Integrated bioinformatical analysis of lncRNA-mRNA co-expression profiles of cervical cancer

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Background: Aberrant gene expression, including protein-coding and non-coding genes (like long non-coding RNA, lncRNA), is associated with cervical cancer development. To reveal the possible molecular mechanisms of cervical carcinogenesis, this study conducted high throughput sequencing along with a bioinformatics analysis. *Methods*: The differentially expressed lncRNAs and mRNAs were assessed using a microarray technique in three pairs of cervical cancer and paracancerous tissues and analyzed using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations. Their co-expression profiles, containing 653 nodes and 400 edges, were constructed and analyzed using Pearson's correlation and lncRNA-mRNA co-expression network analyses. *Results*: There were 242 lncRNAs and 169 mRNAs upregulated and 1204 lncRNAs and 1131 mRNAs downregulated in cervical cancer (fold change ≥ 2 or ≤ -2; $p \leq 0.05$). The KEGG pathway analytic data showed that these differentially expressed mRNAs were primarily enriched in the ubiquitin-mediated proteolysis and cancer pathways. The differentially expressed *cis*-targeted lncRNAs were associated with the TGF-β signaling and cell adhesion molecules, while the *trans*-targeted lncRNAs were related to p53 signaling. Functional analysis of the integrated mRNA-lncRNA co-expression networks were linked to autophagy, regulation of vascular genesis, transcriptional repressor complex, and regulation of gene expression. *Conclusions*: These differentially expressed lncRNAs and mRNAs could be associated with cervical cancer development and/or progression.

Keywords

Cervical cancer; Long non-coding RNA; Microarray; Pathway analysis; Co-expression network

1. Introduction

Cervical cancer is the second leading cause of morbidity and mortality among all gynecological malignancies [1]. Approximately 95% of cervical cancer is caused by infection with a high-risk persistent human papillomavirus (HR-HPV) [2], including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, and 82 HR-HPV subtypes [3]. Molecularly, cervical carcinogenesis, like most other human cancers, is mainly related to the activation of oncogenes or deactivation of tumor suppressor genes, which may also involve the integration of viral DNA with genomic DNA of human chromosome [4, 5]. Over the past 60 years, the simple and economic Papanicolaou (Pap) smear and cervical cytology have been widely used in the early detection of cervical cancer [5]. Traditional chemotherapy and radiotherapy are by far the most common treatments for advanced cervical cancer, but present serious side effects [6, 7]. Thus, research on the pathogenesis and biological functions are urgently needed for the early detection, treatment, and monitoring of cervical cancer.

Genetically, more than 70% of the human genome sequences has been transcribed into non-coding RNAs (ncRNAs) [8]. Long non-coding RNAs (lncRNA), a class of RNA molecules with transcripts longer than 200 nucleotides (nt), have shown to play an important role in human cancer development and progression via the epigenetic, transcriptional, and post-transcriptional regulation of protein-coding genes [9]. In general, the molecular mechanism of lncRNA can be categorized in four aspects, i.e., signals, decoys, guides, and scaffolds [10]. LncRNA interacts with DNA, RNA, and proteins to affect the formation and function of the secondary and tertiary structures of these molecules. When localized in the nucleus, lncRNAs can guide chromatin-modifying complexes or transcription factors, while in the cytoplasm, it can control the stability of mRNA or compete with endogenous mRNA for protein expression mechanisms [11]. miRNAs can also downregulate expression of target gene mRNA in cytoplasm by binding to the seed region of the three prime untranslated region (3'-UTR). LncRNAs can compete for large numbers of miRNAs in the cells, acting like a sponge and reducing or interfering with the ability of the target gene mRNA to encode proteins. Recently, there is growing evidence that shows that lncRNAs and miRNAs possess particular expression in cervical cancer and different pathophysiological stages of tumor growth, confirming the involvement of certain lncRNAs in cervical carcinoma [12–16]. Despite these findings, the expression patterns, targets, and functions of lncRNAs involved in the pathogenesis of cervical cancer remain largely unknown. Therefore, further research is of great importance.

Table 1. Clinical characteristics of the specimens used for microarray analysis.

Sample ID	Histologic diagnosis	Group
Ctl_1_NS	Normal	N_1
Ctl_2_NS	Normal	N_2
Ctl_3_NS	Normal	N_3
S369_TN_3_NS	SCC	SCC-1
S372_TN_2_NS	SCC	SCC-2
S102_TN_5_NS	Adenocarcinoma	Adenocarcinoma-1

MTMR3, a member of the myotubularin family, is an inositol lipid 3-phosphatase that can hydrolyze PtdIns3P (PI3P) [17]. PI3P is required for the process of autophagy [18]. Some studies have reported that the suppressive effect of MTMR3 on PI3P can inhibit autophagosome formation [19]. Nevertheless, whether MTMR3 is involved in the regulation of cervical cancer has not been elucidated. Recently, both lncRNA and mRNA have been identified as a new class of gene expression regulators [20], although the functions of most lncRNAs remain to be elucidated. In contrast, the alteration and function of some differentially expressed mRNAs have also been recognized in cervical cancer. In this study, we assessed the differentially expressed lncRNA in cervical cancer tissue samples using microarray analysis and analyzed the lncRNA-mRNA network to explore the possible molecular mechanisms of cervical carcinogenesis.

2. Materials and methods

2.1 Patients and tissue samples

In this study, we collected three pairs of invasive cervical cancer tissues (S102_TN_5_NS, S369_TN_3_NS, S372_TN_2_NS) and paracancerous tissues (Ctl_1_NS, Ctl_2_NS, and Ctl_3_NS) from the Obstetrics and Gynecology Hospital of Fudan University (Shanghai, China). These patients were histologically diagnosed with squamous cervical carcinoma (SCC) or adenocarcinoma and classified according to the National Comprehensive Cancer Network (NCCN) criteria [21]. Immunohistochemical results showed that these tumor tissues were CK7(+), P16(+), and Ki-67(+). The clinical characteristics of these cases are shown in Table 1. Tissue specimens were collected from the surgery room and snap-frozen for RNA isolation (see next subsection). This study was approved by the Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University (2018-11).

2.2 RNA isolation and purification

Total RNA was isolated using a RNAiso Plus kit (cat. #9109; Takara Bio, Kusatsu, Shiga Prefecture, Japan) according to the manufacturer's instructions, quantified using a spectrophotometer, and then analyzed with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) for RNA integrity. The resulting RNA samples were further purified with the NucleoSpin RNA Clean-up XS kit (Macherey Nagel, Strabe, Germany) and RNase-free DNase set (Qiagen, GmBH, Germany) according to the manufacturers' protocols.

2.3 ceRNA array and analysis

Six human ceRNA arrays (4 × 180 K) were purchased from Shanghai Biotechnology Corporation (SBC; China) and used for the microarray analysis of these tissue samples. In brief, the quantified and quality-controlled RNA samples (see above) were labeled using the Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies) as probes following the kit's instructions. The labeled cRNA samples were purified using a Qiagen RNease Mini kit and then hybridized to the ceRNA microarray slides in a hybridization oven (Agilent Technologies) overnight (1.65 μg Cy3-labeled cRNA for each reaction) using the Gene Expression Hybridization Kit (Agilent Technologies), according to the manufacturer's instructions. After that, the microarrays were intensively washed with the washing buffer from the Wash Buffer Kit (Agilent Technologies) following the kit's protocol. In the end, the microarrays were scanned using the Agilent Microarray Scanner to collect data. For data analysis, Agilent Technologies (Shanghai Biotechnology Corporation) helped us to extract and analyze the scanned microarray data using the Feature Extraction software 12.0 (Agilent Technologies, https://www.agilent.com.cn/zh-cn/product/mirna-microarray-platform/mirna-microarray-software/feature-extraction-software-228496) and limma software packages in the R program. The raw data were normalized using the Quantile algorithm with the limma packages. Changes in gene expression level with a fold-change of ≥ 2 or ≤ 0.5 were used as a cut-off value for the differentially expressed lncRNAs or mRNAs (upregulated vs. downregulated) in cervical cancer vs. normal tissues.

2.4 Gene ontology (GO) and pathway analysis

The microarray data of differentially expressed lncRNAs and mRNAs in cervical cancer tissues vs. normal ones were first analyzed using the GO database (http://www.geneontology.org/) for their functional categories as the specific biological GO terms [22]. Then, we performed the pathway analyses of the differentially expressed lncRNAs and mRNAs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, assisted by the Shanghai Biotechnology Corporation, to examine their potentially related gene pathways in cervical cancer tissues vs. normal ones.

2.5 Prediction and evaluation of the corresponding lncRNAs-targeting mRNAs

We further predicted and evaluated the targeting mRNAs of the differentially expressed lncRNAs for their *cis/trans*-regulatory effects on mRNAs. Specifically, *cis*-targeted mR-

NAs were considered as targets of lncRNAs when the mRNA genes were localized within 10 kb of the paired lncRNAs, while trans-targeting occurred when lncRNAs contained the specific gene sequences. After selecting the complementary or similar gene sequences paired with lncRNAs using the Basic Local Alignment Search Tool (BLAST) online software, we calculated the RNA duplex energy level between the two sequences using the RNA duplex energy parameter of ≤ -30.

2.6 Analysis of the lncRNA-mRNA co-expression networks

To assess the relationship between lncRNAs and mR-NAs, we conducted the lncRNA-mRNA co-expression network analysis of differently expressed lncRNAs (total of 1446, including 242 upregulated and 1204 downregulated) and mRNAs (total of 1300, including 169 upregulated and 1131 downregulated) using Pearson's correlation coefficients (PCCs). The lncRNA-mRNA co-expression network was constructed by Cytoscape software (Version 3.7.1; The Cytoscape Consortium, San Diego, CA, USA) and analyzed statistically. Correlation with a value of $-0.99 \geq x \geq +0.99$ between lncRNAs and mRNAs expression levels was considered meaningful. Overall, we obtained a total of 653 nodes and 400 edges to be included in the network analysis.

2.7 Reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR)

To verify the microarray data, we selected 15 mRNAs and 10 lncRNAs randomly and assessed their expression levels in three cases of cervical cancer and normal tissue specimens using RT-qPCR. Total RNA was isolated from tissue samples and reversely transcribed into cDNA using an RNAiso Plus kit (Takara) and HiScript II Q RT SuperMix (Vazyme Biotech, Nanjing, China), respectively, according to the kits' instructions. These cDNA samples were subjected to qPCR amplification using the SYBR Green RT RCP master mix (Genetech, Shanghai, China) with specific primers in a 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The primers of mRNAs and lncRNAs were designed and synthesized by Sinotech Genomics (Shanghai, China) and are listed in Table 1. The relative fold-change of each mRNA and lncRNA was calculated using the $2^{-\Delta\Delta Ct}$ method after normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. The experiment was performed in triplicate and repeated at least once.

2.8 Statistical analysis

The data were statistically analyzed using SPSS (Version 17.0; SPSS Inc., Chicago, IL, USA) and are presented as the mean $\pm$ standard deviation (SD) or fold-change. The statistical analysis included Student's t-test, where a $p < 0.05$ is considered statistically significant.

3. Results

3.1 Identification of differentially expressed lncRNAs and mRNAs in cervical cancer tissues

To assess the RNA expression profiles in cervical cancer, we first conducted a whole transcriptome microarray analysis in three pairs of cervical tumor and normal tissues using the ceRNA chips (Shanghai Biotechnology, China), which contain 88,371 circRNA probes, 68,423 lncRNA probes, and 18,853 mRNA probes. A total of 1300 DE-mRNA and 1446 DE-lncRNA transcripts were identified in cervical cancer development with a threshold of fold-change ≥ 2 or ≤ -2; $p \leq 0.05$. Particularly, we identified 242 upregulated and 1204 downregulated lncRNAs and 169 upregulated and 1131 downregulated mRNAs. Among these genes, NR_001298 was the most upregulated and lnc-DDT-2:2 the most downregulated lncRNAs, while NM_021983 (HLA-DRB4) was the most upregulated and NM_052892 (PKD1L2) the most downregulated mRNAs. The heat map data and volcano plot data are shown in Fig. 1A–D, respectively.

3.2 Bioinformatical analysis of differentially expressed mRNAs and lncRNAs

To explore the possible functions of lncRNA, we predicted its potential targets in *cis*-regulatory relationships by searching for protein-coding genes 10-kb upstream and downstream of all the identified lncRNAs. To gain further insight into the potential biological and pathophysiological processes that may be moderated by these DE-RNAs in cervical cancer, we performed the GO term and KEGG pathway analyses. Concerning the biological process, we found that DE-lncRNA was mostly enriched in extrinsic apoptotic-signaling pathway via death domain receptors and was involved in nuclear-transcribed mRNA poly(A) tail shortening and metalloaminopeptidase activity. The top 30 GO terms are shown in Fig. 2A. These results suggest that one of the primary roles of lncRNA may be post-transcriptional regulation of gene expression. Undistinguishably, the GO terms of DE-mRNA were mainly enriched in protein K11-linked ubiquitination, mitochondrial fusion, and the anaphase-promoting complex. The KEGG pathway analysis indicates that these up- and downregulated mRNAs function in ubiquitin-mediated proteolysis and molecular pathways involved in cancer, respectively (Fig. 2A). In particular, the lncRNAs and *cis*-targeted mRNAs showed to play in a role in the TGF-β signaling, cell adhesion molecules (CAMs), and cancer pathways (Fig. 2B).

3.3 Identification of the lncRNA-mRNA co-expression networks in cervical cancer

The functions of lncRNA primarily depend on interaction with mRNAs, which may be an important target of the former. It has been reported that the functions of most lncRNAs include the targeting of transcription, translation, and post-translation of their targeting mRNAs. To gain a better understanding of these DE-RNAs in cervical cancer, we determined the potential targets of lncRNAs in *cis*-regulatory relationships and performed a co-expression analysis using Pearson's correlation analysis with Psych pack-
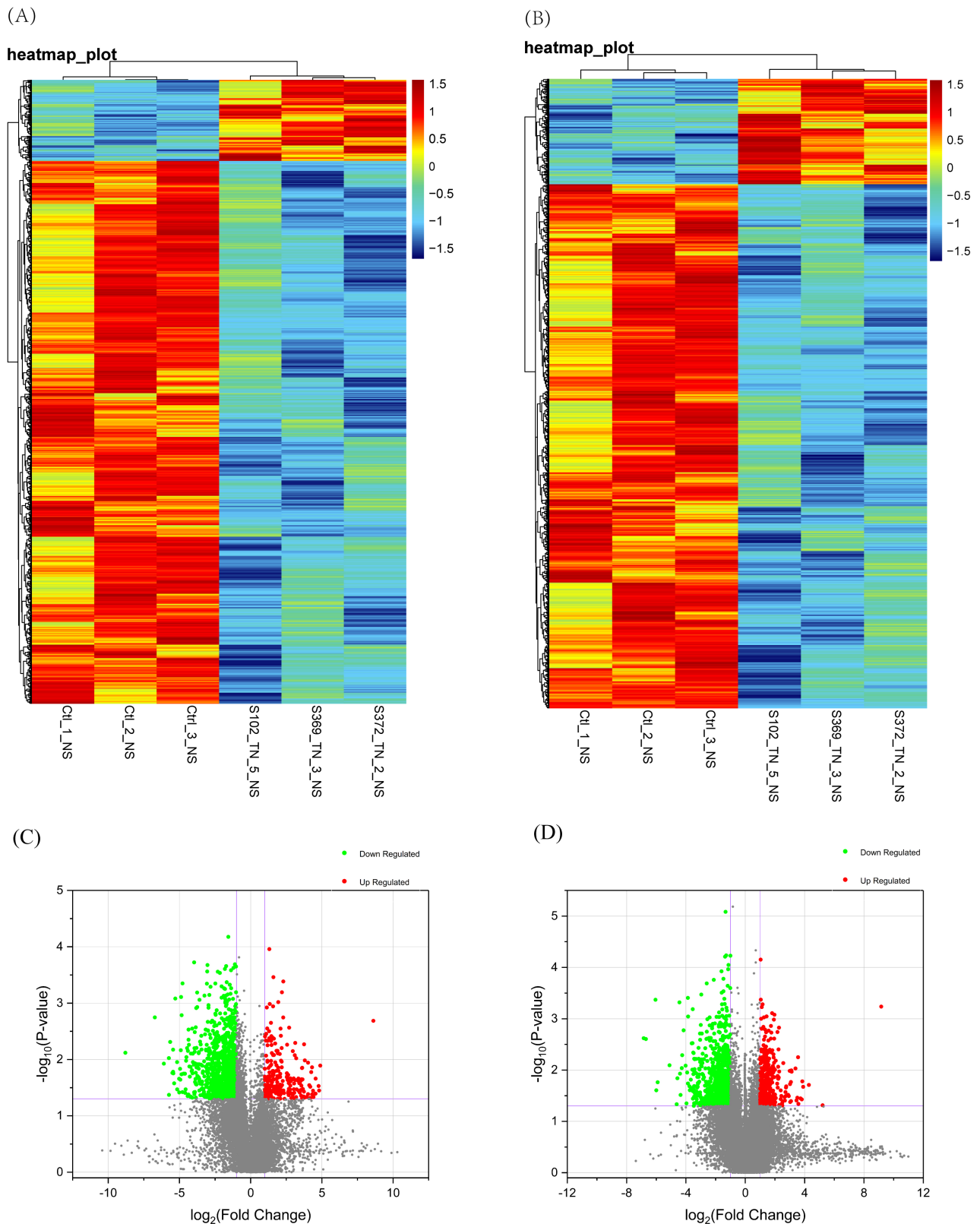
Fig. 1. Identification of differentially expressed mRNAs and lncRNA in cervical cancer. (A) Heat maps of differentially expressed mRNAs. (B) Heat maps of differentially expressed lncRNAs, which are based on the expression values of all lncRNAs and mRNAs detected through the microarray analysis. (C) Volcano plots of differentially expressed mRNAs. (D) Volcano plots of differentially expressed lncRNAs, and cutoff criterion was fold-change of ≥ 2 or ≤ -2; $p \leq 0.05$.
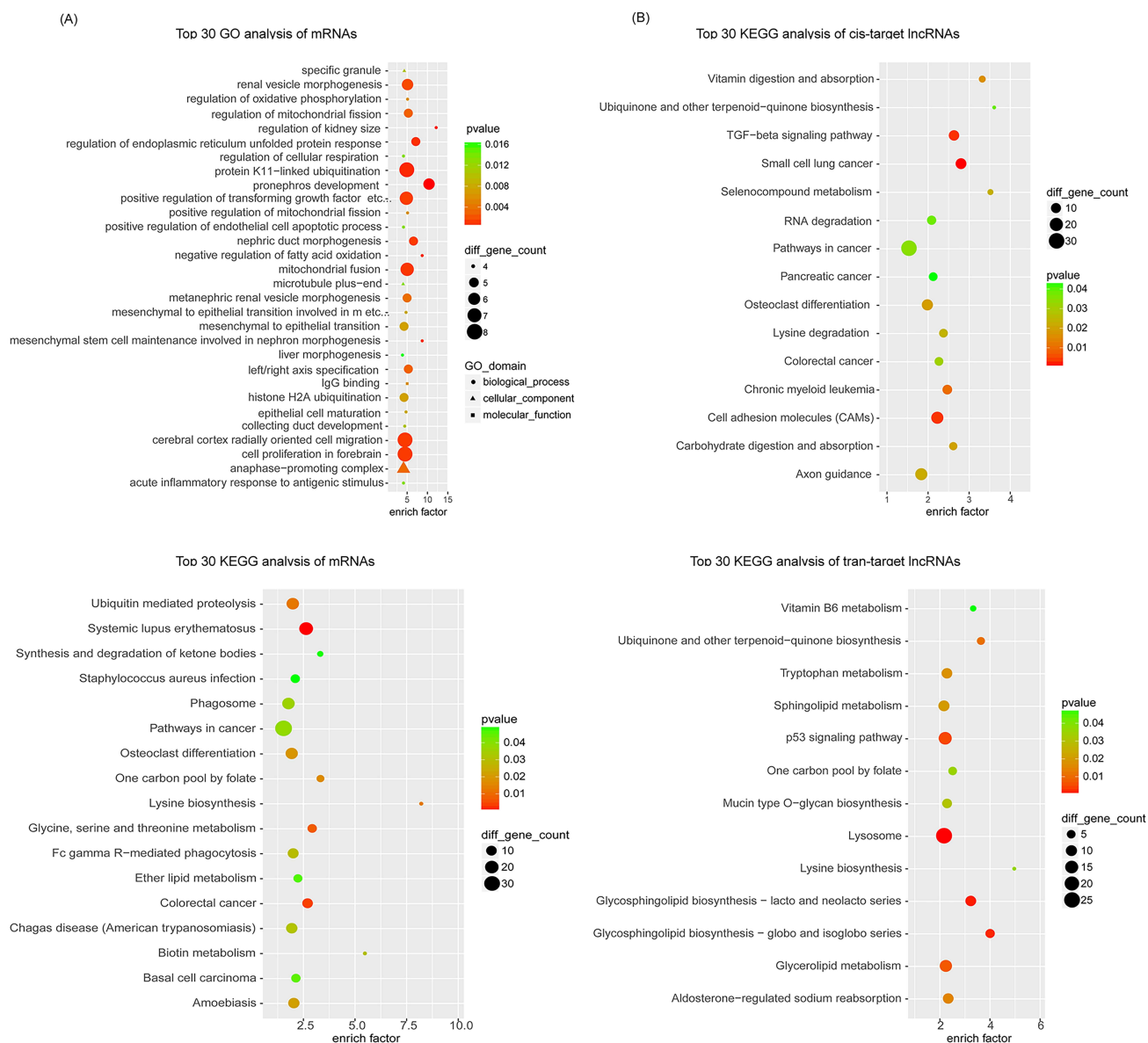
Fig. 2. Data obtained from the Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of differentially expressed mRNAs and lncRNAs in cervical cancer. (A) GO term annotations and KEGG pathway enrichment analysis of mRNAs with top 30 enrichment scores. (B) KEGG pathway enrichment analysis of *cis*-targeted lncRNAs and trans-targeted lncRNAs with the top 30 enrichment scores.

ages (Corr. Test method, parameter CI = F, adjust = BH) in the R program. The screening correlation coefficient was determined to be r ≥ 0.7 with $p < 0.05$, then Cytoscape (https://cytoscape.org/, version 3.7.0) was used to construct the co-expression network. In total, we were able to identify 653 nodes and 400 edges for the network (Fig. 3). It is suggested that an mRNA may be associated with one to six lncRNAs, while one lncRNA is correlated to one to three mRNA. Significantly, six mRNAs were shown to most connect with this network, namely NM_033318 (SMDT1), NM_021090 (MTMR3), NM_021964 (ZNF148), NM_003026 (SH3GL2), NM_018127 (ELAC2), and NM_017934 (PHIP) (Table 2). A functional analysis was conducted to uncover the related biological functions of these mRNAs, revealing that the co-

expressed genes were enriched in 51 GO terms (31 under the biological process, 11 GO under the cellular component, and 9 under the molecular function). Particularly, some of the terms were tumor-associated terms, including autophagy (GO:0006914), regulation of vascular genesis (GO:2001214), transcriptional repressor complex (GO:0017053), and regulation of gene expression (GO:0010629) (Fig. 4A). Furthermore, the co-expressed genes were found to be enriched in Herpes simplex virus 1 infection (Fig. 4B). These findings indicate that lncRNAs also regulate *trans*-target genes.

3.4 Survival analysis using GEPIA2

To assess the prognostic value of these above hub genes, we searched and downloaded data from the online database GEPIA2 (http://gepia2.cancer-pku.cn/#index), which con-
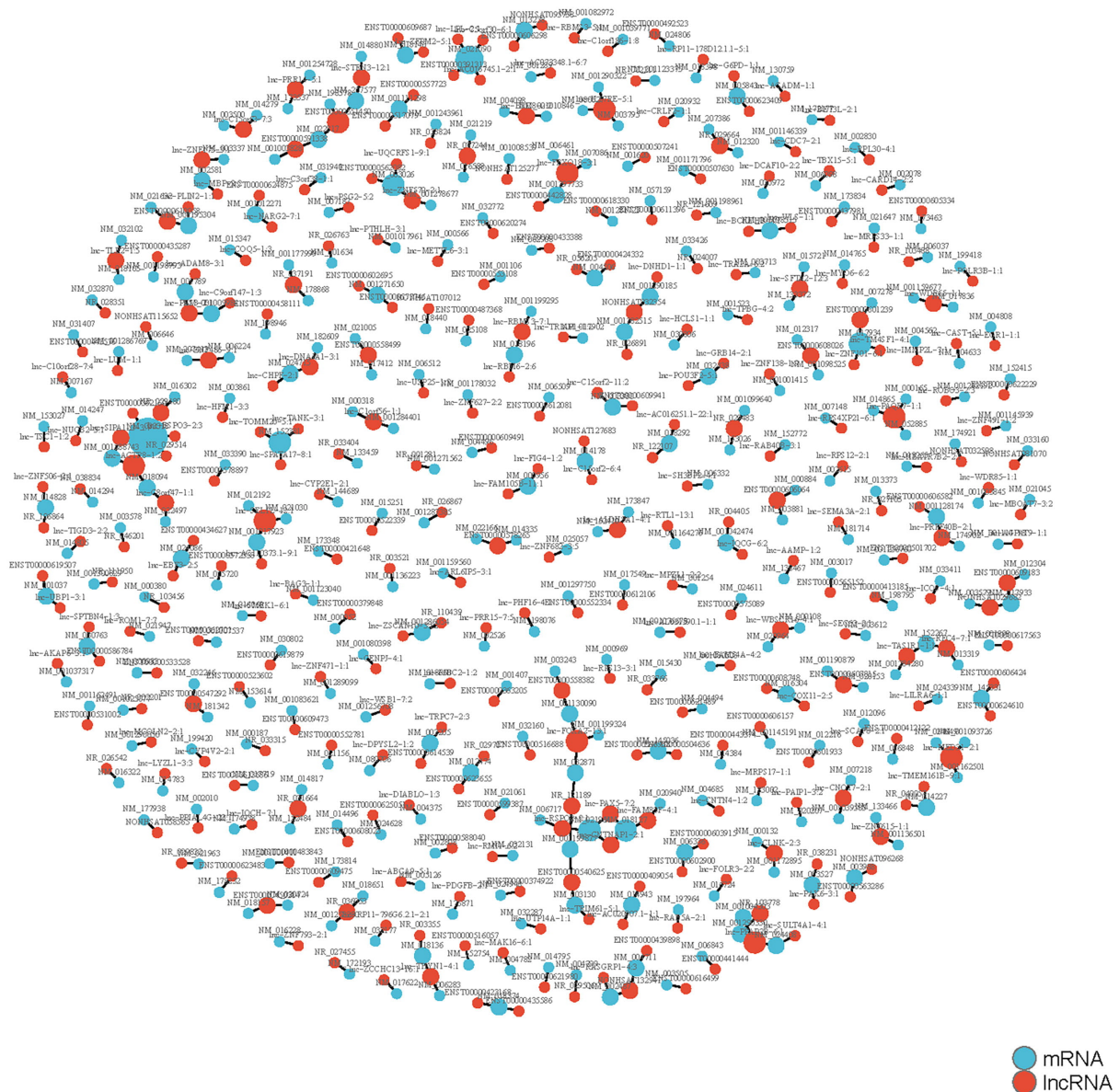
Fig. 3. Data on the differentially expressed lncRNA-mRNA co-expression network. The lncRNA-mRNA co-expression network was constructed using the Cytoscape software (https://cytoscape.org/, version 3.7.0) and analyzed statistically. Correlation values of $-0.7 \geq x \geq +0.7$.

tains gene expression and overall survival data. Importantly, GEPIA2 is an enhanced web server for large-scale gene expression profiling and interactive analysis that includes 198,619 isoforms and 84 cancer subtypes from TCGA and GTEx samples [23]. Our results demonstrate that MTMR3 has a lower expression in cervical squamous cell carcinoma and endocervical adenocarcinoma. Based on the Kaplan-Meier data analysis data, patients with higher MTMR3 expressed tumor had poor overall survival (OS), although the p-value was not statistically significant (HR = 0.94, p-value = 0.78; Fig. 5A). Similarly, the low expression of SMDT1 was also correlated with poor overall survival (OS) (HR = 0.65,

p-value = 0.077; Fig. 5B). As for the other hub genes, association of their expression with survival of patients was not statistically significant (**Supplementary Fig. 1**).

3.5 Validation of the microarray data

To verify the reliability of our microarray analysis results, we performed RT-qPCR validation in 15 randomly selected mRNAs and 10 randomly selected lncRNAs from cervical cancer and normal tissue samples (Primers used were shown in Table 3). We found that two lncR-NAs (lnc-CLRN3-3:1 and lnc-TMEM238-2:1) were upregulated in cervical cancer tissues, whereas ENST00000369391,

Table 2. Co-expression profiles of De-mRNAs and De-lncRNAs.

mRNA	Gene symbol	Connecting RNAs
NM_033318	SMDT1	NR_029480, NR_029514, lnc-SIPA1L3-3:7, lnc-RSPO3-2:3, ENST00000362160, lnc-ACTR8-1:2
NM_021090	MTMR3	lnc-C5orf30-6:1, lnc-AC016745.1-2:1, lnc-LPL-2:1, ENST00000391313
NM_021964	ZNF148	lnc-RSPO2-2:1, lnc-CNTNAP1-2:1, lnc-PAX5-7:2
NM_003026	SH3GL2	lnc-UQCRFS1-9:1, ENST00000562082, lnc-ZNF570-2:1
NM_018127	ELAC2	lnc-CNTNAP1-2:1, lnc-PAX5-7:2, lnc-FAM83F-4:1
NM_017934	PHIP	lnc-ZNF101-6:1, lnc-TM4SF1-4:1, ENST00000601239

Table 3. Primers used for RT-qPCR.

Gene name	DNA sequences	
PIK3R1	5'-TCTTTTGACGAGAGGGAGGA-3'	5'-GTCAAGGAGGCACACTACCC-3'
TCF7L2	5'-ACCATCTTCGTTTCCCCTTT-3'	5'-GGTCAGTCCGGGTCCTAAG-3'
PRICKLE1	5'-CGTCCCACACAAGGGTTT-3'	5'-GCACCAGACCAGGGAATGT-3'
GSN	5'-ACGGCTGAAGGACAAGAAGA-3'	5'-TTCGATCACAAAACGTCCAA-3'
CCNE2	5'-CATGGTGTTCAACCTGTGCT-3'	5'-CATGTCAAGAAAGCCCCAGT-3'
KAT2B	5'-CATAACCCCCTAAAATCCATCA-3'	5'-CAGAGTTGGGAATGGCAGAT-3'
MITF	5'-TGGATTCATTTCAGGGGCTA-3'	5'-GGGAGTGGTGTGGATCATTT-3'
UBE2D3	5'-GTTTGTTGAAATGGCTTAGTTGA-3'	5'-CAAAACATGGCTGAGTTACCC-3'
UBE2S	5'-GCATGAGGGAAGCAAACAGTC-3'	5'-TTTCAGTGGGTTCCGAGGC-3'
TGFBR2	5'-GGGTGGGCTGAGAGTTAAAG-3'	5'-AGGTCAATGGGCAACAGCTA-3'
FN1	5'-AGCATCACCCTGGGAGTTT-3'	5'-CGAAGCAGAACAGGCAATG-3'
INHBA	5'-TGCACCTTTTGGCAACTTCT-3'	5'-TACAATGTCCACCCCCAAAC-3'
SMAD4	5'-ATGCCAGAAGCCAGAGAAGA-3'	5'-CCTGGGACTTTCAACTGACC-3'
APPL1	5'-TGTAAAACCTGCCACCTGAA-3'	5'-CCAAGATCACACCACTGCAC-3'
SAV1	5'-GACGAGGCAGTGGAGAGTTC-3'	5'-CCCTGGTTGTCATTTTTGGT-3'
ENST00000369391	5'-CCAAGCCCAGGAAACAGATA-3'	5'-CCAAGCCCAGGAAACAGATA-3'
ENST00000547898	5'-TGACACCCTCCTTCTCACAG-3'	5'-TCCTCTCCATGCTTTCTTCC-3'
lnc-STOM-1:1	5'-TGCCCAGTGGTGATGAGGTT-3'	5'-GGCCTGAAGGGTTCTTGGA-3'
NR_110418	5'-CCAGCAATAGACCCTCCTTCAA-3'	5'-ACCGGTGGATCCTGCTTTC-3'
ENST00000561507	5'-CCATCATTTTTCCAGCAGTT-3'	5'-AGGGGGTGGCATTAAAAGAG-3'
lnc-SEMA3A-2:1	5'-TCGTGGATGACCTCTCCTATTGT-3'	5'-CCCCCAAATTAGTGCCGTAT-3'
lnc-TMEM238-2:1	5'-AGACCCAGGACACCCAAGTA-3'	5'-ACAAACTCACCACTCGTTGC-3'
lnc-CLRN3-3:1	5'-GATCTTTCAGGTAAAAACGTAGTCT-3'	5'-TTCACAGCAGATAAACAAAGTTAGC-3'
lnc-IGSF11-5:1	5'-AAGGTGCTGGCAGATCTAGTTGT-3'	5'-TTGCCCTGTGAAGACATAGTAAGAA-3'
NR_026867	5'-AAGCCTTCAGGGTCAGTTCA-3'	5'-AGTCCCCAAAATCATCAAATCCA-3'

ENST00000547898, ENST00000561507, lnc-SEMA3A-2:1, lnc-STOM-1:1, NR_110418, and NR_026867 were verified to be downregulated in cervical cancer tissues vs. those of the control tissues (Fig. 6A). Moreover, according to the validation of differentially expressed mRNAs, we discovered that CCNE2, FN1, INHBA, and UBE2S were upregulated, while APPL1, GSN, KAT2B, MITF, PIK3R1, PRICKLE1, SAV1, SMAD4, TCF7L2, TGFBR2, and UBE2D3 were downregulated in cervical cancer tissues vs. control tissues (Fig. 6B). The qPCR results confirm that the expression patterns of these RNAs are consistent with their expression levels calculated from the RNA-seq data (The comparative results were shown in **Supplementary Fig. 2**), which indicates the reliability of high-throughput sequencing.
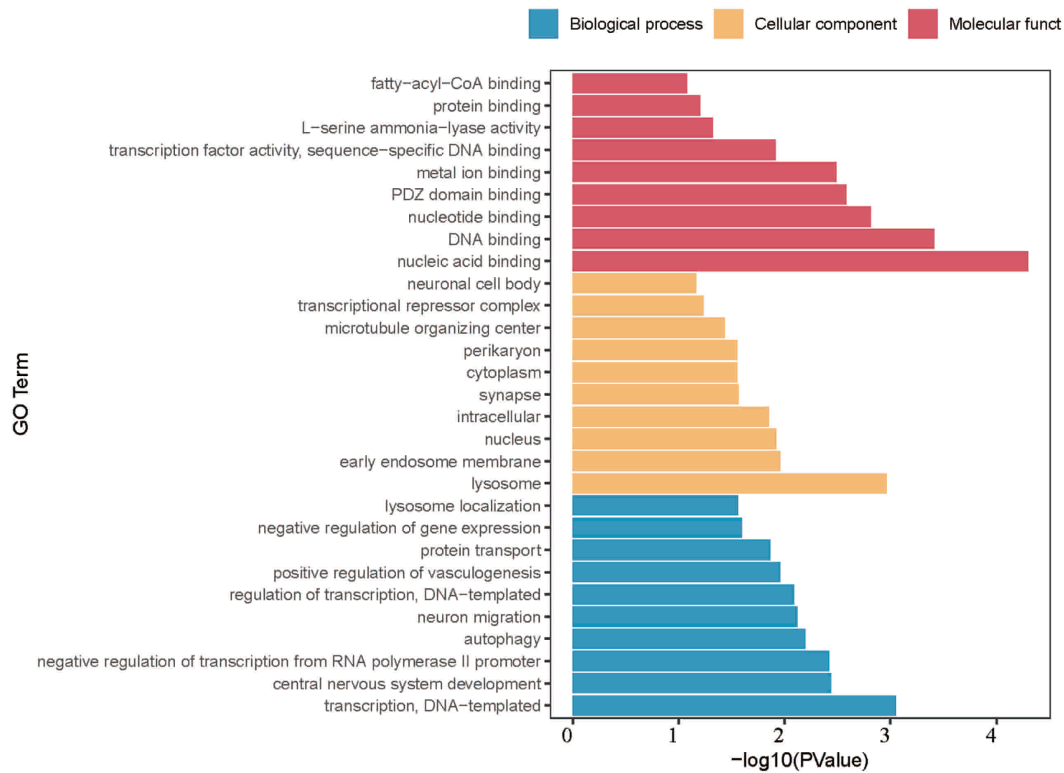
4. Discussion

The risk in developing cervical cancer is higher in developing countries [24], where there is an increase in preva-lence of HVP infection. In addition, the underlying molecular mechanisms of cervical carcinogenesis is still poorly understood, lncRNAs have been reported to be altered in many cases of cervical cancer [1, 25–27]. In the current study, we conducted a microarray analysis of differentially expressed lncRNAs and mRNAs and then performed the integrated bioinformatical analysis of these differentially expressed lncRNAs and mRNAs. In the present study, we identified a total of 2746 differentially expressed lncRNAs and mRNAs using the ceRNA microarray chips. Of these, 242 lncRNAs and 169 mRNAs were found to be significantly upregulated, and 1204 lncRNAs and 1131 mRNAs were downregulated in cervical cancer tissues compared with the control tissues. In addition, various dysregulated lncRNAs and mRNAs were randomly chosen for qRT-PCR validation, and the results confirm the microarray analysis findings to some extent.
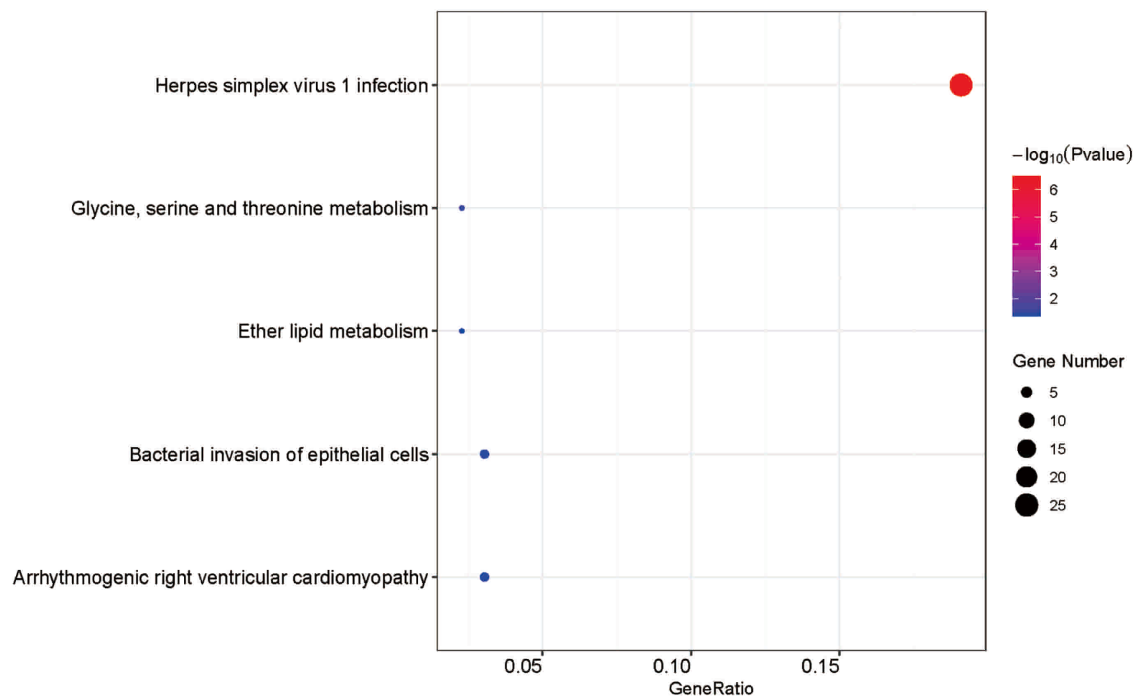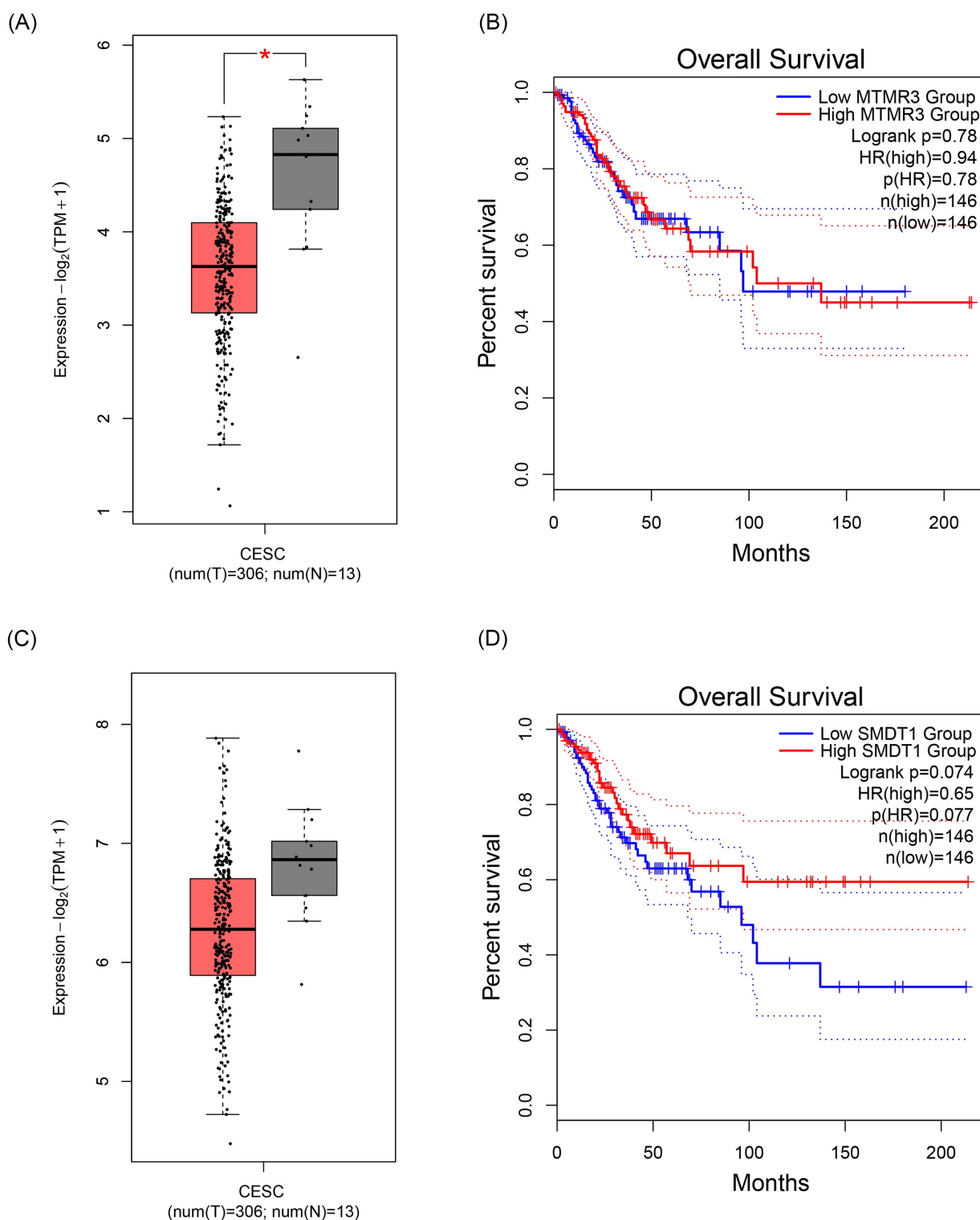
Fig. 4. Data on GO terms and KEGG analysis of genes in the differentially expressed lncRNA-mRNA co-expression network. (A) GO analysis genes in co-expression network. (B) KEGG analysis of genes in co-expression network.

Fig. 5. Different expressions of the hub genes in cervical cancer vs. normal tissues and association with overall survival. (A and C) Expression levels of MTMR3 or SMDT1. (B and D) Overall survival analysis using Kaplan-Meier curves and the log-rank test.

To predict the potential functions of the differentially expressed lncRNAs identified in this study, GO and KEGG pathway analyses were performed using the coding genes associated with the significantly differentially expressed lncRNAs. Our GO term and KEGG pathway analyses revealed that different biological events and signaling pathways may
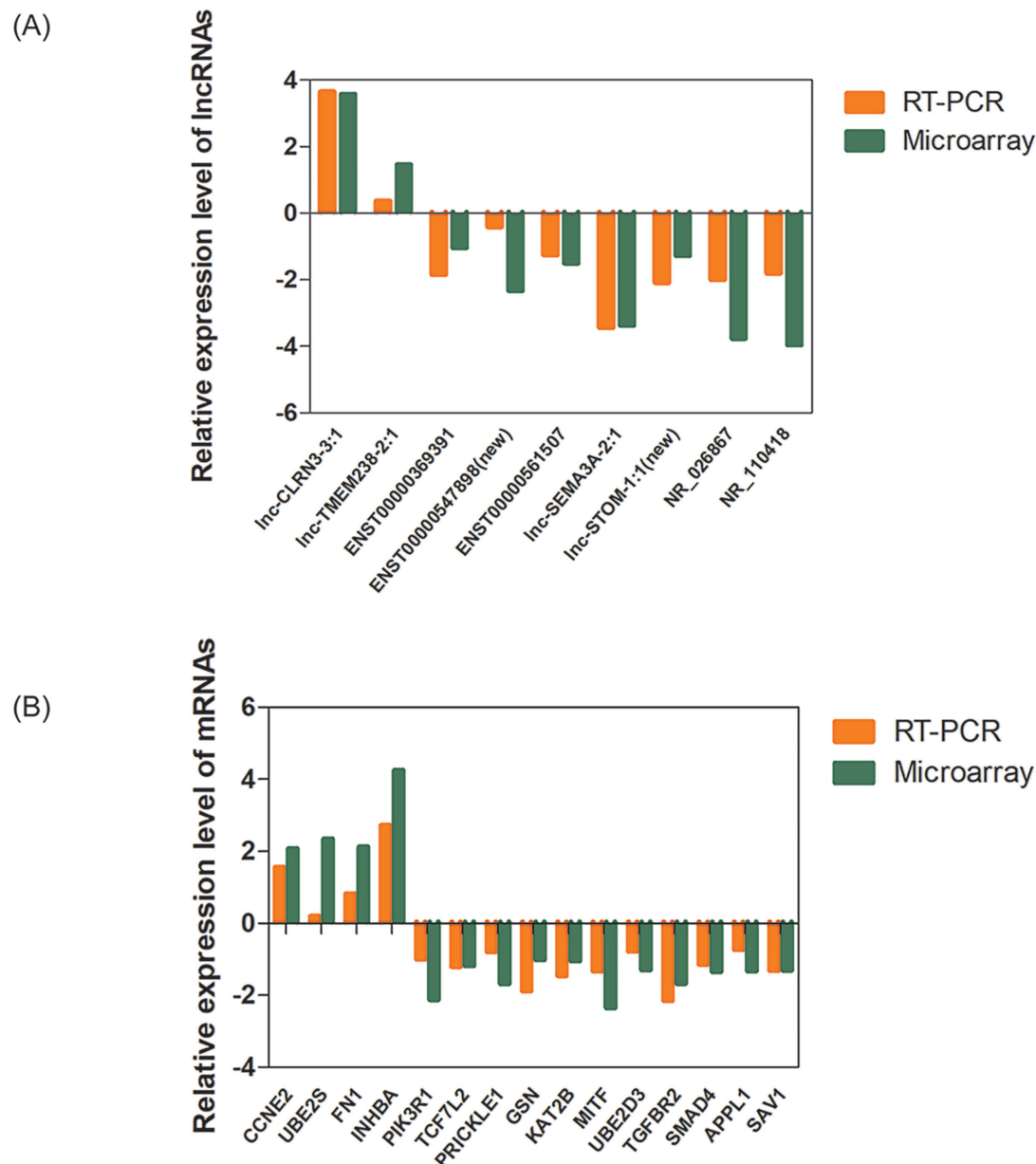
Fig. 6. RT-qPCR validation of microarray data in cervical cancer vs. normal control tissue specimens. (A) Relative expression levels of lncRNAs in cervical cancer tissues. (B) Relative expression levels of mRNAs in cervical cancer tissues.

be associated with cervical carcinogenesis. The GO term analyses divided these differentially expressed mRNAs in cervical cancer tissue specimens into functional modules of cell process, like protein ubiquitination, cell migration, cell proliferation, and immunity. Our KEGG pathway analysis revealed that these differentially expressed mRNAs are involved in ubiquitin-mediated proteolysis and diverse cancer pathways. Protein ubiquitination was also reported to regulate the functions and signaling of a profusion of proteins in various cell pathways [28, 29], while the chains of K11-linked polyubiquitination participate in cell mitosis [30, 31]. Furthermore, our pathway analysis of these differentially

expressed lncRNAs revealed their association with CAMs, TGF-β, notch, p53, and various other cancer signaling pathways. As previously reported, the TGF-β and notch signaling pathways demonstrated to play an essential role in cervical cancer development [2].

Furthermore, we also employed a lncRNA-mRNA network analysis to identify interactions between differentially expressed mRNAs and differentially expressed lncRNAs, as previously described [32, 33]. Our results showed that a total of 328 lncRNAs and 315 mRNAs were included in the co-expression network, which consisted of 653 network nodes and 400 connections. We also found that SMDT1,

an mRNA, was correlated with up to 6 lncRNAs; MTMR3 was correlated with 4 lncRNAs; and ZNF148, SH3GL2, ELAC2 and PHIP were associated with 3 lncRNAs. Particularly, as a member of the myotubularin family, MTMR3 has demonstrated to participate in tumor development in oral [34], gastric [35], and breast cancers [36]. According to the functional analysis, the integrated mRNA-lncRNA co-expression network was linked to autophagy, regulation of vascular genesis, transcriptional repressor complex, and regulation of gene expression. Based on previous studies, vascular genesis and angiogenesis are under the tight regulation of growth factors, like transforming growth factor-β1 (TGF-β1) [37]. Moreover, it has been reported that TGF-β-related signaling promotes tumor vasculature by enhancing the pericyte-endothelium association [38]. As for autophagy, recent studies have proposed that p53 is able to induce autophagy in both transcription-dependent and non-transcription-dependent manners and may also inhibit autophagy [39]. These results are consistent with former functional analysis of DE-lncRNAs and mRNAs in the study.

Overall, the presented study confirms the usefulness of the cDNA microarray to identify the differentially expressed genes in human cancer. Further research is warranted to verify and evaluate these genes as biomarkers for the early detection and prognosis of cervical cancer.

Despite our promising findings, this study is preliminary, and deeper research is needed to confirm the importance of these differentially expressed mRNAs and lncRNAs in cervical cancer development. Therefore, the limitations of this study include the small sample size and limited data for analysis. In conclusion, this study effectively profiled differentially expressed mRNAs and lncRNAs in cancerous tissues and confirmed their association with the development of cervical cancer. Future study of some of these differentially expressed mRNAs and lncRNAs using a larger sized cervical cancer tissue sample is highly recommended.

Author contributions

TJ contributed to the conception of the study, acquisition of the financial support, oversight, and leadership responsibility for the research activity planning and execution. LYY conducted the research, performed the data analyses, and wrote the manuscript. ZJF contributed significantly to the provision of the study's patients and laboratory materials. PJQ helped perform the analysis with constructive discussions.

Ethics approval and consent to participate

Patients have given their written informed consent before enrolled into our study. The ethics committee of the Obstetrics and Gynecology Hospital of Fudan University approved our study protocol with a reference number of 2018-11.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://ceog.imrpress.com/EN/10.31083/j.ceog4806219.

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