$7.31x10^{-6}$
RHOB	LINC00671	0.997708778	$7.87x10^{-6}$
DDB1	RP11-429J17.2	0.997652855	$8.26x10^{-6}$
ORMDL3	MYCBP2-AS2	0.997626959	$8.44x10^{-6}$
OR5B2	RP11-927P21.2	0.99758326	$8.75x10^{-6}$
ADAMTS14	AC004471.9	0.997577319	$8.80x10^{-6}$
TRIM73	RP11-565F19.2	0.997494288	$9.41x10^{-6}$
PEG3	RP11-17E13.2	0.997467797	$9.61x10^{-6}$

lncRNA, long non-coding RNA.

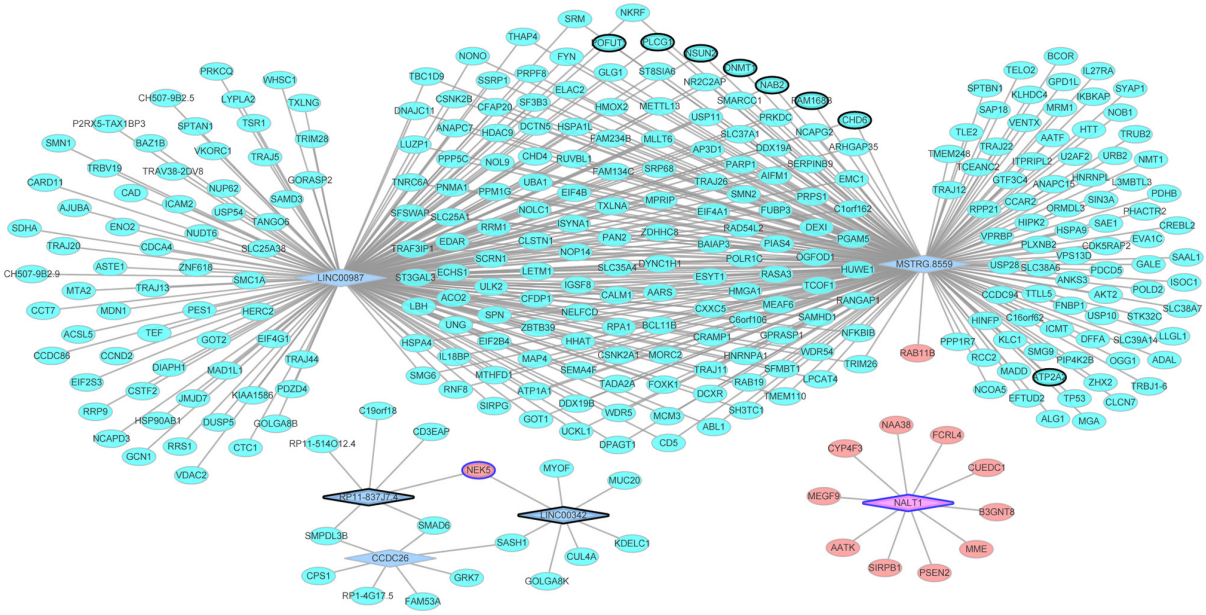

Figure 2. Selected DElncRNA-DEmRNA co-expression network. Ovals and rhombuses represent DEmRNAs and DElncRNAs, respectively, comparing AS patients and normal controls. Shapes with bold black outlines indicate the top 10 DEmRNAs/DElncRNAs when comparing AS and normal controls. AS, ankylosing spondylitis; DEmRNA, differentially expressed mRNA; DElncRNA, differentially expressed long non-coding RNA.

HumanHT-12 V4.0 expression beadchip), which was downloaded from the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/gds). The mRNA and lncRNA expression profile of 16 patients with AS and 16 normal controls (Australian cohort) in the GSE25101 dataset (GPL6947 Illumina HumanHT-12 V3.0 expression beadchip) was also downloaded from the GEO database. The expression levels of the selected DEmRNAs and DElncRNAs between the patients with AS and normal controls in the present study were validated using the GSE73754 and GSE25101 datasets, and the difference in the expression levels was visualized using box plots.

Receiver operating characteristic (ROC) analyses. To assess the diagnostic value of the DEmRNAs in AS, ROC analyses were conducted using pROC package (version 1.13.0) in R language (cran.r-project.org/web/packages/pROC/index.html). The area under the curve (AUC) under the binomial exact confidence interval was calculated. ROC curves were then generated.

Statistical analysis. Values are displayed as the mean ± standard deviation. Student's t-test was used to assess differences among the groups, and $P \leq 0.05$ was considered to indicate a statistically significant difference. Co-expression associations between the lncRNAs and the protein-coding genes were estimated using pairwise PCC analysis using R language (version 3.3.3; stat.ethz.ch/R-manual/R-devel/library/stats/html/hclust.html).

Results

Identification of DEmRNAs and DElncRNAs between patients with AS and normal controls. Compared with the normal controls, 1,072 DEmRNAs (320 upregulated and 752 downregulated) and 372 DElncRNAs (230 upregulated and 142 downregulated) in patients with AS were identified. The top 10 up- and downregulated DEmRNAs and DElncRNAs between the patients with AS and normal controls are shown in Tables I and II, respectively.

MANSC domain containing 1 (MANSC1) and DNMT1 were the most significantly up- and downregulated DEmRNAs between the patients with AS and normal controls, respectively (data not shown). NOTCH1 associated lncRNA in T-cell acute lymphoblastic leukemia 1 (NALT1) and RP11-837J7.4 were the most significantly up- and downregulated DElncRNAs between the patients with AS and normal controls, respectively (data not shown).

AS-specific PPI network construction. The AS-specific PPI network consisted of 159 nodes and 164 edges. The top 10 mRNAs that had the highest degrees were Myc proto-oncogene basic helix-loop-helix transcription factor (MYC; degree=65), heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1; degree=40), spectrin-a non-erythrocytic 1 (SPTAN1; degree=18), TATA-box binding protein associated factor 10 (TAF10; degree=9), ETS proto-oncogene 1 transcription factor (degree=7), NCK adaptor protein 1 (degree=6), eukaryotic translation initiation factor 4B (degree=5), KIAA1033 (degree=5), extended synaptotagmin 1 (degree=5) and tumor protein 53 (degree=5); of these, MYC, HNRNPA1, SPTAN1 and TAF10 were hub proteins of the AS-specific PPI network (Fig. 1).

AS-specific weighted DEmRNA-DElncRNA co-expression network analysis. A total of 3,505 lncRNA-mRNA pairs were identified, which included 302 DElncRNAs and 602 DEmRNAs with |PCC value ≥0.90| and $P<0.001$. Based on these lncRNA-mRNA pairs, the negatively and positively co-expressed DEmRNA-DElncRNA interaction networks were constructed, respectively. The top 100 co-expressed DEmRNA-DElncRNA interaction network is presented in Table III. Based on the positively co-expressed DEmRNA-DElncRNA interaction network, MSTRG.8559 (degree=226) and long intergenic

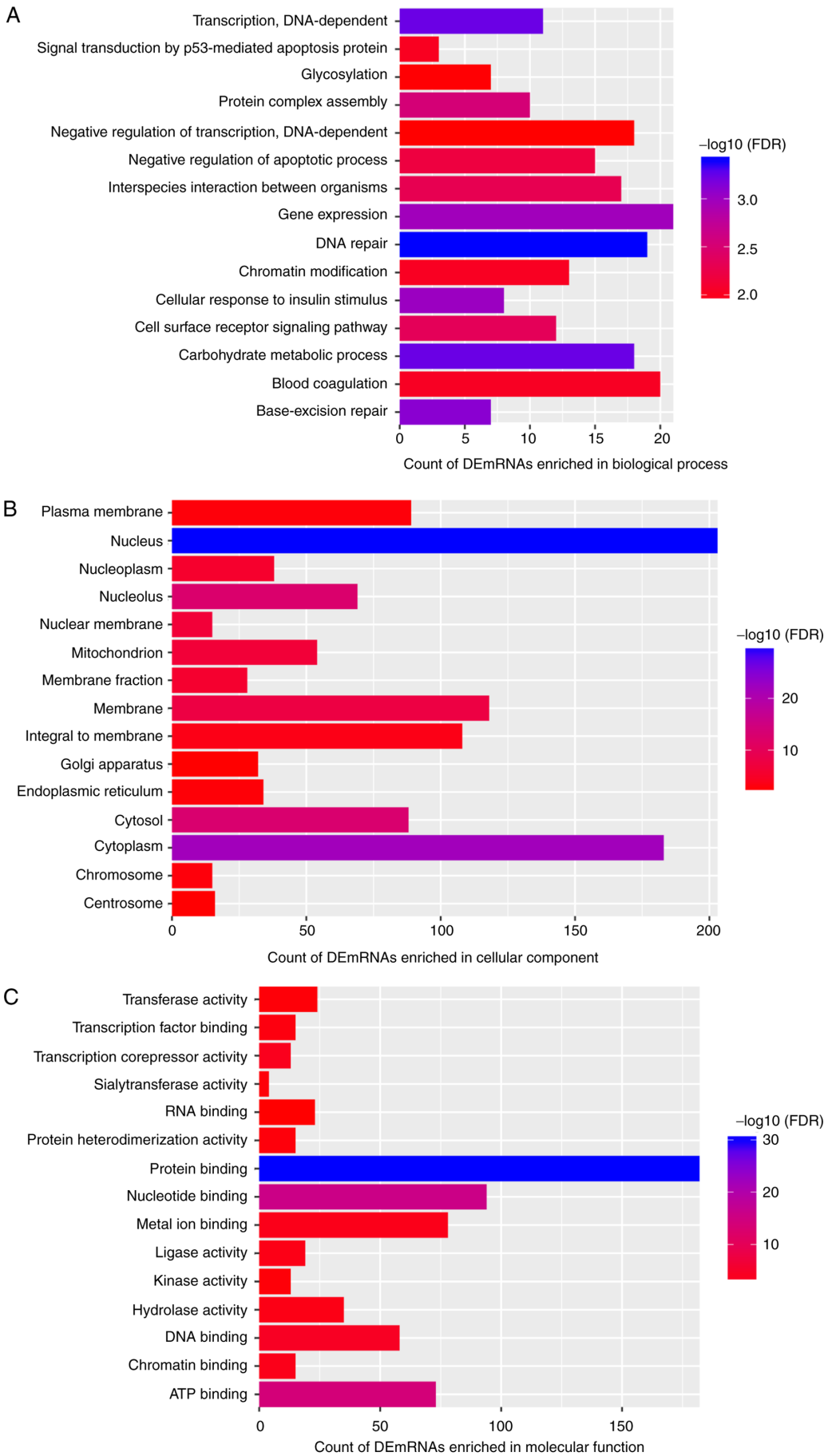

Figure 3. Top 15 most significantly enriched GO terms of DEmRNAs co-expressed with DElncRNAs. The x-axis presents the number of DEmRNAs enriched in the GO terms and the y-axis presents the GO terms. (A) Biological process; (B) Cellular component; (C) Molecular function. GO, Gene Ontology; DEmRNA, differentially expressed mRNA; DElncRNA, differentially expressed long non-coding RNA; FDR, false discovery rate.

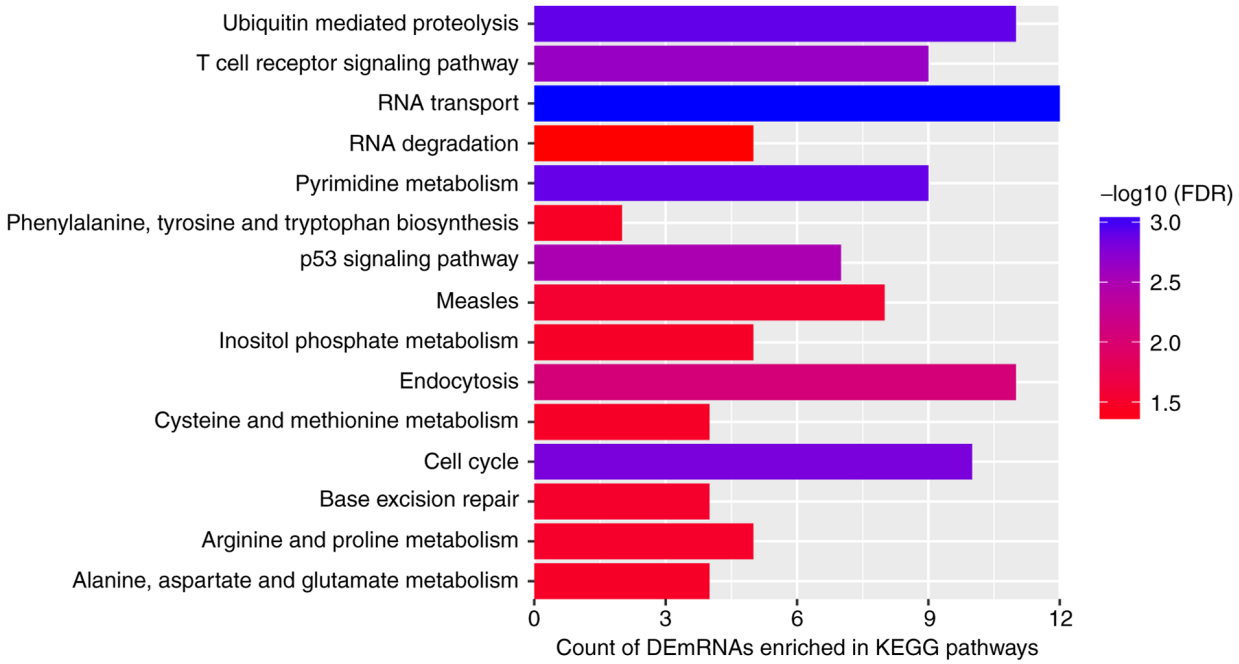

Figure 4. Top 15 most significantly enriched KEGG pathways of DEmRNAs co-expressed with DElncRNAs. The x-axis presents the number of DEmRNAs enriched in KEGG pathways and the y-axis presents the KEGG pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEmRNA, differentially expressed mRNA; DElncRNA, differentially expressed long non-coding RNA; FDR, false discovery rate.

non-protein coding RNA 987 (LINC00987; degree=209) were the top 2 DElncRNAs that were co-expressed with the greatest number of DEmRNAs (Fig. 2). The selected co-expression DEmRNA-DElncRNA interaction network is presented in Fig. 2.

Functional analysis of DEmRNAs co-expressing DElncRNAs between patients with AS and normal controls. Based on the GO enrichment analysis (Fig. 3) of the 602 DEmRNAs that were co-expressed with DElncRNAs in patients with AS and normal controls, the significantly enriched GO terms were as follows: 'DNA repair' (FDR=3.94x10^{-4}; Fig. 3A), 'carbohydrate metabolic process' (FDR=5.66x10^{-4}; Fig. 3A), 'nucleus' (FDR=3.34x10^{-30}; Fig. 3B) and 'protein binding' (FDR=7.54x10^{-31}; Fig. 3C). 'T-cell receptor signaling pathway' (FDR=2.29x10^{-3}; Fig. 4) and 'cell cycle' (FDR=1.52x10^{-3}; Fig. 4) were the significantly enriched KEGG pathways.

DEmRNAs close to DElncRNAs with cis-regulatory effects. A total of 84 DElncRNAs and nearby cis target DEmRNA pairs, which included 73 DElncRNAs and 82 DEmRNAs, were obtained (Table IV).

Validation of the expression of DEmRNAs and DElncRNAs using the GSE73754 and GSE25101 datasets. A total of 4 DEmRNAs (DNMT1, PDCD1, CARD11 and PLCG1) were selected to perform expression validation using the GSE73754 dataset (Fig. 5). The expression of all four DElncRNAs was downregulated in patients with AS when compared with the normal controls, which was generally consistent with the RNA sequencing data (DNMT1, P<0.05; CARD11, P<0.05; PDCD1, P>0.05; PLCG1, P>0.05). Three lncRNAs (cat eye syndrome chromosome region candidate 7, hydatidiform mole associated and imprinted and KIAA0125) and seven DEmRNAs [MANSC1, adenosine triphosphatase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (ATP2A2), myeloid zinc finger 1 (MZF1), PDCD1, DNMT1, CARD11 and PLCG1] were selected to perform expression validation using the GSE25101 dataset (Fig. 6). The expression of KIAA0125 (P>0.05), MANSC1 (P>0.05), ATP2A2 (P>0.05), MZF1 (P>0.05), PDCD1 (P>0.05), DNMT1 (P<0.0001), CARD11 (P<0.05) and PLCG1 (P>0.05) was generally consistent with the RNA sequencing data.

ROC curve analysis. ROC curve analyses and the AUC were used to assess the discriminatory ability of the four DEmRNAs (DNMT1, PDCD1, CARD11 and PLCG1) among the 52 patients with AS and 20 normal controls of the GSE73754 dataset. The AUCs of PDCD1 and PLCG1 were <0.7 (data not shown). The AUCs of CARD11 and DNMT1 were 0.782 and 0.737, respectively (Fig. 7). For AS diagnosis, the sensitivity (proportion of true positive) and 1-specificity (proportion of false positive) of CARD11 were 70.0 and 80.8%, respectively, and for DNMT1 were 75.0 and 69.2%, respectively (Fig. 7).

Discussion

AS is a type of autoimmune disorder that is associated with HLB-27 and T-cells; however, its etiology and pathogenesis remain unclear (18). The delays in the diagnosis of AS and the insufficient responses to the currently available therapeutics supports the requirement for a greater understanding of its pathogenesis.

A previous microarray study identified four lncRNAs, lnc-ZNF354A-1, lnc-LIN54-1, lnc-FRG2C-3 and lnc-USP50-2, that are involved in the abnormal osteogenic differentiation

Table IV. Nearby targeted DEmRNAs of DElncRNAs between ankylosing spondylitis and normal controls.

	DElncRNA			Nearby DEmRNA		
Chr	Symbol	Start -100 kb	End +100 kb	Symbol	Start	End
chr1	PIK3CD-AS1	9,552,610	9,754,586	CLSTN1	9,729,026	9824526
chr14	LINC01588	49,827,571	50,192,643	SOS2	50,117,120	50,231,558
chr22	AC004471.9	19,021,529	19,224,503	SLC25A1	19,175,575	19,178,830
chr2	AC007879.5	207,139,864	207,629,795	KLF7	207,074,137	207,167,267
ChrX	INE1	47,104,921	47,305,865	UBA1	47,190,861	472,151,28
ChrX	INE1	47,104,921	47,305,865	USP11	47,232,690	47,248,328
chr1	TAF1A-AS1	222,489,825	222,693,032	MIA3	222,618,086	222,668,012
chr7	AC007285.6	29,888,600	30,125,660	SCRN1	29,920,103	29,990,289
chr1	RP11-318C24.2	175,804,762	176,020,513	RFWD2	175,944,831	176,207,493
chr5	AC008781.7	141,518,414	141,726,481	DIAPH1	141,515,016	141,619,055
chr3	U73166.2	50,160,303	50,363,358	HYAL3	50,292,831	50,299,468
ChrX	RP13-314C10.5	23,672,992	23,882,956	PRDX4	23,664,262	23,686,399
chr1	RP11-195C7.1	176,107,648	176,329,330	RFWD2	175,944,831	176,207,493
chr21	AP001056.1	44,075,489	44,276,453	PWP2	44,107,290	44,131,181
chr21	AP001056.1	44,075,489	44,276,453	TRAPPC10	44,012,319	44,106,552
chr1	RP11-7O11.3	43,844,370	44,046,551	ST3GAL3	43,705,824	43,931,165
chr3	RP11-379K17.4	169,839,353	170,066,734	GPR160	170,037,929	170,085,403
chr2	AC092620.2	138469090	138,674,458	SPOPL	138,501,801	138,573,547
chr3	LINC00969	195,558,062	195,839,964	MUC20	195,720,882	195,741,123
chr4	RP11-15B17.1	99,850,006	100,295,099	LAMTOR3	99,878,336	99,894,490
chr5	SCAMP1-AS1	78,242,365	78,460,507	AP3B1	78,000,525	78,294,755
chr15	RP11-30K9.6	58,668,072	58,870,974	ADAM10	58,588,807	58,749,978
chr8	BAALC-AS1	103,056,990	103,398,772	SLC25A32	103,39,8635	103,415,189
chr5	RP11-159F24.5	43,415,274	43,625,310	NNT	43,602,692	43,707,405
chr5	LINC01187	170,091,579	170,299,141	FOXI1	17,010,5897	170,109,725
chr8	LINC01151	122,570,385	122,794,106	ZHX2	122,781,394	122,974,512
chr11	RP11-727A23.4	83,080,144	83,284,520	PCF11	83,156,988	83,187,451
chr8	GS1-393G12.12	144,214,590	144,415,138	BOP1	144,262,102	144,291,370
chr12	RP11-561P12.5	8,448,361	8,667,613	CLEC4E	8,533,305	8,540,963
chr12	RP11-561P12.5	8,448,361	8,667,613	CLEC4D	8,509,475	8,522,366
chr12	RP11-996F15.2	29,180,418	29,417,848	ERGIC2	29,337,352	29,381,189
chr14	RP11-44N21.1	104,993,609	105,199,004	CDCA4	105,009,573	105,021,148
chr14	RP11-44N21.1	104,993,609	105,199,004	C14 or f79	104,985,775	105,010,482
chr12	RP11-753H16.3	54,253,661	54,597,688	HNRNPA1	54,280,193	54,287,088
chr14	RP11-193F5.1	60,779,714	61,082,585	SLC38A6	60,981,114	61,083,733
chr14	RP5-1021I20.1	73,687,360	73,903,270	PNMA1	73,711,783	73,714,372
chr3	AC099668.5	49,584,480	49,784,983	MST1	49,683,947	49,689,501
chr6	RP11-425D10.10	109,282,795	109,483,666	SMPD2	109,440,763	109,443,919
chr14	CTD-2547L24.4	91,158,299	91,359,003	CCDC88C	91,271,323	91,417,844
chr14	RP11-524O1.4	21,284,292	21,484,920	CHD8	21,385,194	21,456,126
chr14	RP11-524O1.4	21,284,292	21,484,920	SUPT16H	21,351,472	21,384,266
chr16	RP11-459F6.3	58,029,529	58,259,133	CFAP20	58,113,588	58,129,450
chr16	RP11-264B17.3	28,874,804	29,090,775	LAT	28,984,826	28,990,783
chr1	RP11-196G18.22	149,744,498	149,949,024	HIST2H2BF	149,782,689	149,812,373
chr16	LA16c-306E5.3	3,358,071	3,615,564	NLRC3	3,539,033	3,577,400
chr16	RP11-461A8.4	3,550,636	3,751,703	NLRC3	3,539,033	3,577,400
chr17	RP1-59D14.5	2,275,061	2,479,306	TSR1	2,322,503	2,337,507
chr17	RP1-59D14.5	2,275,061	2,479,306	METTL16	2,405,562	2,511,891
chr17	RP1-59D14.5	2,275,061	2,479,306	SMG6	2,059,839	2,303,771
chr17	RP11-927P21.2	64,799,766	65,000,716	LRRC37A3	64,854,312	64,919,480

Table IV. Continued.

	DElncRNA			Nearby DEmRNA		
Chr	Symbol	Start -100 kb	End +100 kb	Symbol	Start	End
chr17	RP11-1094M14.11	35,468,109	35,674,843	SLFN14	35,548,125	35,558,098
chr17	CTD-2534I21.8	44,847,912	45,048,939	EFTUD2	44,849,943	44,899,662
chr19	PTOV1-AS1	49,738,639	49,951,676	NUP62	49,906,825	49,929,763
chr20	RP4-591C20.9	63,761,212	63,964,293	UCKL1	63,939,829	63,956,415
chr19	AC007292.3	4,256,637	4,458,448	CCDC94	4,247,079	4,269,090
chr19	RAB11B-AS1	8,274,373	8,490,685	RAB11B	8,389,981	8,404,434
chr5	CTD-2033C11.1	65,824,629	66,025,135	ERBIN	65,926,475	66,082,549
chr4	RP11-572O17.1	1,612,821	1,813,622	FAM53A	1,617,915	1,684,302
chr4	RP11-572O17.1	1,612,821	1,813,622	LETM1	1,811,479	1,856,247
chr1	RP4-736L20.3	10,329,881	10,530,677	DFFA	10,456,522	10,472,526
chr10	RP11-574K11.29	73,603,735	73,813,581	USP54	73,497,538	73,625,953
chr1	RP11-156E8.1	244,869,350	245,071,088	EFCAB2	244,969,705	245,127,164
chr3	RP11-767C1.2	12,732,219	12,932,728	IQSEC1	12,897,220	13,073,117
chr1	RP11-11N7.4	244,764,738	244,965,272	HNRNPU	244,840,638	244,864,560
chr3	RP11-778D9.13	184,032,942	184,233,561	AP2M1	184,174,689	184,184,091
chr12	RP1-197B17.4	47,631,908	47,832,351	HDAC7	47,782,722	47,833,132
chr17	AC142472.6	45,046,730	45,248,470	NMT1	45,051,610	45,109,016
chr15	CTD-2562G15.3	75,352,964	75,553,947	SIN3A	75,369,379	75,455,842
chr17	RP11-333J10.3	36,898,598	37,100,034	AATF	36,948,875	37,056,871
chr15	CTD-2382E5.6	41,808,204	42,008,714	JMJD7	41,828,085	41,837,581
chr18	RP11-405M12.3	74,970,197	75,171,091	ZNF407	74,597,870	75,065,671
chr17	HEXDC-IT1	82,325,498	82,527,310	NARF	82,458,180	82,490,537
chr17	RP11-159D12.6	57,906,674	58,108,187	CUEDC1	57,861,243	57,955,323
chr15	RP11-1H8.5	34315,450	34,520,273	LPCAT4	34,358,618	34,367,278
chr15	RP11-1H8.5	34,315,450	34,520,273	SLC12A6	34,229,996	34,338,060
chr5	CTC-487M23.6	112,794,933	112,996,531	SRP19	112,861,222	112,869,788
chr5	CTC-487M23.6	112,794,933	112,996,531	DCP2	112,976,702	113,020,970
chr5	AC005593.2	131,697,415	131,897,929	FNIP1	131,641,714	131,797,063
chr17	RP13-638C3.5	82,548,849	82,750,657	FN3KRP	82,716,683	82,730,328
chr14	RP11-298I3.6	22,923,083	23,124,217	AJUBA	22,971,174	22,982,642
chr16	RP11-451N19.3	58,605,799	58,806,297	SLC38A7	58,665,109	58,685,104
chr16	RP11-451N19.3	58,605,799	58,806,297	GOT2	58,707,131	58,734,357
chr19	CTD-2233K9.1	46,846,535	47,049,156	ARHGAP35	46,918,676	47,005,077
chr6	XXbac-BPG283O16.9	30,182,349	30,386,054	RPP21	30,345,131	30,346,884
chr6	XXbac-BPG283O16.9	30,182,349	30,386,054	TRIM26	30,184,455	30,213,427

Chr, chromosome; DEmRNAs, differentially expressed mRNAs; DElncRNA, differentially expressed long non-coding RNA.

of mesenchymal stem cells (MSCs) in patients with AS. The expression of these four lncRNAs was positively correlated with that of bone morphogenetic protein 2 and Noggin in MSCs from healthy donors (8). A recent study reported that lncRNA-AK001085 was downregulated in patients with AS and served as a potential diagnostic indicator, thus, lncRNA-AK001085 was considered to be a potential suppressor of AS (9). Due to the lack of research, the regulatory mechanism of the majority of lncRNAs in AS remains unknown. In the present study, the key DEmRNAs and DElncRNAs associated with AS were identified and their functions in AS were investigated using RNA sequencing and bioinformatics analysis.

LINC00342 was demonstrated to be upregulated in patients with non-small cell lung cancer and its expression was positively correlated with lymph node metastasis and the Tumor-Node-Metastasis stage (19). Coiled-coil domain containing 26 (CCDC26) is also a tumor-associated lncRNA that regulates the growth of glioma, pancreatic cancer and myeloid leukemia cells (20-22). In the present study, LINC00342 and CCDC26 were downregulated in patients with AS, which suggests that they may serve roles in AS.

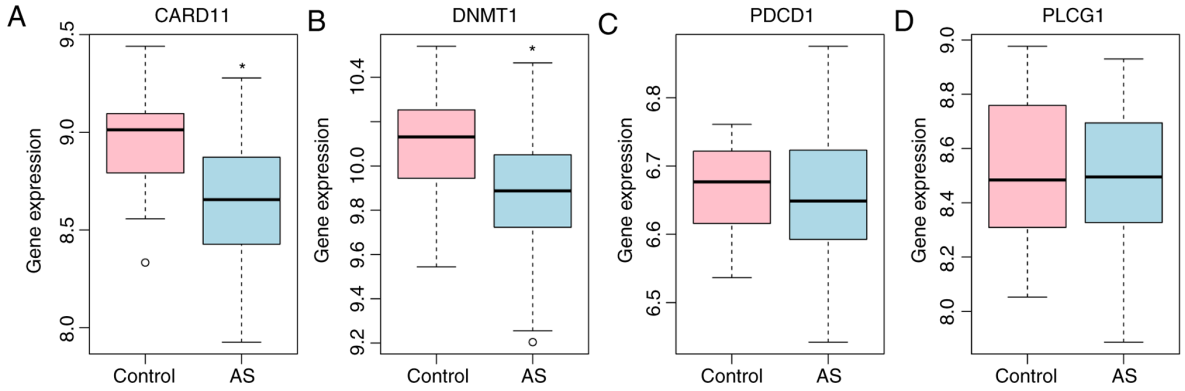

Figure 5. Validation the expression of selected DEmRNAs between patients with AS and normal controls in the GSE73754 dataset. The x-axis presents the AS and normal control groups and the y-axis presents the expression levels of (A) CARD11, (B) DNMT1, (C) PDCD1 and (D) PLCG1. Data are presented as the median and the 75th and 25th percentiles. *P<0.05 vs. control. AS, ankylosing spondylitis; DEmRNA, differentially expressed mRNA; CARD11, caspase recruitment domain-containing protein 11; DNMT1, DNA methyltransferase 1; PDCD1, programmed cell death 1; PLCG1, phospholipase Cγ1.

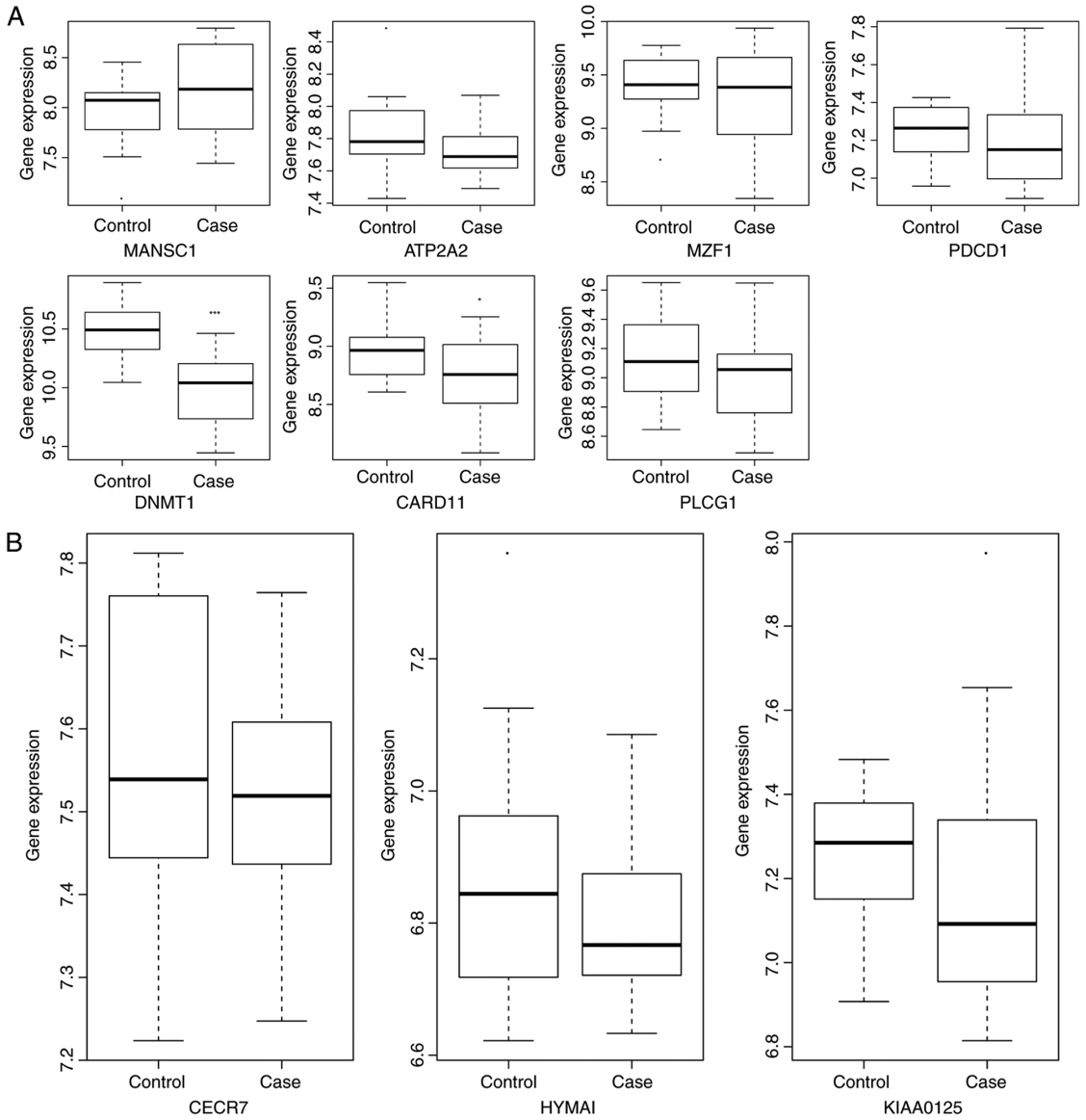

Figure 6. Validation of the expression of selected DEmRNAs and DElncRNAs between patients with AS and the normal controls in the GSE25101 dataset. The x-axis presents the AS and normal control groups and the y-axis presents the expression levels. (A) The selected DEmRNAs and (B) The selected DElncRNAs. AS, ankylosing spondylitis; DEmRNA, differentially expressed mRNA; DElncRNA, differentially expressed long non-coding RNA; CARD11, caspase recruitment domain-containing protein 11; DNMT1, DNA methyltransferase 1; PDCD1, programmed cell death 1; PLCG1, phospholipase Cγ1; MANSC1, MANSC domain containing 1; ATP2A2, adenosine triphosphatase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2; MZF1, myeloid zinc finger 1; CECR7, cat eye syndrome chromosome region candidate 7; HYMAI, hydatidiform mole associated and imprinted.

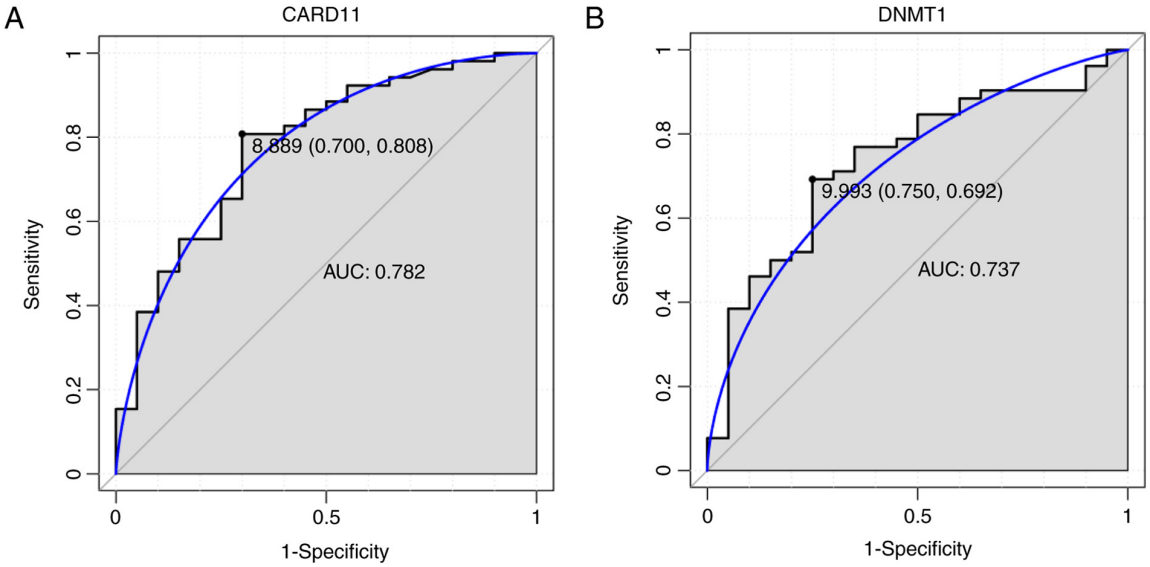

Figure 7. ROC curves of CARD11 and DNMT1 comparing patients with ankylosing spondylitis and normal controls in the GSE73754 dataset. ROC curves displayed the diagnostic ability of differentially expressed long non-coding RNA with sensitivity (the proportion of true positive) and 1-Specificity (the proportion of false positive). The x-axis presents 1-specificity and y-axis presents sensitivity. (A) CARD11 and (B) DNMT1. ROC, receiver operating characteristic; CARD11, caspase recruitment domain-containing protein 11; DNMT1, DNA methyltransferase 1; AUC, area under the curve.

Further studies are required in order to identify their precise function in AS.

Furthermore, the results of the present study indicated that many novel DElncRNAs may be involved in AS. In order to investigate their functions in AS, a weighted DElncRNA-DEmRNA co-expression network was constructed and functional annotation of the DElncRNAs co-expressed with DElncRNAs was performed. A total of 3,505 DElncRNA-DEmRNA co-expression pairs, which included 302 DElncRNAs and 602 DEmRNAs, were obtained. Based on the DEmRNAs co-expressed with DElncRNAs, 'T-cell receptor signaling pathway' was a significantly enriched pathway. T-cells have been demonstrated by previous studies to serve important roles in the pathology of AS (23-25). HLA-B27-reactive cluster of differentiation-4$^+$ T-cells have been reported to be involved in the pathogenesis of spondyloarthropathies (26). The number of peripheral T-helper (Th)-2 and Th17 lymphocytes has been demonstrated to be increased in AS, which is suggestive of their potential roles in AS (27,28). Therefore, the results of the present study support those of previous studies as well as the importance of the T-cell receptor signaling pathway in AS. Furthermore, the DEmRNAs that were enriched in the T-cell receptor signaling pathway, including NF-κB inhibitor β, CARD11, P21 (Ras-related C3 botulinum toxin substrate 1) activated kinase 6, protein kinase Cθ, PLCG1, PDCD1, FYN proto-oncogene Src family tyrosine kinase, protein kinase B2 and Vav guanine nucleotide exchange factor 3, may be involved with AS by regulating the T-cell receptor signaling pathway.

Among these DEmRNAs, PDCD1 is a known AS-associated gene. PDCD1 is a member of the immunoglobulin superfamily that is expressed on the surface of peripheral T-cells, and regulates T-cell responses and the maintenance of peripheral tolerance (29,30). A previous study demonstrated that the expression levels of PDCD1 on activated T-cells were decreased in patients with AS (1). Downregulation of PDCD1 may be involved in AS by stimulating the activity of T-cells and elevating the production of cytokines, which promotes spinal inflammation and destruction in patients with AS (1). Downregulated PDCD1 was also identified in patients with AS in the present study, which provides evidence to support the results of the previous study.

CARD11 is a shared member of the CARD and CARD-containing membrane-associated guanylate kinase protein 1 families that has been reported to serve a vital role in regulation of inflammation and the immune response (31). Although to the best of our knowledge, there have been no studies that have reported on the association between CARD11 and AS, downregulated CARD11 has been implicated in another type of autoimmune disease, rheumatoid arthritis, via NF-κB activation, reduced Th17 responses and the decreased production of proinflammatory cytokines, including IL-1β, IL-6 and IL-17 (10,32,33). Joint inflammation and destruction were demonstrated to be attenuated by CARD11 small interfering RNA treatment in mice (10). Considering the crucial roles of NF-κB activation, Th17 cells and proinflammatory cytokines in AS, it was hypothesized that CARD11 may also be a key regulator of AS.

Similar to CARD11, PLCG1 has been reported to be associated with other types of autoimmune disease, including multiple sclerosis and lymphoproliferative syndrome (11,12). Furthermore, the interaction between PLCG1 and linker for activation of T-cells (LAT) was revealed to be involved with the activation and proliferation of T-cells (11,12). The production of IL-6 by T-cells is also regulated by PLCG1-LAT (11,12). Therefore, downregulated PLCG1 in the patients with AS in the present study may serve important roles in the progression of AS by regulating T-cells and the production of IL-6.

DNMT1 encodes an enzyme that establishes and regulates patterns of methylated cytosine residues (13). A previous study observed downregulated and hypermethylated DNMT1 in the peripheral blood mononuclear cells of patients with AS when compared with normal controls, which suggests that DNMT1 may be a potential biomarker of AS (13). Thus, the downregu-

lation of DNMT1 observed in patients with AS in the present study is consistent with this previous study.

Based on the ROC analysis in the present study, CARD11 and DNMT1 may have great diagnostic value for AS, and therefore may be potential biomarkers.

LINC00987 was a downregulated lncRNA in the patients with AS in the present study, and was co-expressed with DNMT1, CARD11 and PLCG1. In addition, DNMT1 and PLCG1 were co-expressed with another downregulated lncRNA, MSTRG.8559, in the patients with AS. Furthermore, LINC00987 and MSTRG.8559 were two hub lncRNAs of the positively co-expressed DElncRNA-DEmRNA network, which regulates the majority of the DEmRNAs in AS. It was hypothesized that these two DElncRNAs may serve crucial roles by regulating the expression of DNMT1, the T-cell receptor signaling pathway and its associated genes. Further studies are required to further investigate the biological functions of LINC00987 and MSTRG.8559, particularly those in AS.

RP11-837J7.4 and NALT1 were the most significantly down- and upregulated, respectively, DElncRNAs in patients with AS in the present study; however, their biological functions remain known. Further studies are required in order to identify whether these two DElncRNAs could serve as diagnostic biomarkers for AS.

Previous studies have revealed the prevalence of lncRNA-mediated cis regulation on nearby transcription (34-36). The 84 AS-specific DElncRNA and nearby cis target DEmRNA pairs obtained in the present study provide novel information for understanding the biological functions of lncRNAs in AS.

In conclusion, the present study obtained lncRNA and mRNA expression profiles from patients with AS and normal controls using RNA sequencing. A number of key genes, including PDCD1, DNMT1, CARD11 and PLCG1, that are involved in AS were identified. In addition, the results indicated that numerous novel DElncRNAs may be involved in AS. Furthermore, the functions of DElncRNAs in AS were investigated using functional annotation of DEmRNAs co-expressed with DElncRNAs and through the identification of nearby target DEmRNAs of DElncRNAs. These results may support further studies on the potential roles of lncRNAs in AS. However, the sample size for RNA-seq was small, which is a limitation of the present study, therefore, studies with larger sample sizes are required in order to confirm this conclusion.

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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

ZX and GC conceived and designed the experiments. ZX and HL performed the experiments. ZX and QC analyzed the data. HL and QC were significant contributors in the manuscript. All authors revised the manuscript and have agreed to the publication of this manuscript.

Ethics approval and consent to participate

All of the participants submitted written informed consent and the present study was approved by the Ethics Committee of the 2nd Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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