Co-expressed differentially expressed genes and long non-coding RNAs involved in the celecoxib treatment of gastric cancer: An RNA sequencing analysis

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Abstract. The aim of the present study was to investigate the mechanisms of long non-coding RNAs (lncRNAs) in a gastric cancer cell line treated with celecoxib. The human gastric carcinoma cell line NCI-N87 was treated with 15 μM celecoxib for 72 h (celecoxib group) and an equal volume of dimethylsulfoxide (control group), respectively. Libraries were constructed by NEBNext Ultra RNA Library Prep kit for Illumina. Paired-end RNA sequencing reads were aligned to a human hg19 reference genome using TopHat2. Differentially expressed genes (DEGs) and lncRNAs were identified using Cuffdiff. Enrichment analysis was performed using GO-function package and KEGG profile in Bioconductor. A protein-protein interaction network was constructed using STRING database and module analysis was performed using ClusterONE plugin of Cytoscape. *ATP5G1*, *ATP5G3*, *COX8A*, *CYC1*, *NDUFS3*, *UQCRC1*, *UQCRC2* and *UQCRFS1* were enriched in the oxidative phosphorylation pathway. *CXCL1*, *CXCL3*, *CXCL5* and *CXCL8* were enriched in the chemokine signaling and cytokine-cytokine receptor interaction pathways. *ITGA3*, *ITGA6*, *ITGB4*, *ITGB5*, *ITGB6* and *ITGB8* were enriched in the integrin-mediated signaling pathway. DEGs co-expressed with lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1, lnc-AP000974.1-1:1 and lnc-RAB3IL1-2:1 were enriched in the pathways associated with cancer, such as the basal cell carcinoma pathway in cancer. In conclusion, these DEGs and differentially expressed lncRNAs may be important in the celecoxib treatment of gastric cancer.

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Abbreviations: lncRNAs, long non-coding RNAs; DEGs, differentially expressed genes; PPI, protein-protein interaction; ncRNAs, non-coding RNAs; miRNAs, microRNAs; DMSO, dimethylsulfoxide; QC, quality control; NGS, next generation sequencing; GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function; mPTP, mitochondrial permeability transition pore

Key words: celecoxib, gastric cancer, differentially expressed genes, enrichment analysis

Introduction

Despite the mortality rate for gastric carcinoma reducing 3.1% annually and the overall 5-year relative survival rate increasing to 28% over the past 10 years, the mortality rate for gastric carcinoma remains >50% worldwide (1). The most effective treatment for resectable gastric cancer is surgery, which presents good survival rates. The majority of cases of gastric cancer are diagnosed at an advanced stage or as a relapse after surgery (2). Therefore, a further understanding of the molecular mechanisms of gastric cancer is of clinical importance and it is required in order to improve the early diagnosis and therapeutic strategies of gastric cancer.

Over the last decade, the majority of the potential therapeutic targets reported and the diagnostic markers for gastric cancer are protein-coding genes identified from large-scale DNA microarray analysis, including the novel genes *KLF5*, *FAT4*, *KMT2C*, *GATA4*, *MLL* and *GATA6* (3-6). The majority of studies on non-coding RNAs (ncRNAs) are focused on short ncRNAs called microRNAs, while alterations in the structure, expression levels and cognate RNA-binding proteins of long ncRNAs (lncRNAs) with a length of >200 nucleotides (nt) have been associated with cancer, and appear to be gaining prominence as further studies are conducted (7). In addition, growing evidence has confirmed that lncRNAs that are capable of regulating tumor suppression or that exhibit oncogenic effects may be considered as novel biomarkers and therapeutic targets for cancer (8,9). Furthermore, it has been demonstrated that differentially expressed long non-coding RNAs (DE-lncRNAs), including H19 and uc001lsz, may present potential roles in the development and occurrence of gastric cancer (10). In a study by Hu *et al* (11), a novel lncRNA GAPLINC (924 bp) was highly expressed in gastric cancer specimens and it was capable of controlling the expression levels of CD44 to regulate cell invasion by competing for miR211-3p.

Table I. Differentially expressed lncRNAs in the celecoxib and the control groups.

lncRNAs ID	Celecoxib	Control	Fold change	q value
Upregulated				
lnc-IGFL3-2:1	18.43	44.60	1.27	$1.07x10^{-7}$
lnc-PTMS-1:3	427.83	613.56	0.52	$1.71x10^{-6}$
lnc-SCD-1:13	105.06	152.80	0.54	$1.71x10^{-6}$
lnc-TNS4-2:1	6.28	15.94	1.34	$1.71x10^{-6}$
lnc-TTLL10-3:1	7.71	11.67	0.60	$2.91x10^{-5}$
lnc-CKMT1A-1:1	50.37	97.92	0.96	$5.80x10^{-5}$
lnc-LRR1-1:2	4422.95	5914.51	0.42	$6.36x10^{-5}$
lnc-RAB3IL1-2:1	2279.25	3231.60	0.50	$7.18x10^{-4}$
lnc-JUNB-1:1	372.34	562.08	0.59	$2.00x10^{-3}$
lnc-RP11-259P6.1.1-2:1	29.78	39.57	0.41	$3.20x10^{-3}$
lnc-IGFL2-2:1	105.84	167.35	0.66	$5.76x10^{-3}$
lnc-S100P-3:1	142.85	240.91	0.75	$6.65x10^{-3}$
lnc-SRGAP3-1:29	0	1.73	1.80e+308	$1.19x10^{-2}$
lnc-RAB44-3:1	8.43	14.88	0.82	$1.25x10^{-2}$
lnc-GLTSCR2-2:7	18.58	26.75	0.53	$1.26x10^{-2}$
lnc-PDZD7-3:2	0	3.41	1.80e+308	$2.41x10^{-2}$
lnc-CEACAM6-1:1	33.20	57.59	0.79	$2.51x10^{-2}$
lnc-SPNS3-1:3	18.58	27.11	0.54	$4.89x10^{-2}$
lnc-UNC5B-1:1	12.62	18.20	0.53	$4.89x10^{-2}$
Downregulated				
lnc-C9orf16-2:1	875.46	425.36	-1.04	0
lnc-C9orf16-3:1	352.54	156.14	-1.17	0
lnc-TRIM31-1:2	47.17	21.45	-1.14	$4.10x10^{-8}$
lnc-DDX47-3:1	211.38	145.19	-0.54	$1.46x10^{-7}$
lnc-PCK1-3:1	13.92	5.48	-1.35	$8.52x10^{-7}$
lnc-MYO16-7:1	349.12	200.98	-0.80	$1.71x10^{-6}$
lnc-YPEL5-5:1	71.82	44.54	-0.69	$1.71x10^{-6}$
lnc-TNK2-8:1	16.60	2.33	-2.83	$4.54x10^{-6}$
lnc-AC069257.9.1-4:73	124.40	66.92	-0.90	$6.69x10^{-5}$
lnc-CCDC80-1:4	18.79	3.17	-2.57	$1.74x10^{-3}$
lnc-AC069257.9.1-4:72	151.58	81.26	-0.90	$5.89x10^{-3}$
lnc-KRT36-1:1	45.00	18.61	-1.27	$6.65x10^{-3}$
lnc-CCDC33-1:1	32.45	19.88	-0.71	$8.64x10^{-3}$
lnc-CXCL3-1:1	5.06	1.51	-1.74	$2.51x10^{-2}$
lnc-PDZK1IP1-3:1	25.26	11.57	-1.13	$3.28x10^{-2}$
lnc-SUSD3-4:2	19.48	9.17	-1.09	$3.37x10^{-2}$
lnc-AC069257.9.1-4:53	99.18	57.43	-0.79	$3.59x10^{-2}$
lnc-AP000974.1-1:1	36.09	16.13	-1.16	$4.79x10^{-2}$

Celecoxib and control columns indicate the average expression values of the lncRNAs in the celecoxib and the control group, respectively. lncRNA, long non-coding ribonucleic acids.

A previous study demonstrated that celecoxib induced apoptosis and autophagy of gastric cancer SGC-7901 cells via the PI3K/Akt signaling pathway (12). According to a study by Lan *et al* (13), celecoxib inhibited *Helicobacter pylori*-induced invasion in gastric cancer via the adenine nucleotide translocator-dependent pathways. Furthermore, the activated Notch1 signaling pathway may contribute to the pathogenesis of gastric cancer, at least partly through *COX-2* (14). Treatment with celecoxib, a *COX-2* inhibitor, can significantly reduce the incidence of gastric cancer in rats (15). In addition, an elevated *COX-2* expression level is an independent prognostic factor indicative of poor prognosis and it is associated with reduced survival in patients with gastric cancer (16). Pang *et al* (17) reported that the Akt/GSK3β/NAG-1 signaling pathway may be considered as the major mechanism of the *COX-2*-independent

Table II. Top five enriched gene ontology terms in biological process, cellular component and molecular function categories for upregulated DEGs.

A, Biological process

GO_ID	Term	Count	P-value	DEGs
GO:0044281	Small molecule metabolic process	99	1.87×10^{-9}	*ABCC3, ACAA1, B3GNT3, CD320, DDX11, ECHS1, FA2H, GAPDH, UQCRFS1, WNT11*[a]
GO:0055114	Oxidation-reduction process	44	9.79×10^{-9}	*ACAA1, ACSS2, COX8A, ECHS1, FA2H, HMOX1, UQCRC1, UQCRC2, UQCRFS1, VAT1*[a]
GO:0044710	Single-organism metabolic process	137	3.01×10^{-8}	*ABCC3, ACAA1, B3GNT3, BMP4, PCBD1, PSMD8, RHOB, VAT1, WNT11, XRCC6*[a]
GO:0043436	Oxoacid metabolic process	44	6.07×10^{-8}	*ABCC3, ACAA1, B3GNT3, CKMT1A, ECHS1, SOD1, SULT2B1, TPI1, TST, UGT1A6*[a]
GO:0006082	Organic acid metabolic process	44	9.50×10^{-8}	*ABCC3, ACAA1, B3GNT3, CKMT1A, GOT1, SERINC2, SLC2A1, TPI1, TST, UGT1A6*[a]

B, Cellular component

GO_ID	Term	Count	P-value	DEGs
GO:0005576	Extracellular region	138	3.64×10^{-23}	*ADIRF, BMP4, CAPG, IL1RN, ITGA3, ITGA6, ITGB4, ITGB5, VAT1, VDAC1*[a]
GO:0031982	Vesicle	129	5.68×10^{-20}	*ADIRF, AHNAK2, ENO1, ITGA3, ITGB4, ITGB5, SFN, UQCRC2, VASP, VAT1*[a]
GO:0031988	Membrane-bounded vesicle	126	4.25×10^{-18}	*ADIRF, ATP6AP1, BAIAP2L2, CAPG, EPS8L1, FTH1, FURIN, GAPDH, UQCRC2, VASP*[a]
GO:0043230	Extracellular organelle	118	2.21×10^{-18}	*ADIRF, GOT1, ITGA3, ITGB4, ITGB5, KLK14, UGT1A6, UPK3B, UQCRC2, VASP*[a]
GO:0044421	Extracellular region	131	8.27×10^{-13}	*ADIRF, HMOX1, IL1RN, ITGA3, ITGA6, ITGB4, ITGB5, KLK14, TXN, WNT11*[a]

C, Molecular function

GO_ID	Term	Count	P-value	DEGs
GO:0005515	Protein binding	182	7.34×10^{-7}	*AATK, HSP90AA1, IRF2BP1, ITGA3, ITGA6, ITGB4, ITGB5, UQCRFS1, VASP, VDAC1*[a]
GO:0016491	Oxidoreductase activity	31	9.29×10^{-7}	*ACAA1, GAPDH, HMOX1, HPDL, HR, LDHA, MAOB, NDUFS3, PCBD1, PIR*[a]
GO:0043236	Laminin binding	6	7.96×10^{-6}	*ECM1, GPC1, ITGA3, ITGA6, LGALS1, LYPD3*
GO:0050840	Extracellular matrix binding	7	2.07×10^{-5}	*ECM1, GPC1, GPR56, ITGA3, ITGA6, LGALS1, LYPD3*
GO:0008106	Alcohol dehydrogenase ($NADP^+$) activity	4	3.68×10^{-5}	*AKR1B1, AKR1C2, AKR1C3, ALDH3A1*

GO, gene ontology; DEGs, differentially expressed genes; NADP, nicotinamide adenine dinucleotide phosphate. [a]Not all of the gene names were included in the table.

effects of celecoxib on gastric cancer cells. *COX-2* has been indicated to regulate E-cadherin expression via the NF-κB and Snail signaling pathway in gastric cancer (18). It has also been reported that celecoxib has the potential for clinical use in gastric cancer treatment by the mechanism of activating miR-29c (19). Although various advances have been made in the study of mechanisms of lncRNAs in gastric cancer, the understanding of the expression patterns and functional roles of lncRNAs in gastric cancer treated with celecoxib requires further investigation.

In the present study, the RNA sequencing data of NCI-N87 human gastric carcinoma cells treated with or

Table III. Top five enriched gene ontology terms in the biological process, cellular component and molecular function categories for downregulated DEGs.

A, Biological process

GO_ID	Term	Count	P-value	DEGs
GO:0009888	Tissue development	42	4.66×10^{-8}	*ADAM9, ALDH1A3, FNDC3B, NTN4, PKP2, RIPK4, TNFRSF19, TRIM16, TSC22D3, WNT7B*[a]
GO:0048513	Organ development	58	1.90×10^{-7}	*ADAM9, EGLN1, LTBP3, MAP3K1, MDK, NRIP1, TNFRSF19, TNS3, TRIM16, TSC22D3*[a]
GO:0048731	System development	70	6.10×10^{-7}	*ADAM9, SGPL1, TNFAIP2, TNFRSF19, TNS3, TRIM16, TRIO, TSC22D3, WNT7B, ZSWIM6*[a]
GO:0048518	Positive regulation of biological process	74	6.85×10^{-7}	*ADAM9, GLIS3, HSPB1, IGFBP3, IRF1, ITGB8, KLK6, TRIM16, TRIO, WNT7B*[a]
GO:0009653	Anatomical structure morphogenesis	49	7.77×10^{-7}	*ADAM9, MAP1B, MAP2, NTN4, PKP2, PTPRJ, RIPK4, SAT1, SEMA7A, SGPL1*[a]

B, Cellular component

GO_ID	Term	Count	P-value	DEGs
GO:0044421	Extracellular region	73	1.02×10^{-10}	*ADAM9, CCDC80, CLIC5, FRAS1, SNX18, SOSTDC1, ST6GAL1, SULF2, TNFAIP2, VWA2*[a]
GO:0005615	Extracellular space	35	1.02×10^{-8}	*ADAM9, HSPG2, IGFBP3, MUC4, PLAT, POTEF, SERPINA3, TNFAIP2, VWA2, WNT7B*[a]
GO:0005576	Extracellular region	77	1.78×10^{-8}	*ADAM9, KRT15, LCN2, SLC7A5, SNX18, SOSTDC1, ST6GAL1, SULF2, TACSTD2, TGM2*[a]
GO:0043230	Extracellular organelle	56	1.92×10^{-8}	*ADAM9, IVL, KRT13, MYOF, PLAT, POTEF, SLC7A5, SNX18, ST6GAL1, TACSTD2*[a]
GO:0065010	Extracellular organelle, membrane-bound	56	1.92×10^{-8}	*ADAM9, IGFBP3, LTBP3, MARCKS, SELENBP1, SNX18, ST6GAL1, TGM2, THSD4, VWA2*[a]

C, Molecular function

GO_ID	Term	Count	P-value	DEGs
GO:0005080	Protein kinase C binding	4	1.31×10^{-3}	*ADAM9, HSPB1, MARCKS, PKP2*
GO:0008009	Chemokine activity	4	1.42×10^{-3}	*CXCL1, CXCL3, CXCL5, CXCL8*
GO:0019838	Growth factor binding	6	1.43×10^{-3}	*BMPR2, CTGF, IGFBP3, IGFBP6, LTBP3, TRIM16*
GO:0031994	Insulin-like growth factor I binding	2	2.28×10^{-3}	*IGFBP3, IGFBP6*
GO:0055106	Ubiquitin-protein transferase regulator activity	2	2.28×10^{-3}	*CDKN2A, TRIB1*

GO, gene ontology; DEGs, differentially expressed genes. [a]Not all of the gene names were included in the table.

without celecoxib were prepared and analyzed using bioinformatics methods. Briefly, differentially expressed genes (DEGs) and lncRNAs were identified for pathway enrichment analysis. A protein-protein interaction (PPI) network for DEGs was constructed and module analysis was performed. Finally, co-expression analysis of DEGs and lncRNAs was performed. The results of the data in the present study may provide novel insight into the roles of celecoxib in gastric cancer.

Materials and methods

Cell culture and celecoxib treatment. The human gastric carcinoma cell line NCI-N87 was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Inc.) in a

Table IV. Top ten enriched pathways for upregulated differentially expressed genes and seven enriched pathways for downregulated DEGs.

Pathway	Count	P-value	Gene symbol
Upregulated			
Glycolysis/gluconeogenesis	10	$1.03x10^{-6}$	*ACSS2, ALDH3A1, ALDOA, ENO1, ENO2, GAPDH, LDHA, PGM1, PKM, TPI1*
Metabolic pathways	43	$6.04x10^{-5}$	*ACAA1, ACSL5, ACSS2, AGPAT2, AK1, AKR1B1, ALDH1A1, ALDH3A1, ALDOA, ALPP, ALPPL2, ATP5G1, ATP5G3, ATP6AP1, B3GNT3, CKMT1A, CKMT1B, COX8A, CYC1, ECHS1, ENO1, ENO2, GAPDH, GOT1, ITPK1, LDHA, MAOB, MGAT3, NDUFS3, NT5E, PGM1, PGP, PIK3C2B, PKM, PLA2G4B, PLCE1, PRDX6, TPI1, TST, UGT1A6, UQCRC1, UQCRC2, UQCRFS1*
Phenylalanine metabolism	4	$4.00x10^{-4}$	*ALDH3A1, GOT1, MAOB, PRDX6*
Parkinson's disease	10	$4.67x10^{-4}$	*ATP5G1, ATP5G3, COX8A, CYC1, NDUFS3, SLC25A5, UQCRC1, UQCRC2, UQCRFS1, VDAC1*
Huntington's disease	12	$5.52x10^{-4}$	*ATP5G1, ATP5G3, CLTB, COX8A, CYC1, NDUFS3, SLC25A5, SOD1, UQCRC1, UQCRC2, UQCRFS1, VDAC1*
Prion diseases	5	$8.46x10^{-4}$	*EGR1, HSPA1A, MAPK3, SOD1, STIP1*
Oxidative phosphorylation	9	$2.11x10^{-3}$	*ATP5G1, ATP5G3, ATP6AP1, COX8A, CYC1, NDUFS3, UQCRC1, UQCRC2, UQCRFS1*
Alzheimer's disease	10	$3.16x10^{-3}$	*ATP5G1, ATP5G3, COX8A, CYC1, GAPDH, MAPK3, NDUFS3, UQCRC1, UQCRC2, UQCRFS1*
Metabolism of xenobiotics by cytochrome P450	6	$4.14x10^{-3}$	*AKR1C2, AKR1C3, ALDH3A1, CYP1B1, EPHX1, UGT1A6*
Cardiac muscle contraction	6	$6.17x10^{-3}$	*ATP1A1, COX8A, CYC1, UQCRC1, UQCRC2, UQCRFS1*
Downregulated			
Epithelial cell signaling in *H. pylori* infection	3	$2.92x10^{-2}$	*CXCL1, CXCL8, MAP3K14*
Complement and coagulation cascades	3	$3.03x10^{-2}$	*C3, PLAT, SERPINA1*
Histidine metabolism	2	$3.29x10^{-2}$	*ALDH1A3, AOC1*
Arrhythmogenic right ventricular cardiomyopathy	3	$3.62x10^{-2}$	*ITGB6, ITGB8, PKP2*
Axon guidance	4	$3.80x10^{-2}$	*EFNB2, NFAT5, NTN4, SEMA7A*
Chemokine signaling pathway	5	$3.80x10^{-2}$	*BCAR1, CXCL1, CXCL3, CXCL5, CXCL8*
Cytokine-cytokine receptor interaction	6	$4.55x10^{-2}$	*BMPR2, CXCL1, CXCL3, CXCL5, CXCL8, TNFRSF19*

DEGs, differentially expressed genes.

humidified air incubator (Thermo Fisher Scientific, Inc.) at 37˚C and with 5% CO_2. The cells were passaged at 80-90% confluence with 0.25% trypsin (Thermo Fisher Scientific, Inc.).

Cells at the exponential growth phase with a density of $1x10^6$ were seeded in a cell culture dish (Corning Inc., NY, USA) with a diameter of 6 cm and incubated in 5 ml serum-free Dulbecco's modified Eagle medium (Thermo Fisher Scientific, Inc.) overnight. Celecoxib (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich), and the cells were treated with 15 μM celecoxib for 72 h (celecoxib group). Cells treated with an equal volume of DMSO were used as a control group.

RNA sequencing data. The total RNA was extracted using TRIzol (Thermo Fisher Scientific, Inc.) following the manufacturer's protocol, and were quantified with a 721 spectrophotometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). Next, libraries were prepared by the NEBNext Ultra RNA Library Prep kit for Illumina (#E7530; New England BioLabs, Inc., Ipswich, MA, USA) according to the manufacturer's instructions. Briefly, RNA fragments ~200 nt in length were generated and then double-stranded cDNA was synthesized and end-repaired. Following the adaptor ligation, PCR amplification was performed as follows: A library was added with 10 μl 5X HF Buffer, 1 μl 10 μM reverse PCR primer 2-1: 5'-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' and primer 2-2: 5'-CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3', primer 2-3: 5'-CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3', primer 2-4: 5'-CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGA

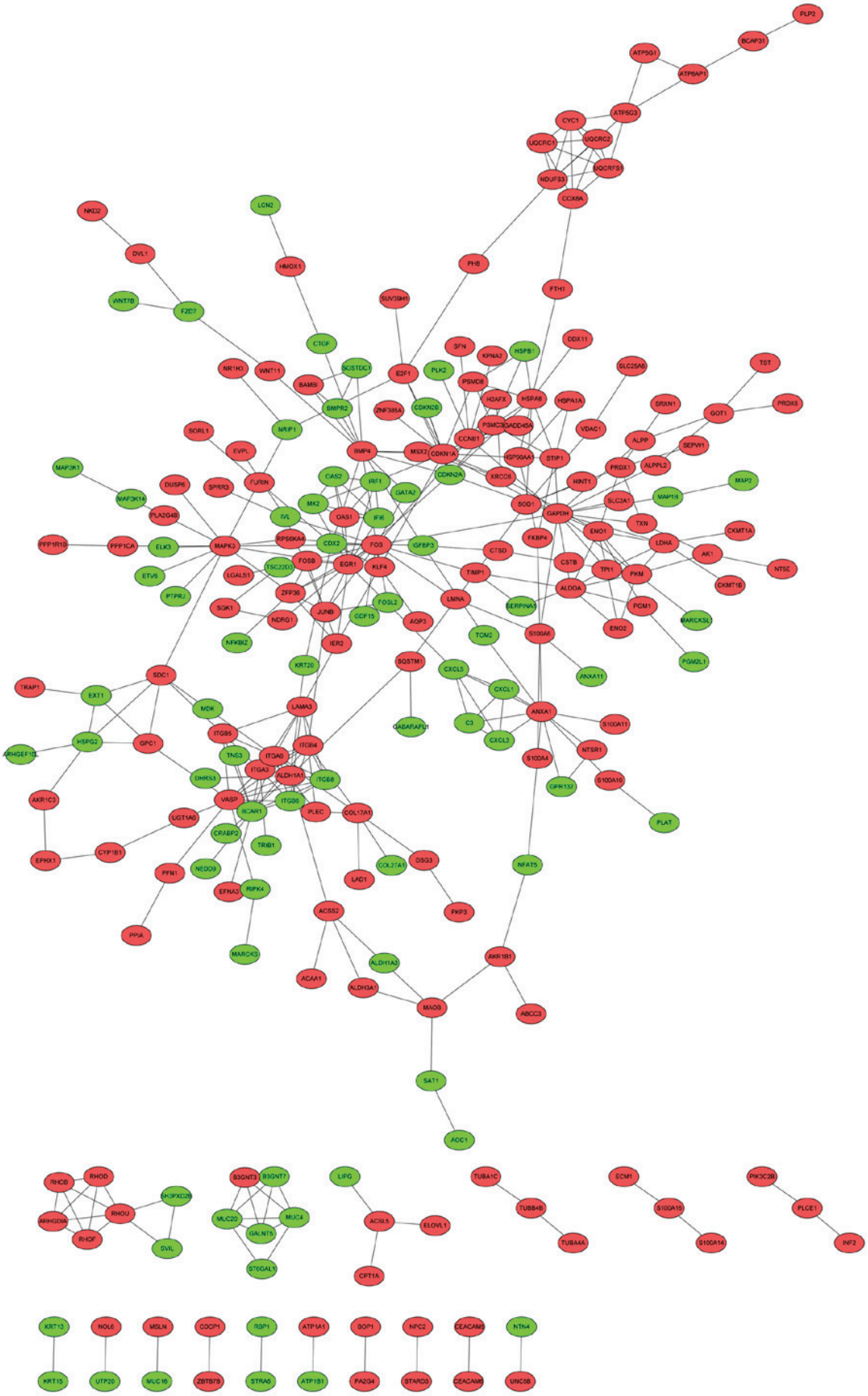

Figure 1. Constructed protein-protein interaction network for the differentially expressed genes. Red and green nodes indicate the up- and downregulated differentially expressed genes, respectively.

Table V. Top five enriched gene ontology terms in biological process, cellular component and molecular function categories for DEGs in module 1.

A, Biological process

GO_ID	Term	Count	P-value	DEG
GO:0007229	Integrin-mediated signaling pathway	7	$1.34x10^{-14}$	*ITGB6, BCAR1, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0030198	Extracellular matrix organization	7	$3.66x10^{-10}$	*ITGB6, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0043062	Extracellular structure organization	7	$3.73x10^{-10}$	*ITGB6, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0007155	Cell adhesion	8	$1.30x10^{-8}$	*ITGB6, BCAR1, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0022610	Biological adhesion	8	$1.35x10^{-8}$	*ITGB6, BCAR1, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*

B, Cellular component

GO_ID	Term	Count	P-value	DEG
GO:0008305	Integrin complex	6	$3.33x10^{-15}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0098636	Protein complex involved in cell adhesion	6	$3.33x10^{-15}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0043235	Receptor complex	6	$2.87x10^{-9}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0030055	Cell-substrate junction	6	$2.02x10^{-8}$	*BCAR1, VASP, ITGA6, ITGB4, ITGB5, ITGA3*
GO:0009986	Cell surface	6	$4.88x10^{-7}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*

C, Molecular function

GO_ID	Term	Count	P-value	DEG
GO:0005178	Integrin binding	4	$3.15x10^{-7}$	*ITGB6, ITGA6, ITGB5, ITGA3*
GO:0050839	Cell adhesion molecule binding	4	$2.35x10^{-6}$	*ITGB6, ITGA6, ITGB5, ITGA3*
GO:0005102	Receptor binding	7	$2.36x10^{-6}$	*ITGB6, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
GO:0043236	Laminin binding	2	$1.47x10^{-4}$	*ITGA6, ITGA3*
GO:0050840	Extracellular matrix binding	2	$4.39x10^{-4}$	*ITGA6, ITGA3*

GO, gene ontology; DEGs, differentially expressed genes.

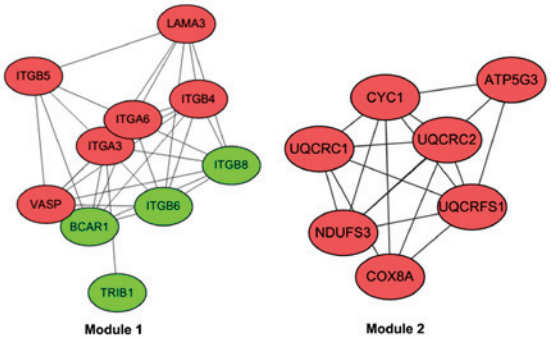

Figure 2. Two modules selected from the protein-protein interaction network. Red and green nodes indicate the up- and downregulated differentially expressed genes, respectively.

Table VI. Top five enriched gene ontology terms in biological process, cellular component and molecular function categories for DEGs in module 2.

A, Biological process

GO_ID	Term	Count	P-value	DEGs
GO:0022904	Respiratory electron transport chain	6	4.60×10^{-13}	*CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0022900	Electron transport chain	6	5.17×10^{-13}	*CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0045333	Cellular respiration	6	6.05×10^{-12}	*CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0015980	Energy derivation by oxidation of organic compounds	6	5.80×10^{-10}	*CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0006091	Generation of precursor metabolites and energy	6	2.32×10^{-9}	*CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*

B, Cellular component

GO_ID	Term	Count	P-value	DEGs
GO:0005743	Mitochondrial inner membrane	7	1.67×10^{-12}	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0019866	Organelle inner membrane	7	3.57×10^{-12}	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0070469	Respiratory chain	5	2.12×10^{-11}	*CYC1, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0031966	Mitochondrial membrane	7	2.30×10^{-11}	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
GO:0005740	Mitochondrial envelope	7	3.57×10^{-11}	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*

C, Molecular function

GO_ID	Term	Count	P-value	DEGs
GO:0015078	Hydrogen ion transmembrane transporter activity	4	4.15×10^{-8}	*ATP5G3, COX8A, UQCRC1, UQCRFS1*
GO:0008121	Ubiquinol-cytochrome-c reductase activity	2	3.57×10^{-6}	*UQCRC1, UQCRFS1*
GO:0016681	Oxidoreductase activity, acting on diphenols and related substances as donors, cytochrome as acceptor	2	3.57×10^{-6}	*UQCRC1, UQCRFS1*
GO:0016679	Oxidoreductase activity, acting on diphenols and related substances as donors	2	4.76×10^{-6}	*UQCRC1, UQCRFS1*
GO:0015077	Monovalent inorganic cation transmembrane transporter activity	4	6.29×10^{-6}	*ATP5G3, COX8A, UQCRC1, UQCRFS1*

DEG, differentially expressed genes; GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

GTTCAGACGTGTGCTCTTCCGATCT-3', 1.5 μl dNTP, 0.5 μl Phusion High-Fidelity DNA Polymerase (2 U/μl) and 5 μl ddH_2O, and then incubated at 98˚C for 40 sec, 65˚C for 30 sec and 72˚C for 30 sec. Next, 1 μl of 10 μM forward PCR primer (5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3') was added and incubated at 98˚C for 10 sec, 10 cycles at 65˚C for 30 sec, 72˚C for 30 sec, and 72˚C for 3 min. Finally, the library was dissolved in 20 μl ddH_2O after being purified by 50 μl AMPure XP magnetic beads. A 1 μg input for 15 cycles and a 5 μg input for 12 cycles was used and the library quality was assessed on a 2100 Electrophoresis Bioanalyzer instrument

Table VII. The 13 and 6 enriched pathways for differentially expressed genes in modules 1 and 2, respectively.

Pathway	Count	P-value	Gene symbol
A, Module 1			
Focal adhesion	9	$5.20x10^{-14}$	*ITGB6, BCAR1, VASP, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
ECM-receptor interaction	7s	$3.66x10^{-12}$	*ITGB6, LAMA3, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
Arrhythmogenic right ventricular cardiomyopathy	6	$2.67x10^{-10}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
Hypertrophic cardiomyopathy	6	$5.41x10^{-10}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
Dilated cardiomyopathy	6	$8.90x10^{-10}$	*ITGB6, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
Regulation of actin cytoskeleton	7	$2.55x10^{-9}$	*ITGB6, BCAR1, ITGA6, ITGB4, ITGB5, ITGA3, ITGB8*
Small cell lung cancer	3	$2.31x10^{-4}$	*LAMA3, ITGA6, ITGA3*
Hematopoietic cell lineage	2	$7.47x10^{-3}$	*ITGA6, ITGA3*
Pathways in cancer	3	$1.11x10^{-2}$	*LAMA3, ITGA6, ITGA3*
Leukocyte transendothelial migration	2	$1.27x10^{-2}$	*BCAR1, VASP*
Toxoplasmosis	2	$1.63x10^{-2}$	*LAMA3, ITGA6*
Cell adhesion molecules	2	$1.65x10^{-2}$	*ITGA6, ITGB8*
B, Module 2			
Parkinson's disease	7	$2.23x10^{-12}$	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
Oxidative phosphorylation	7	$2.49x10^{-12}$	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
Alzheimer's disease	7	$1.33x10^{-11}$	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
Huntington's disease	7	$2.56x10^{-11}$	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*
Cardiac muscle contraction	5	$7.02x10^{-9}$	*CYC1, COX8A, UQCRC1, UQCRC2, UQCRFS1*
Metabolic pathways	7	$9.66x10^{-6}$	*ATP5G3, CYC1, COX8A, UQCRC1, NDUFS3, UQCRC2, UQCRFS1*

ECM, extracellular matrix.

(Agilent Technologies, Inc., Santa Clara, CA, USA). Finally, sequencing was conducted on a HiSeq 2500 System (Illumina, Inc., San Diego, CA, USA).

Data preprocessing and sequence alignment. Quality control (QC) of obtained next generation sequencing (NGS) data was conducted with an NGS QC Toolkit (version 2.3.3; www.nipgr.res.in/ngsqctoolkit.html) in order to remove low quality reads with default parameters (20). Reads with ≥10% low quality bases (Phred quality score <20) were filtered.

The paired-end RNA sequencing reads were aligned to the human hg19 reference genome using TopHat2 (ccb.jhu.edu/software/tophat) (21), and the human hg19 reference genome and its annotation files were obtained from the University of California Santa Cruz Genome Browser (genome.ucsc.edu) (22). The '-no-mixed' option was handled and other parameters were set to default.

Identification of DEGs and lncRNAs. Following sequence alignment and refseq annotation, Cuffdiff (23) was applied to screen DEGs with a cut-off criteria of q<0.05. DE-lncRNAs were identified with the combination of lncRNA annotation by LNCipedia 3.0 (www.lncipedia.org) (24). q<0.05 was considered as the threshold value.

Functional and pathway enrichment analysis for DEGs. Gene ontology (GO) terms in the biological process (BP), cellular component (CC) and molecular function (MF) categories were enriched for DEGs using the GO-function package in Bioconductor (www.bioconductor.org) (25). KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was also conducted by the KEGG profile in Bioconductor. The enrichment thresholds were P<0.05 and the gene counts ≥2.

Construction of the PPI network and module analysis. The Search Tool for the Retrieval of Interacting Genes (STRING; www.string-db.org) database not only provides uniquely comprehensive coverage but also contains predicted, experimental, transferred and text-mined interactions (26). The PPIs for DEGs were predicted using version 9.1 of the STRING database with a combined score >0.7 (26). Cytoscape software version 2.8 (27) was used to visualize the PPI network (www.cytoscape.org).

The ClusterONE plugin of Cytoscape (28) was used to perform module analysis for the PPI network with default parameters. In addition, functional and pathway enrichment analysis of DEGs in the two modules with the highest significance was performed with the cut-off criteria of P<0.05 and gene counts ≥2.

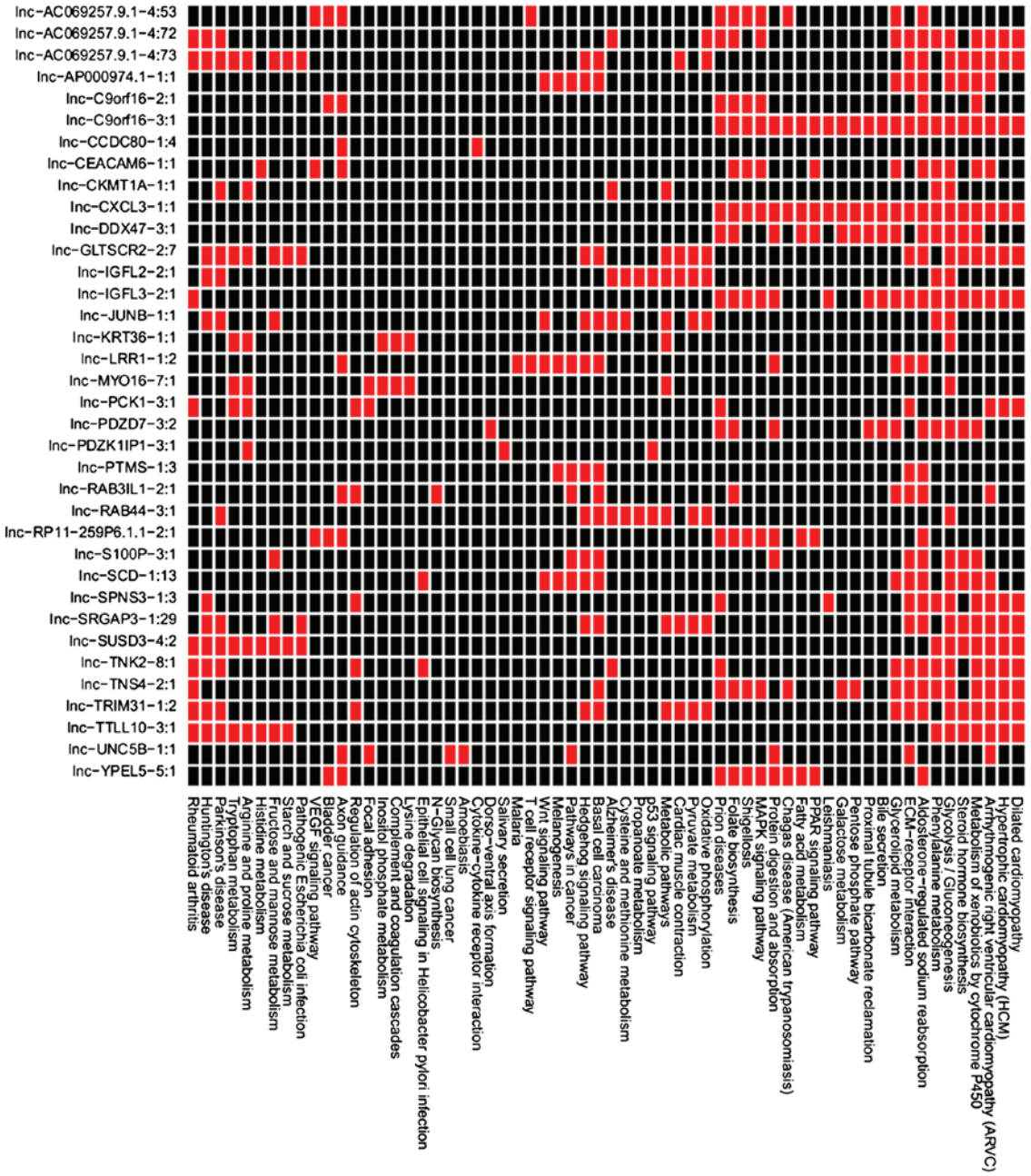

Figure 3. Enriched pathways for the differentially expressed genes co-expressed with each differentially expressed lncRNAs. Horizontal and vertical axis respectively indicate the enriched pathways and differentially expressed lncRNAs. lncRNAs, long non-coding RNAs.

Co-expression analysis of DEGs and lncRNAs. Pearson correlation coefficients between DEGs and lncRNAs were calculated. The co-expressed genes and lncRNA pairs were selected with a Pearson correlation coefficient >0.98. Pathway enrichment analysis was conducted for the DEGs co-expressed with each DE-lncRNA, with thresholds of P<0.05 and gene counts ≥2.

Results

DEGs and lncRNAs. A total of 490 DEGs, of which 302 were upregulated and 188 downregulated genes, were identified in the celecoxib and the control groups. A total of 37 DE-lncRNAs, of which 19 were upregulated and 18 downregulated, were screened (Table I).

Functional and pathway enrichment analysis for DEGs. GO enrichment analysis demonstrated that 672, 108 and 120 terms in the BP, CC and MF categories, respectively, were identified as upregulated genes (Table II), and 453, 45 and 67 terms were identified for downregulated genes (Table III). The most enriched GO terms in the categories for upregulated genes were as follows: BP, CC and MF categories for upregulated genes were small molecule metabolic processes ($P=1.87x10^{-9}$), extracellular region ($P=3.64x10^{-23}$) and protein binding ($P=7.34x10^{-7}$), respectively (Table II). The most enriched GO terms in the BP, CC and MF categories for downregulated genes were tissue development ($P=4.66x10^{-8}$); extracellular region ($P=1.02x10^{-10}$) and protein kinase C binding ($P=1.31x10^{-3}$), respectively (Table III).

According to the pathway enrichment analysis, 28 and 7 pathways were identified for the upregulated and downregulated genes, respectively (Table IV). The upregulated genes were significantly enriched in the glycolysis/gluconeogenesis ($P=1.03x10^{-6}$), metabolic pathways ($P=6.04x10^{-5}$), phenylalanine metabolism ($P=4.00x10^{-4}$), oxidative phosphorylation ($P=2.11x10^{-2}$) and the metabolism of xenobiotics by cytochrome P450 ($P=4.14x10^{-3}$) (Table IV).

The downregulated genes were enriched in epithelial cell signaling in *Helicobacter pylori* infection (involving, *CXCL1* and *CXCL8*; $P=2.92x10^{-2}$), complement and coagulation cascades ($P=3.03x10^{-2}$), arrhythmogenic right ventricular cardiomyopathy ($P=3.62x10^{-2}$), chemokine signaling pathway (involving *CXCL1*, *CXCL3*, *CXCL5* and *CXCL8*; $P=3.80x10^{-2}$) and cytokine-cytokine receptor interaction (involving *CXCL1*, *CXCL3*, *CXCL5* and *CXCL8*; $P=4.55x10^{-2}$) (Table IV).

PPI network and module analysis. After the PPIs of DEGs were predicted using the STRING database, the PPI network was visualized (Fig. 1). Based on the ClusterONE plugin, two modules with the highest significance (module 1, $P=9.96x10^{-5}$, nodes=10; module 2, $P=8.98x10^{-4}$, nodes=7) were selected (Fig. 2).

The DEGs in module 1 (including, *ITGB6*, *ITGA6*, *ITGB4*, *ITGB5*, *ITGA3* and *ITGB8*) were most significantly associated with functions of the integrin complex (CC, $P=3.33x10^{-15}$), the protein complex involved in cell adhesion (CC, $P=3.33x10^{-15}$) and the integrin-mediated signaling pathway (BP, $P=1.34x10^{-14}$) (Table V). In module 2, DEGs were involved in the respiratory electron transport chain (BP, $P=4.60x10^{-13}$) and the electron transport chain (BP, $P=5.17x10^{-13}$) (Table VI).

The DEGs in module 1 were most significantly enriched in the focal adhesion pathway ($P=5.20x10^{-14}$) and the extracellular matrix (ECM)-receptor interaction pathway ($P=3.66x10^{-12}$) (Table VII). In addition, the DEGs in module 2 were enriched in Parkinson's disease ($P=2.23x10^{-12}$), oxidative phosphorylation ($P=2.49x10^{-12}$), Alzheimer's disease ($P=1.33x10^{-11}$), Huntington's disease ($P=2.56x10^{-11}$) and metabolic pathways ($P=9.66x10^{-6}$) (Table VII).

Co-expression analysis of DEGs and DE-lncRNAs. The pairs of co-expressed genes and lncRNAs were obtained and the enriched pathways for the DEGs co-expressed with each DE-lncRNAs are presented in Fig. 3. The DEGs co-expressed with lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1, lnc-AP000974.1-1:1 and lnc-RAB3IL1-2:1 were enriched in the pathways associated with cancer, such as basal cell carcinoma, pathways in cancer and ECM-receptor interaction (Table VIII). The DEGs co-expressed with lnc-SCD-1:13, lnc-LRR1-1:2 and lnc-S100P-3:1 were enriched in the Wnt signaling pathway (Table VIII). The DEGs co-expressed with lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1 and lnc-AP000974.1-1:1 were enriched in the Hedgehog signaling pathway (Table VIII).

Discussion

In the present study, the RNA sequencing data between gastric cancer cells treated with celecoxib and those treated with DMSO was used to explore the mechanism of celecoxib treatment in gastric cancer cells. It has been previously demonstrated that altered patterns of DNA methylation associated with *Helicobacter pylori* infection of gastric epithelial cells may contribute to the risk of gastric cancer (29). Following *Helicobacter pylori* infection, the significant expression of CXCL5 and CXCL8 was observed in primary human gastric epithelial cells (30). Verbeke *et al* (31) also reported that CXC chemokines may contribute to the transition of chronic inflammation in esophageal and gastric cancer. In addition, CXC chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8) could promote the migration and proliferation of endothelial cells by interacting with CXCR2 (32). Furthermore, the overexpression of CXCL1 and CXCR2 may be involved in the tumor invasion in gastric cancer (33). The study by Park *et al* (34) demonstrated that the overexpression of CXCL5 may contribute to the pathogenesis of gastric cancer.

The results of the present study revealed that some DEGs (CXCL1 and CXCL8) were enriched in the epithelial cell signaling pathway in *Helicobacter pylori* infection whereas other DEGs (CXCL1, CXCL3, CXCL5 and CXCL8) were enriched in both the chemokine signaling and cytokine-cytokine receptor interaction pathways, which were consistent with the previous reports. Based on these results, CXCL1, CXCL3, CXCL5 and CXCL8 were suggested to contribute to the development of gastric cancer through multiple pathways.

ITGA3 is known to be involved in the development of gastric cancer (35). The MPS-1/ITGB4 signaling axis mediates cell migration and invasiveness, which may be used as targets during the therapy of gastric cancer (36). Song *et al* (35) revealed that the polymorphisms of microRNA-binding sites in the 3'UTR region of the integrin genes (ITGA3, ITGA6, ITGB3, ITGB4 and ITGB5) were associated with the susceptibility of gastric cancer. Pathway enrichment analysis revealed that integrin genes (ITGA3, ITGA6, ITGB4, ITGB5, ITGB6 and ITGB8) in module 1 were enriched in the integrin-mediated signaling pathway. Altogether, we could speculate that these integrin genes may participate in the celecoxib treatment of gastric cancer via the integrin-mediated signaling pathway.

Co-expression analysis revealed that the DEGs co-expressed with lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1, lnc-AP000974.1-1:1 or lnc-RAB3IL1-2:1 were enriched in a number of pathways, including ECM-receptor interaction, Wnt signaling and Hedgehog signaling pathways. A number of studies reported that lncRNAs are important in the pathogenesis of gastric cancer (37-39). Chang *et al* (40) revealed that the genes in the ECM-receptor interaction pathway were involved in the metastasis and aggression of gastric cancer. In addition, Tang *et al* (41) demonstrated that miR-200b and miR-22 could synergistically inhibit the growth of gastric cancer through the Wnt-1 signaling pathway. Furthermore, Yan *et al* (42) reported that the activated Hedgehog signaling pathway was involved in the progression of gastric cancer. These results implied that lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1, lnc-AP000974.1-1:1 and lnc-RAB3IL1-2:1 may be important in the celecoxib treatment of gastric cancer via different pathways. However, the correlation between COX-2 and DEGs or DE-lncRNAs remains unclear, and needs to be confirmed by further experiments.

Table VIII. The DEGs co-expressed with differentially expressed lncRNAs associated with pathways in cancer.

lncRNA/pathway	DEG
lnc-SCD-1:13	
Wnt signaling pathway	*DVL1*, *FZD7*, *NFAT5*, *WNT11*, *WNT7B*
Hedgehog signaling pathway	*BMP4*, *WNT11*, *WNT7B*
Basal cell carcinoma	*BMP4*, *DVL1*, *FZD7*, *WNT11*, *WNT7B*
ECM-receptor interaction	*HSPG2*, *ITGA3*, *ITGB4*, *LAMA3*, *SDC1*
Glycolysis/gluconeogenesis	*ALDH1A3*, *ALDOA*, *PKM*
Aldosterone-regulated sodium reabsorption	*ATP1A1*, *SFN*, *SGK1*
Glycerolipid metabolism	*AGPAT2*, *AKR1B1*, *LIPG*
Metabolism of xenobiotics by cytochrome P450	*AKR1C2*, *ALDH1A3*, *CYP1B1*
Steroid hormone biosynthesis	*AKR1C2*, *CYP1B1*, *SULT2B1*
Epithelial cell signaling in *H. pylori* infection	*ATP6AP1*, *CXCL8*, *MAP3K14*
Pathways in cancer	*BMP4*, *CXCL8*, *DVL1*, *FOS*, *FZD7*, *ITGA3*, *LAMA3*, *WNT11*, *WNT7B*
Arrhythmogenic right ventricular cardiomyopathy	*ITGA3*, *ITGB4*, *PKP2*
Melanogenesis	*DVL1*, *FZD7*, *WNT11*, *WNT7B*
lnc-LRR1-1:2	
Wnt signaling pathway	*DVL1*, *NFAT5*, *WNT11*, *WNT7B*
Axon guidance	*EFNA3*, *NFAT5*, *RHOD*, *UNC5B*
ECM-receptor interaction	*ITGA3*, *LAMA3*, *SDC1*
Basal cell carcinoma	*BMP4*, *DVL1*, *WNT11*, *WNT7B*
Aldosterone-regulated sodium reabsorption	*ATP1A1*, *SGK1*
Hedgehog signaling pathway	*BMP4*, *WNT11*, *WNT7B*
Malaria	*CXCL8*, *SDC1*
Glycerolipid metabolism	*AGPAT2*, *AKR1B1*
T cell receptor signaling pathway	*FOS*, *MAP3K14*, *NFAT5*
Pathways in cancer	*BMP4*, *CXCL8*, *DVL1*, *FOS*, *ITGA3*, *LAMA3*, *SLC2A1*, *WNT11*, *WNT7B*
Melanogenesis	*DVL1*, *WNT11*, *WNT7B*
Protein digestion and absorption	*ATP1A1*, *KCNE3*, *SLC1A5*
lnc-PTMS-1:3	
Basal cell carcinoma	*BMP4*, *DVL1*, *WNT11*, *WNT7B*
Aldosterone-regulated sodium reabsorption	*SFN*, *SGK1*
Hedgehog signaling pathway	*BMP4*, *WNT11*, *WNT7B*
ECM-receptor interaction	*ITGA3*, *LAMA3*, *SDC1*
Pathways in cancer	*BMP4*, *DVL1*, *FOS*, *ITGA3*, *LAMA3*, *SLC2A1*, *WNT11*, *WNT7B*
Melanogenesis	*DVL1*, *WNT11*, *WNT7B*
lnc-S100P-3:1	
ECM-receptor interaction	*ITGA3*, *LAMA3*, *SDC1*
Basal cell carcinoma	*BMP4*, *DVL1*, *WNT11*, *WNT7B*
Glycolysis/gluconeogenesis	*ACSS2*, *ALDH1A3*, *ALDOA*, *ENO2*
Aldosterone-regulated sodium reabsorption	*ATP1A1*, *SFN*, *SGK1*
Hedgehog signaling pathway	*BMP4*, *WNT11*, *WNT7B*
Metabolism of xenobiotics by cytochrome P450	*AKR1C2*, *ALDH1A3*, *CYP1B1*
Steroid hormone biosynthesis	*AKR1C2*, *CYP1B1*, *SULT2B1*
Pathways in cancer	*BMP4*, *CXCL8*, *DVL1*, *FOS*, *ITGA3*, *LAMA3*, *SLC2A1*, *WNT11*, *WNT7B*
Fructose and mannose metabolism	*AKR1B1*, *ALDOA*
Protein digestion and absorption	*ATP1A1*, *KCNE3*, *SLC1A5*
lnc-AP000974.1-1:1	
Wnt signaling pathway	*DVL1*, *FZD7*, *NFAT5*, *WNT11*, *WNT7B*
Hedgehog signaling pathway	*BMP4*, *WNT11*, *WNT7B*
Basal cell carcinoma	*BMP4*, *DVL1*, *FZD7*, *WNT11*, *WNT7B*
ECM-receptor interaction	*HSPG2*, *ITGA3*, *ITGB4*, *LAMA3*, *SDC1*
Glycolysis/gluconeogenesis	*ALDH1A3*, *ALDOA*, *ENO2*, *PKM*

Table VIII. Continued.

lncRNA/pathway	DEG
Aldosterone-regulated sodium reabsorption	*ATP1A1, SFN, SGK1*
Glycerolipid metabolism	*AGPAT2, AKR1B1, LIPG*
Metabolism of xenobiotics by cytochrome P450	*AKR1C2, ALDH1A3, CYP1B1*
Steroid hormone biosynthesis	*AKR1C2, CYP1B1, SULT2B1*
Pathways in cancer	*BMP4, CXCL8, DVL1, FOS, FZD7, ITGA3, LAMA3, WNT11, WNT7B*
Arrhythmogenic right ventricular cardiomyopathy	*ITGA3, ITGB4, PKP2*
Melanogenesis	*DVL1, FZD7, WNT11, WNT7B*
lnc-RAB3IL1-2:1	
Axon guidance	*NFAT5, SEMA7A, UNC5B*
Basal cell carcinoma	*DVL1, FZD7*
Extracellular matrix-receptor interaction	*HSPG2, ITGA3, ITGA6*
Aldosterone-regulated sodium reabsorption	*ATP1A1, SGK1*
Folate biosynthesis	*ALPP, ALPPL2*
Glycerolipid metabolism	*AGPAT2, LIPG*
N-Glycan biosynthesis	*MGAT3, ST6GAL1*
Regulation of actin cytoskeleton	*FGD3, ITGA3, ITGA6, PFN1*
Pathways in cancer	*CXCL8, DVL1, FZD7, ITGA3, ITGA6*
Arrhythmogenic right ventricular cardiomyopathy	*ITGA3, ITGA6, PKP2*

lncRNA, long non-coding ribonucleic acid; DEGs, differentially expressed genes.

In conclusion, a total of 490 DEGs and 37 DE-lncRNAs were identified in the celecoxib group. Several DEGs (including CXCL1, CXCL3, CXCL5, CXCL8 and integrin genes) and DE-lncRNAs (including lnc-SCD-1:13, lnc-LRR1-1:2, lnc-PTMS-1:3, lnc-S100P-3:1, lnc-AP000974.1-1:1 and lnc-RAB3IL1-2:1) may affect celecoxib treatment of gastric cancer through different pathways. However, these results were obtained by bioinformatics analysis and require further validation.

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