Original Article

Expression profiling analysis reveals molecular mechanism of Lnc00675 downregulation promoting cell apoptosis in acute myeloid leukemia U937 cells

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Background: Acute myeloid leukemia (AML), an aggressive malignancy with poor prognosis, is the most common in adult leukemia. Long non-coding RNA (lncRNA) could affect the regulation of protein-coding genes, cell proliferation and apoptosis, tumor cell resistance to radio- and chemotherapy and pathological processes. Lnc00675 is a lncRNA also known as transmembrane protein 238 like (TMEM238L), which identified as a marker of tumor promoter and unfavorable prognosis in patients with pancreatic ductal adenocarcinoma, glioma and cervical cancer. However, the association between Lnc00675 and hematological tumors has not been previously reported.

Methods: Expression profile gene chip technology was used to screen for differentially expressed genes (DEGs) through comparing Lnc00675 overexpression and Lnc00675 downregulation. Gene ontology (GO) analysis was performed to identify the biologic implications of the DEGs. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to identify biologically important pathways associated with the DEGs. Cell Counting Kit-8 (CCK-8) assay and flow cytometric analysis were utilized to detect the cell proliferation rate and the cell apoptosis rate, respectively.

Results: Comparing Lnc00675 overexpression and Lnc00675 downregulation, a total of 866 and 1,115 DEGs were upregulated and downregulated, respectively. Bioinformatics analysis indicated that Lnc00675 might affect U937 cells proliferation and apoptosis through JAK-STAT signaling pathway and PI3K-Akt signaling pathway. The cell proliferation rate in si-Lnc00675 group was significantly lower than those of si-NC group and Lnc00675 group ($P<0.05$). The cell apoptosis rate of si-Lnc00675 group (22.93%±2.24%) was significantly higher than those of si-NC group (0.37%±0.88%) and Lnc00675 group (0.73%±0.35%) ($P<0.01$).

Conclusions: Downregulation of lnc00675 expression inhibited proliferation and promoted apoptosis in human leukemia U937 cells.

Keywords: Lnc00675; U937 cells; acute myeloid leukemia (AML); JAK-STAT signaling pathway; PI3K-Akt signaling pathway; expression profile analysis

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Introduction

Acute myeloid leukemia (AML), an aggressive malignancy with poor prognosis, is the most common in adult leukemia. The therapeutic efficacy of patients with AML remains poor, with only 40% young patients (<60 years old) or 10% patients (>60 years old) achieving long-term survival (1). The pathological mechanism of AML is a series of events including changes in cell proliferation, differentiation, and apoptosis caused by pathogenic factors, such as somatic mutations, cytogenetic abnormalities, epigenetic changes (2,3).

Long non-coding RNAs (lncRNAs) are e a class of RNAs longer than 200 nucleotides, which don't have the function of encoding proteins (4). LncRNA could affect protein-coding gene regulation, cell proliferation and apoptosis, tumor cell resistance to radio- and chemotherapy and pathological processes by participating in transcriptional regulation and post-transcriptional regulation (5-8). Accumulating evidence supports that misregulation of lncRNA-based epigenetic networks contribute to many types of cancer (9,10). Lnc00675 is a lncRNA also known as transmembrane protein 238 like (TMEM238L), and is identified as a marker of tumor promoter and unfavorable prognosis in patients with pancreatic ductal adenocarcinoma (11), glioma (12) and cervical cancer (13). In spite of the aforementioned link between Lnc00675 and cancer, very few researches have been carried out to find the molecular mechanism of Lnc00675 in cancer metastasis. Li *et al.* reported the positively correlation between Lnc00675 expression and TRIP6 protein expression in glioma tissues and cell lines (12). Ma *et al.* reported that LINC00675 promoted cervical tumorigenesis by modulating the Wnt/β-catenin pathway (13).

However, the association between Lnc00675 and hematological tumors has not been previously reported. In the current study, we analyzed the effect of Lnc00675 on proliferation and apoptosis in human leukemia U937 cells, and the other aim of the current study was to investigate molecular mechanism of Lnc00675 using expression profiling analysis. Our results probably identify Lnc00675 as a novel therapeutic target and provide a new perspective for molecular mechanisms of AML.

Methods

Cell culture and transfection

Human leukemia U937 cells (RRID: CVCL_0007) was cultured in 90% RPMI-1640 (Hyclone, USA) + 10% FBS (Gibco, USA) + penicillin (100 U/mL) and streptomycin (100 g/mL). Cells were cultured under 5% CO_2 and 95% air in an incubator set at 37 ℃. U937 cells in logarithmic growth phase were divided into three groups, such as Lnc00675 group, si-Lnc00675 group and si-NC group. U937 cells were seeded in 25 cm^2 cell culture flasks.

Cell transfection

Transfections were performed using Lipofectamine™ 2000 (Invitrogen, USA). U937 cells suspended in serum-free RPMI-1640 were inoculated in 25 cm^2 cell culture flasks to undergo transfection with Lnc00675 overexpression vector (Lnc00675 group), Lnc00675 siRNA vector (si-Lnc00675 group), and Lnc00675 siRNA negative control vector (si-NC group), respectively. All nucleotide vectors were purchased from Shanghai Genechem Co., Ltd. (China).

Microarray analysis

U937 cells of Lnc00675 group and si-Lnc00675 were isolated, pelleted cells by centrifugation, respectively. Used 1 mL of TRIzol Reagent (Invitrogen, USA) to lyse 1×10^7 U937 cells by repetitive pipetting. Microarray experiments were conducted by Shanghai KangChen Biotech (China) with Agilent Human 4x44K Gene Expression Microarray chips with 444,000 probes, the Agilent One-Color Microarray-Based Gene Expression Analysis protocol was used, including total RNA Clean-up and RNA QC, purify the labeled/amplified RNA and labeled cRNA QC, hybridization, microarray Wash, Scanning, extract data using Agilent Feature Extraction software. Bioconductor DESeq2 version 1.12.3 (https://www.rdocumentation.org/packages/DESeq2) was used to identify differentially expressed genes (DEGs) using a fold change (FC) >2 for significant upregulation or significant downregulation and a false discovery rate (FDR) <0.05. A scatter plot was drawn according to the analysis of the DEGs. Gene ontology (GO, www.geneontology.org) analysis was performed to identify the biologic implications of the DEGs. Fisher's exact test was used to identify the significant GO terms with FDR-adjusted P values. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to

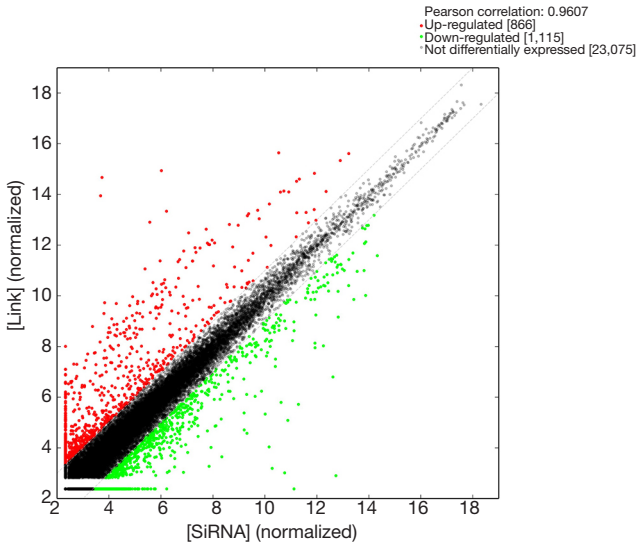


Figure 1 Scatter plot of upregulated and downregulated differentially expressed genes comparing between Lnc00675 group and si-Lnc00675.

identify biologically important pathways associated with the DEGs. Fisher's exact test was used to select the significant pathways based on P values (P<0.05) and FDR (FDR <0.27).

Cell apoptosis detection by flow cytometry

The cells of Lnc00675 group, si-Lnc00675 group and si-NC group were seeded in 6-well cell culture plate, respectively. After 48 h of transfection, the U937 cells were washed with PBS. Flow cytometry was used to detect the apoptosis rates of the three groups. The staining was performed by Annexin V-FITC/PI double staining method (GenStar, China). Binding buffer was used to resuspend cells, 5 μL of Annexin V-FITC was added, then incubated at room temperature for 15 minutes in the dark. PI staining (5 μL) was added for 5 minutes before detection. A FACS Calibur cell analyzer (BD Biosciences) was used to analyze cell apoptosis and apoptosis rate. The percentages of apoptotic cells including early apoptotic cells (Annexin V^+/PI^- cells) and late stage apoptotic cells (Annexin V^+/PI^+ cells) were calculated.

Cell Counting Kit-8 (CCK-8) assay

The viability of U937 cells was detected using CCK-8 assay (Coffit, China). U937 cells (1×10^5 cells/mL) in the logarithmic growth phase were prepared as cell suspensions using RPMI-1640 containing 10% FBS. Cell suspension (100 μL) was inoculated into a well of 96-well plates. 96-well plate was incubated at 37 ℃ and 5% CO_2 for 24, 48 or 72 h after transfection. CCK-8 solution (10 μL) was added to each well and incubated for 2 h at 37 ℃. The absorbance of each well was measured by microplate reader (Shanghai Flash Spectrum Biotechnology, China) at a wavelength of 450 nm. The proliferation rate was calculated using the equation: proliferation rate (%) = $(OD_{treatment} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100\%$.

Statistical analysis

GO and KEGG analyses were performed using the online database DAVID 6.8 (https://david.ncifcrf.gov/). The difference between 2 groups was determined by unpaired Student's *t*-test using GraphPad prism 8.0 software. The differences were considered statistically significant at P<0.05. All experimental results are presented as the mean ± SD.

Results

Differential gene expression

By comparing Lnc00675 group with si-Lnc00675 group, the microarray analysis determined a total of 1,981 DEGs (FC ≥2) (*Figure 1*): 866 genes were upregulated and the remaining 1,115 genes were downregulated. *Tables 1,2* showed the TOP50 upregulated genes and the TOP50 downregulated genes, respectively.

GO analysis of the DEGs

GO analysis contained three domains that represent gene function based on cellular component, biological process and molecular function. A total of 1,385 DEGs were associated with the cell composition domain, of which 608 were upregulated (*Figure 2A*) and 777 genes were downregulated (*Figure 2B*). The TOP5 enrichment score biological process terms were "non-membrane-bounded organelle", "intracellular non-membrane-bounded organelle", "cytoplasmic vesicle", "intracellular vesicle" and "cytoplasmic part". A total of 1,320 DEGs were associated with the biological process domain, of which 581 were

Table 1 The TOP50 upregulated genes (Lnc00675 *vs.* si-Lnc00675)

NO.	Gene symbol	Description	Probe Name	GenBank accession	Fold change
1	CCIN	Calicin	A_23_P60227	NM_005893	1,964.76
2	LOC100129931	Uncharacterized LOC100129931	A_33_P3277883	NR_033828	1,235.66
3	CCDC64B	Coiled-coil domain containing 64B	A_33_P3335590	NM_001103175	161.49
4	CEP104	Centrosomal protein 104 kDa	A_33_P3405754	BC050721	59.59
5	RAB7A	Member RAS oncogene family	A_33_P3226492	AF119891	51.25
6	SFN	Stratifin	A_33_P3389286	NM_006142	51.13
7	UTP18	UTP18 small subunit processome component	A_23_P130020	NM_016001	42.96
8	LINC01123	Long intergenic non-protein coding RNA 1123	A_33_P3228609	NR_046110	42.82
9	LINC01061	Long intergenic non-protein coding RNA 1061	A_24_P691775	NR_037596	42.68
10	KRTAP1-4	Keratin associated protein 1-4	A_33_P3213006	NM_001257305	42.59
11	FAM178B	Family with sequence similarity 178 member B	A_33_P3287119	NM_001122646	34.59
12	EFTUD1	Elongation factor Tu GTP binding domain containing 1	A_24_P754817	NM_024580	33.30
13	MAGIX	MAGI family member, X-linked	A_24_P66105	NM_024859	31.73
14	DHRS4L1	Dehydrogenase/reductase SDR family member 4 like 1	A_33_P3359368	NM_001277864	29.70
15	SHISA5	Shisa family member 5	A_33_P3270636	NM_001272068	29.61
16	SLC51B	Solute carrier family 51, beta subunit	A_23_P436284	NM_178859	28.48
17	TBC1D31	TBC1 domain family, member 31	A_23_P334218	NM_145647	28.18
18	PPP1R14A	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	A_33_P3401647	NM_033256	27.34
19	JAKMIP2	Janus kinase and microtubule interacting protein 2	A_33_P3255290	NM_014790	26.58
20	RAP1GAP2	RAP1 GTPase activating protein 2	A_24_P36890	NM_002885	26.24
21	OR52E8	Olfactory receptor, family 52, subfamily E, member 8	A_33_P3281990	NM_001005168	25.55
22	SSPO	SCO-Spondin	A_33_P3277178	AK093431	25.43
23	PPP1R1A	Protein phosphatase 1, regulatory (inhibitor) subunit 1A	A_33_P3383471	AK123969	25.30
24	MAGEB6	Melanoma antigen family B, 6	A_33_P3368755	NM_173523	24.11
25	BCR	Breakpoint cluster region	A_24_P127235	NM_004327	24.05
26	DCLRE1B	DNA cross-link repair 1B	A_24_P54131	NM_022836	23.81
27	RNF150	Ring finger protein 150	A_24_P350589	NM_020724	23.74
28	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6	A_33_P3315779	NM_001165136	23.19
29	CUL4A	Cullin 4A	A_33_P3322909	NM_001278513	23.00
30	SCOC-AS1	SCOC antisense RNA 1	A_24_P145019	NR_033939	22.94

Table 1 (*continued*)

Table 1 (*continued*)

NO.	Gene symbol	Description	Probe Name	GenBank accession	Fold change
31	ATXN3L	Ataxin 3-like	A_23_P361744	NM_001135995	22.73
32	BTN3A1	Butyrophilin, subfamily 3, member A1	A_33_P3388466	NM_007048	22.47
33	AKAP12	A kinase (PRKA) anchor protein 12	A_23_P111311	NM_144497	21.77
34	CDCA7	Cell division cycle associated 7	A_33_P3296169	NM_031942	21.75
35	ZDHHC3	Zinc finger, DHHC-type containing 3	A_33_P3327479	NM_016598	21.66
36	STARD13	StAR-related lipid transfer (START) domain containing 13	A_23_P342727	NM_178006	21.51
37	CTNND1	Catenin, delta 1	A_33_P3209716	NM_001206885	21.20
38	PPAN-P2RY11	PPAN-P2RY11 readthrough	A_33_P3239759	NM_001198690	21.06
39	REP15	RAB15 effector protein	A_33_P3247624	NM_001029874	20.87
40	MECP2	Methyl CpG binding protein 2	A_33_P3339036	NM_001110792	20.70
41	PIK3R5	Phosphoinositide-3-kinase, regulatory subunit 5	A_23_P66543	NM_014308	20.65
42	THOC2	THO complex 2	A_33_P3235690	NM_001081550	20.63
43	ZCCHC13	Zinc finger, CCHC domain containing 13	A_32_P11096	NM_203303	20.20
44	EGFR	Epidermal growth factor receptor	A_33_P3351944	NM_201283	20.01
45	FBXO2	F-box protein 2	A_23_P45999	NM_012168	19.78
46	BICC1	Bicc family RNA binding protein 1	A_33_P3293913	NM_001080512	19.61
47	PCM1	Pericentriolar material 1	A_24_P555510	NM_006197	19.56
48	SPECC1L	Sperm antigen with calponin homology and coiled-coil domains 1-like	A_33_P3214027	NM_001254732	19.26
49	RIMS3	Regulating synaptic membrane exocytosis 3	A_23_P319583	NM_014747	19.16
50	TRHDE-AS1	TRHDE antisense RNA 1	A_33_P3311493	NR_026836	19.03

upregulated (*Figure 3A*) and 739 were down-regulated (*Figure 3B*). The TOP5 enrichment score biological process terms were "oxoacid metabolic process", "oxidation-reduction process", "organic acid metabolic process", "carboxylic acid metabolic process" and "small molecule metabolic process". A total of 1,324 DEGs were associated with the molecular function domain, of which 580 were upregulated (*Figure 4A*) and 744 were down−regulated (*Figure 4B*). The five most enriched molecular function terms were "oxidoreductase activity", "protein binding", "steroid dehydrogenase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor", "protein binding" and "oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen".

Pathway analysis of the DEGs

Pathway analysis of the DEGs allows the identification of DEGs related to specific cell pathways. Pathway analysis revealed that DEGs were significantly enriched in 73 pathways (*Figure 5A*,*B*). The upregulated genes were involved in 23 pathways and the downregulated DEGs were involved in 50 pathways. The upregulated DEGs were mainly involved in "JAK-STAT signaling pathway", "Cell cycle", "Amoebiasis", "Necroptosis", "Nucleotide excision repair", "Inflammatory bowel disease (IBD)", "Adrenergic signaling in cardiomyocytes", "ErbB signaling pathway", "PI3K-Akt signaling pathway" and "Renal cell carcinoma". The downregulated DEGs were mainly involved in "Steroid biosynthesis", "Glycosaminoglycan degradation", "Adherens junction", "Lysosome, Ferroptosis", "HIF-1 signaling

Table 2 The TOP50 downregulated genes (Lnc00675 *vs.* si-Lnc00675)

NO.	Gene symbol	Description	Probe name	GenBank accession	Fold change
1	DBF4B	DBF4 zinc finger B	A_24_P253780	NM_145663	910.03
2	KAT2B	K(lysine) acetyltransferase 2B	A_32_P159651	NM_003884	426.94
3	FAM50B	Family with sequence similarity 50, member B	A_23_P8240	NM_012135	214.33
4	CPSF4L	Cleavage and polyadenylation specific factor 4-like	A_33_P3265194	–	135.99
5	LOC651337	Uncharacterized LOC651337	A_33_P3617190	AK124119	69.68
6	NOC3L	Nucleolar complex associated 3 homolog	A_23_P202496	NM_022451	56.46
7	MED23	Mediator complex subunit 23	A_23_P330999	NM_015979	55.58
8	DPH2	DPH2 homolog	A_24_P393844	NM_001384	47.56
9	FOXN2	Forkhead box N2	A_32_P140898	NM_002158	28.38
10	SLCO3A1	Solute carrier organic anion transporter family, member 3A1	A_24_P336276	NM_013272	26.61
11	TMEM63A	Transmembrane protein 63A	A_23_P200489	NM_014698	25.86
12	RBM5	RNA binding motif protein 5	A_23_P18276	NM_005778	25.57
13	MAP4	Microtubule-associated protein 4	A_23_P211814	NM_002375	22.55
14	IRF3	Interferon regulatory factor 3	A_23_P27677	NM_001571	18.69
15	SUGCT	Succinyl-CoA:glutarate-CoA transferase	A_23_P145711	NM_024728	15.75
16	IQSEC2	IQ motif and Sec7 domain 2	A_23_P330788	NM_015075	15.01
17	ELMOD3	ELMO/CED-12 domain containing 3	A_33_P3297302	NM_001135021	14.54
18	MEF2BNB	MEF2B neighbor	A_33_P3354771	AK057161	14.32
19	UNC80	Unc-80 homolog	A_33_P3410251	AK090815	14.23
20	LRRC8C	Leucine rich repeat containing 8 family, member C	A_33_P3406030	NM_032270	14.13
21	ELF4	E74-like factor 4	A_24_P340066	NM_001421	13.86
22	METTL20	Methyltransferase like 20	A_33_P3318966	NM_173802	12.63
23	C2CD4C	C2 calcium-dependent domain containing 4C	A_33_P3215412	NM_001136263	12.40
24	ZBTB7C	zinc finger and BTB domain containing 7C	A_33_P3402304	NM_001039360	12.04
25	NEUROD2	Neuronal differentiation 2	A_32_P25295	NM_006160	11.83
26	RASD3	RASD family member 3	A_33_P3349912	NM_001257357	10.53
27	PCDHGC4	Protocadherin gamma subfamily C, 4	A_23_P303101	NM_032406	10.50
28	TMEM254	Transmembrane protein 254	A_23_P97853	NM_025125	10.23
29	ANK2	Ankyrin 2	A_33_P3287967	NM_001148	9.69
30	LOC101928000	Uncharacterized LOC101928000	A_33_P3258712	XR_243583	9.43
31	SLC31A1	Solute carrier family 31, member 1	A_24_P321068	NM_001859	9.39
32	LOC100133985	Uncharacterized LOC100133985	A_33_P3422654	NR_024444	9.36
33	LINC01349	Long intergenic non-protein coding RNA 1349	A_33_P3300067	NR_038914	9.27

Table 2 (*continued*)

Table 2 (*continued*)

NO.	Gene symbol	Description	Probe name	GenBank accession	Fold change
34	CHERP	Calcium homeostasis endoplasmic reticulum protein	A_23_P16139	NM_006387	9.26
35	SPRR2C	Small proline-rich protein 2C	A_23_P126089	NR_003062	9.12
36	NDRG1	N-myc downstream regulated 1	A_23_P20494	NM_006096	9.11
37	SLC9A4	Solute carrier family 9, subfamily A, member 4	A_33_P3396270	NM_001011552	9.06
38	GSTM2P1	Glutathione S-transferase mu 2 pseudogene 1	A_23_P58869	NR_002932	8.68
39	OR2A2	Olfactory receptor, family 2, subfamily A, member 2	A_33_P3394312	NM_001005480	8.68
40	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	A_24_P206604	NM_004566	8.47
41	TPSAB1	Tryptase alpha/beta 1	A_23_P37702	NM_003294	7.97
42	KIF2C	Kinesin family member 2C	A_23_P34788	NM_006845	7.88
43	SLC6A8	Solute carrier family 6, member 8	A_23_P159937	NM_005629	7.74
44	ITGA11	Integrin, alpha 11	A_33_P3353791	NM_181501	7.58
45	ANXA2R	Annexin A2 receptor	A_33_P3299279	NM_001014279	7.49
46	GINS1	GINS complex subunit 1	A_33_P3340025	NM_021067	7.49
47	LOC283887	Uncharacterized LOC283887	A_33_P3677061	XR_132607	7.39
48	FAM178A	Family with sequence similarity 178, member A	A_23_P356139	NM_018121	7.24
49	CLEC12B	C-type lectin domain family 12, member B	A_33_P3303519	NM_205852	7.11
50	GCRG224	Gastric cancer-related gene GCRG224	A_33_P3398867	AF438406	7.09

pathway", "Central carbon metabolism in cancer", "Carbon metabolism", "Glycolysis/Gluconeogenesis", "Amino sugar and nucleotide sugar metabolism" and "Fatty acid metabolism".

Effects of Lnc00675 on proliferation and apoptosis in U937 cells

The proliferation rate of si-Lnc00675 group was significantly lower than those of si-NC group and Lnc00675 group at all three time points ($P<0.05$). There was no significant difference in proliferation rate between si-NC group and Lnc00675 group ($P>0.05$) (*Figure 6A*). Flow cytometric analysis indicated that the downregulation of Lnc00675 significantly promoted cell apoptosis. The cell apoptosis rate of si-Lnc00675 group (22.93±2.24) was significantly higher than those of si-NC group (0.37±0.88) and Lnc00675 group (0.73±0.35) ($P>0.01$) (*Figure 6B*).

Discussion

With the increasing understanding of the lncRNA, the association between tumorigenesis and lncRNA has attracted more and more attention. Notably, Multiple AML researches had shown that the high expression of lncRNA could lead to promote cell proliferation, repress apoptosis, worse prognosis and poor treatment outcomes, such as ZEB2-AS1 (14), lnc-SOX6-1 (15), lnc-CRNDE (16), lnc-HOTAIR (17). With regard to glioma, the high expression of Lnc00675 was dramatically associated with large tumor and advanced World Health Organization grade size (12). The high expression of Lnc00675 positively correlated with poor survival, perineural invasion and lymph node metastasis in patients with pancreatic ductal adenocarcinoma (11). But there is currently no research results available for correlation between Lnc00675 and AML. In the present study, we first reported that the downregulation of Lnc00675 expression

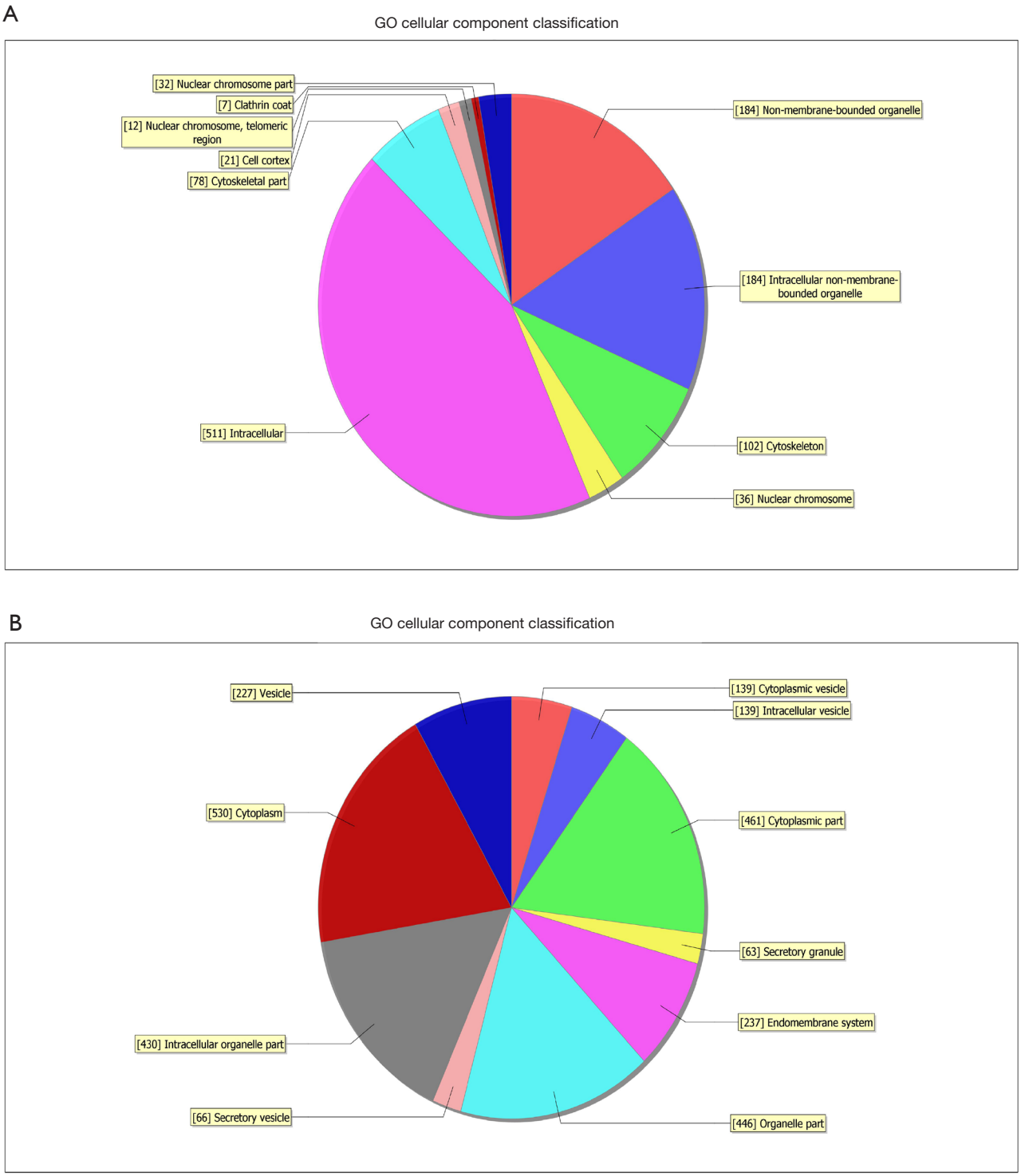


Figure 2 Gene ontology cellular component classification. (A) Cellular component classification of upregulated differentially expressed genes; (B) cellular component classification of downregulated differentially expressed genes.

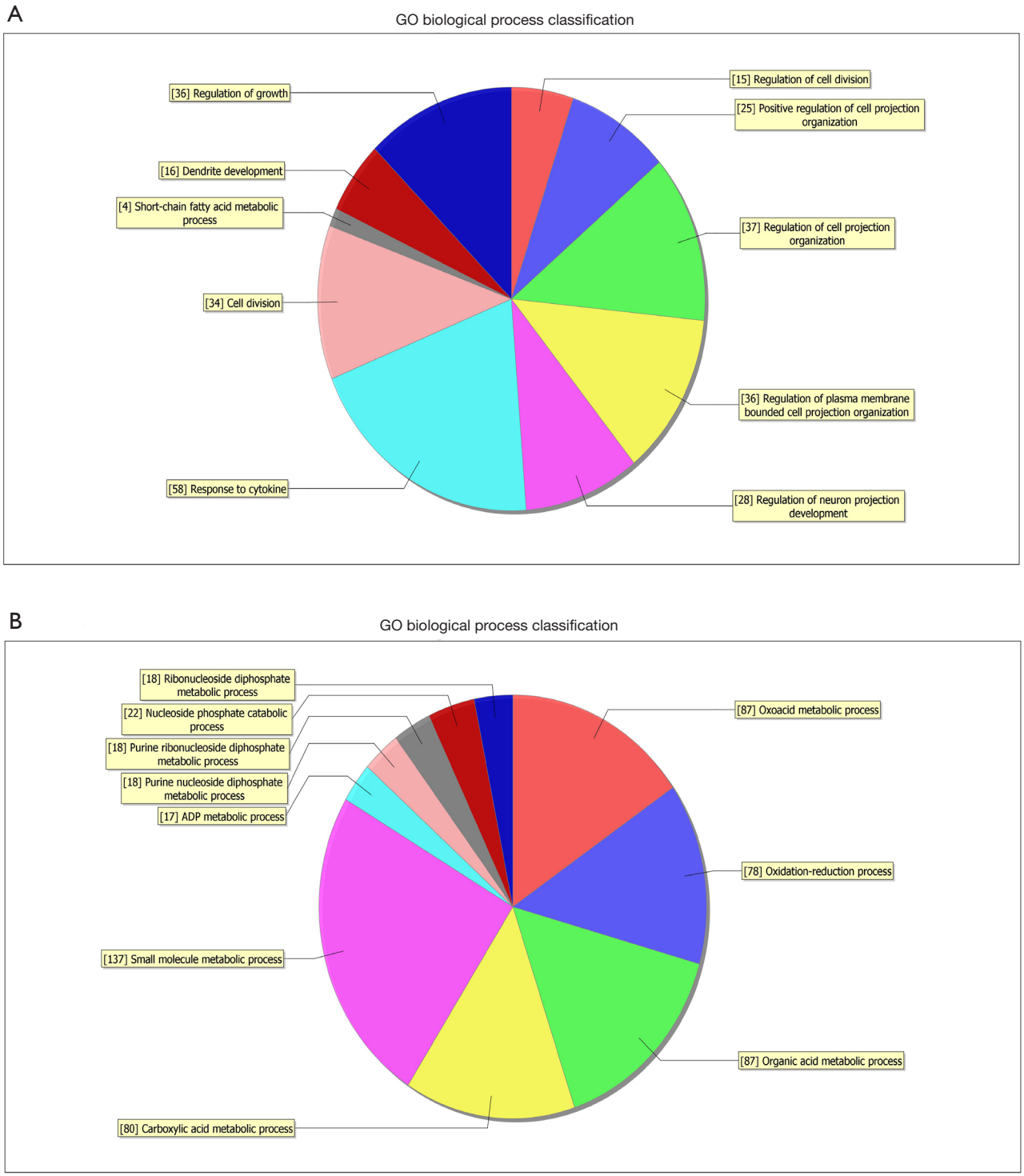
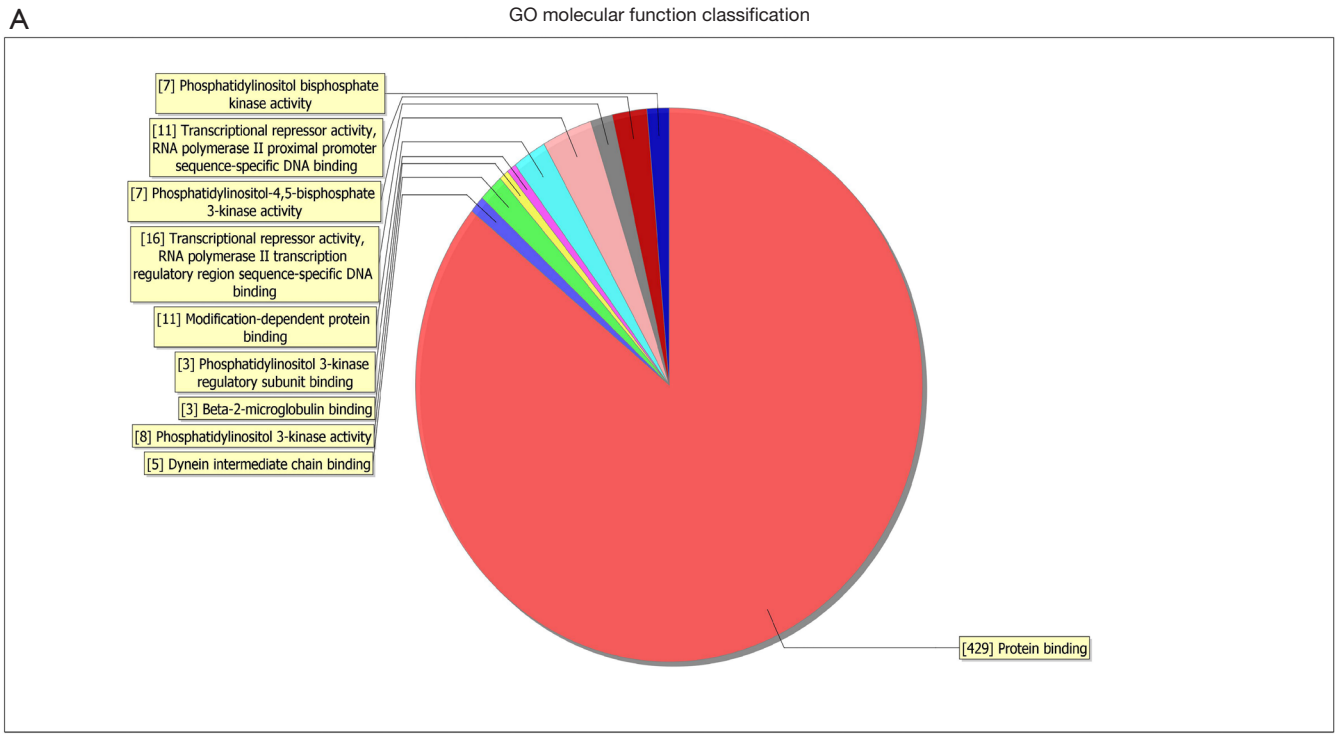


Figure 3 Gene ontology biological process classification. (A) Biological process classification of upregulated differentially expressed genes; (B) biological process classification of downregulated differentially expressed genes.



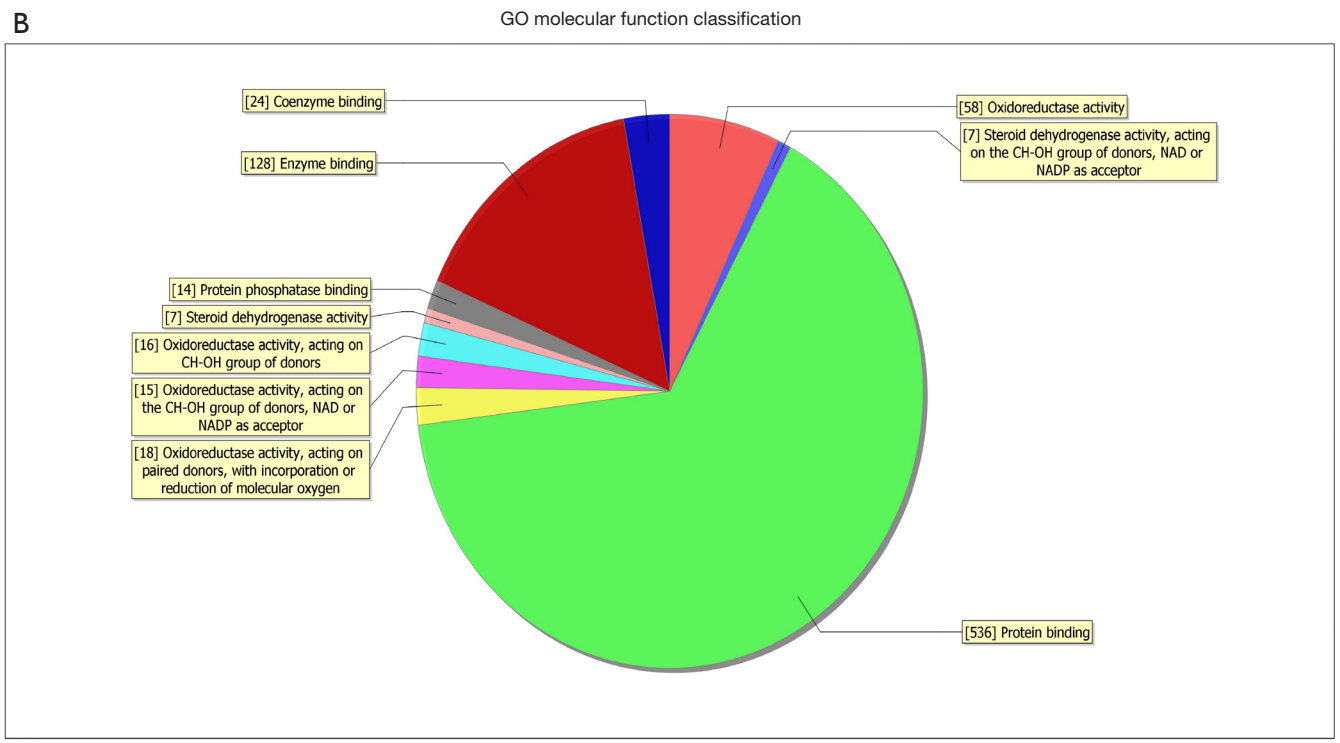


Figure 4 Gene ontology molecular function classification. (A) Molecular function classification of upregulated differentially expressed genes; (B) molecular function classification of downregulated differentially expressed genes.

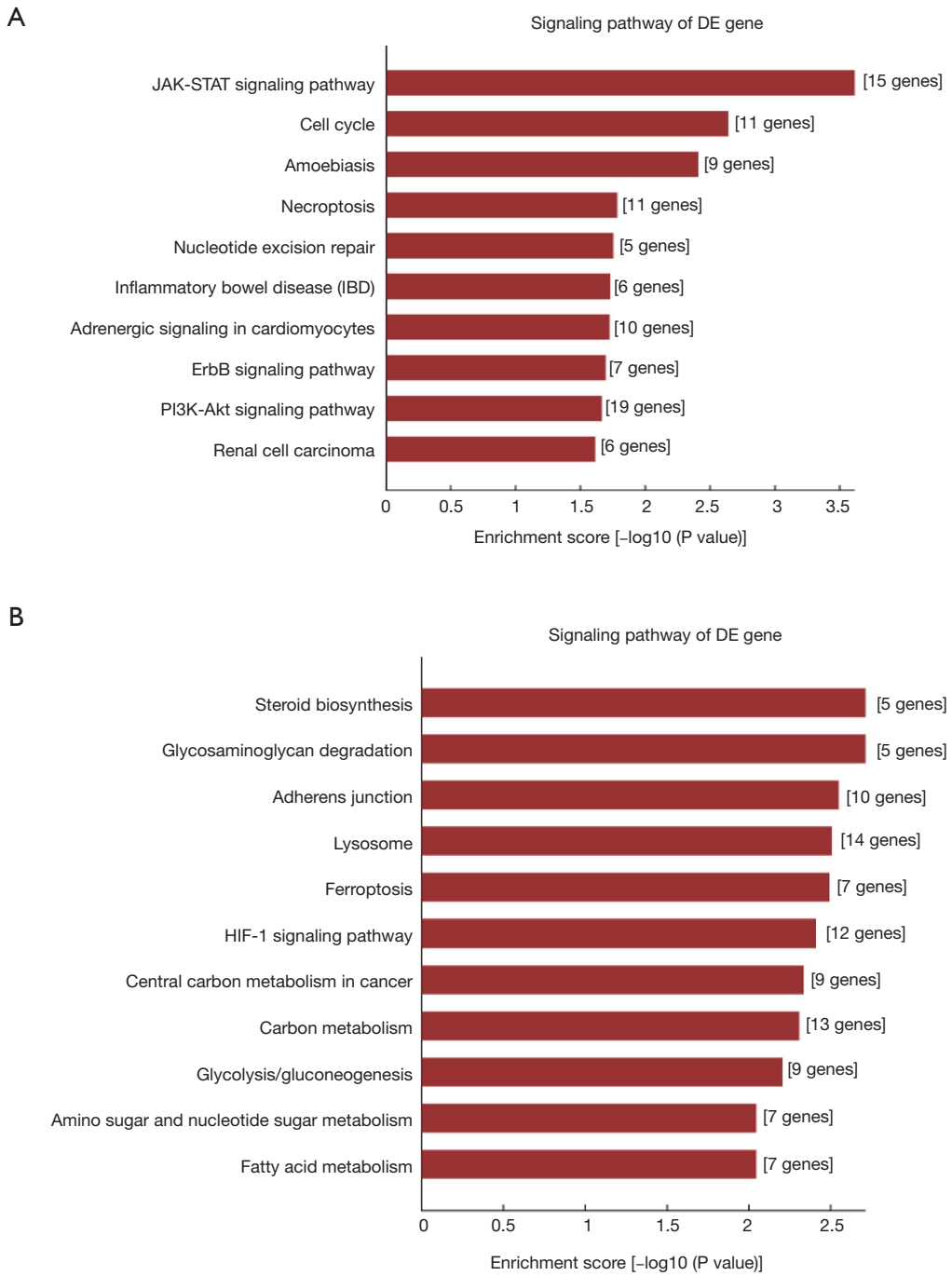


Figure 5 Significantly enrichment pathway analysis of differentially expressed (DE) genes. (A) Upregulated DE genes involved in the Top10 pathways; (B) downregulated DE genes involved in the Top10 pathways.

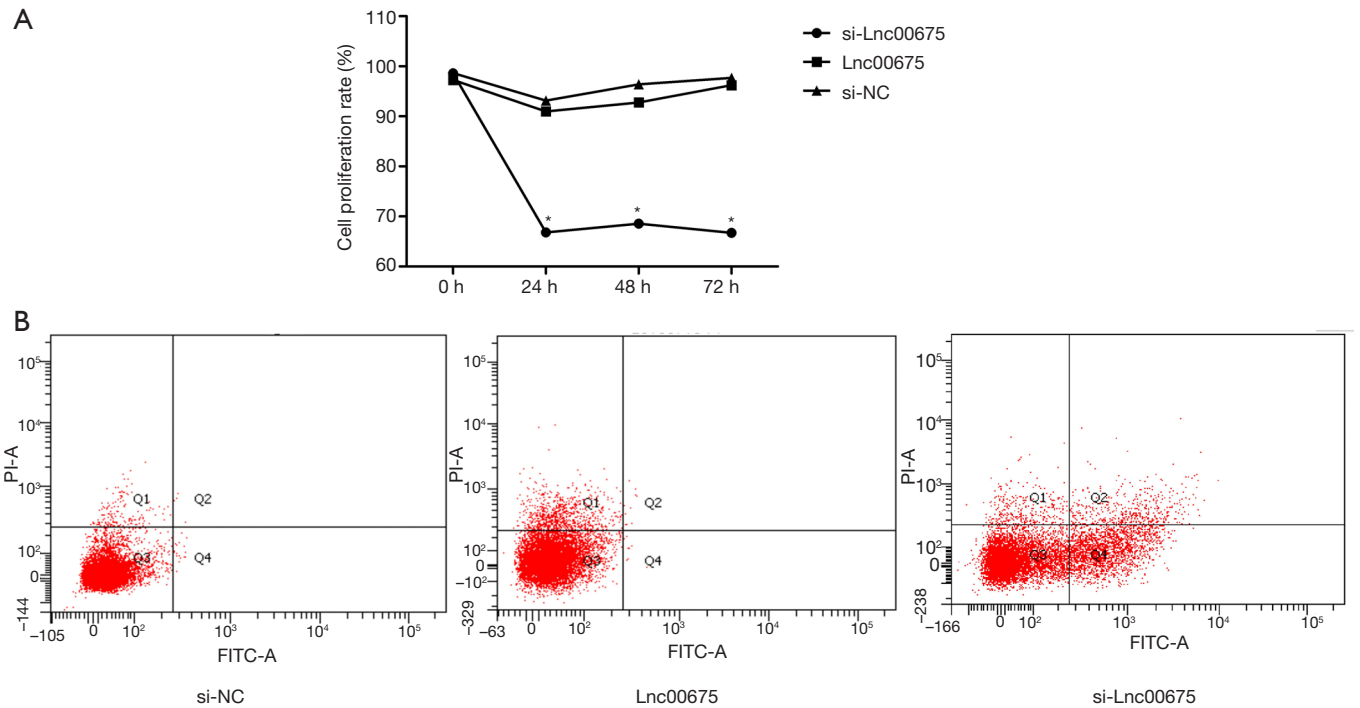


Figure 6 Downregulation of Lnc00675 expression inhibited proliferation and induced cell apoptosis in U937 cells. (A) Cell proliferation was measured by CCK8 assay in U937 cells of Lnc00675 group, si-Lnc00675 group and si-NC group; (B) flow cytometry assay was performed to examine cell apoptosis in U937 of Lnc00675 group, si-Lnc00675 group and si-NC group. *, P<0.05 between si-Lnc00675 group and si-NC group or Lnc00675 group.

resulted in inhibiting cell proliferation and inducing cell apoptosis in U937 cells, but overexpression of Lnc00675 had no effect on the proliferation and apoptosis in U937 cells.

The Wingless (Wnt)/β-catenin signaling pathway has been associated with metabolic reprogramming of cancer cells, cancer stem cells, tumorigenesis and tumor plasticity (18). Ma *et al.* reported that Lnc00675 inhibited apoptosis and promoted proliferation, migration and invasion though the Wnt/β-catenin pathway in cervical cancer cells, and lithium chloride could attenuate the effects of Lnc00675 knockdown (13). Shan *et al.* revealed that Lnc00675 downregulated miR-942 expression in colorectal cancer cells, and miR-942 bound to 3'UTR of glycogen synthase kinase-3β (GSK-3β, a kinase mediating β-catenin phosphorylation in Wnt/β-catenin pathway) by dual-luciferase reporter assay (19). At present, there are no studies investigating the molecular mechanism of Lnc00675 in AML cells. In this regard, KEGG pathway analysis were performed using standard enrichment calculation methods to reveal the molecular mechanism. The result of pathway analysis indicated that Lnc00675 involved in JAK-STAT signaling pathway and PI3K-Akt signaling pathway. The activation of JAK-STAT signaling pathway was implicated in the pathogenesis of AML (20,21), and targeting of this pathway was an effective therapeutic strategy for AML (22,23). Dos Santos *et al.* demonstrated that the PI3K-Akt signaling pathway was constitutively activated in approximately 60% of AML patients cells (24). PI3K-Akt signaling pathway inhibitors, which used alone or with other drugs, have been proven effective for suppressing cell proliferation and promoting apoptosis in AML patients, cell lines or animal models (25).

Epidermal growth factor receptor (EGFR) and interleukin 2 receptor subunit alpha (IL2RA) are involved in both JAK-STAT signaling pathway and PI3K-Akt signaling pathway. Comparing upregulation of Lnc00675 with downregulation of Lnc00675, we found that the expressions of EGFR (FC =20.01) and IL2RA (FC =10.56) were drastically upregulated. EGFR is a cell membrane receptor tyrosine kinase, and mutant EGFR are meaningful serological markers for diagnosis of AML (26). EGFR small

molecule inhibitors have been reported to induce complete and durable remission in AML patients (27). Researches indicated a strong association of IL2RA expression with tyrosine kinases pathways. Upregulation of IL2RA expression was correlated with upregulation expressions of fms related receptor tyrosine kinase 3 (FLT3) (28) and inhibitor of DNA binding 1 (ID1) (29), a key target of tyrosine kinases contributing to leukemia transformation. High expression of IL2RA mRNA was an independent and adverse prognostic factor in AML (30).

The present study, to best of our knowledge, was the first to reveal that downregulation of Lnc00675 expression inhibited proliferation and promoted apoptosis in human leukemia U937 cells. By comparing upregulation of Lnc00675 and downregulation of Lnc00675. We identified 866 upregulated DEGs and 1,115 downregulated DEGs, and indicated that Lnc00675 probably affected U937 cells proliferation and apoptosis through JAK-STAT signaling pathway and PI3K-Akt signaling pathway. We will elucidate molecular mechanism of Lnc00675 in AML and further validate the Lnc00675-mediated signaling pathways in our following researches. The results obtained in the current study may aid in the elucidation of molecular mechanisms of Lnc00675 in AML and contribute to the development of target therapies to treat AML.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-1714). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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