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Differential expression profiles and functional prediction of circular RNAs in lung cancer patients with chronic obstructive pulmonary disease: a pilot study

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Abstract. Chronic obstructive pulmonary disease (COPD), characterized by clinical sub-phenotypes such as emphysema (E) and chronic bronchitis (CB), is associated with a greater risk of lung cancer (LC). This study aimed to assess the expression patterns of circRNA and their potential functional involvement in LC patients with COPD. A circRNA microarray was used to characterize differentially expressed circRNAs (DEcircRNAs) profiles. A total of 176, 240, 163, and 243 DEcircRNAs were identified in comparisons between CB *vs.* LC patients (Con), E *vs.* Con, E *vs.* CB, and CBE *vs.* Con, respectively. DEcircRNAs in all comparison groups were primarily associated with immune-related GO terms and were also enriched in immune and inflammatory pathways. In total, 49 DEcircRNAs were significantly correlated with the infiltration of multiple immune cells. Among them, hsa-MROH9_0001 and hsa-RP11-35J10_0013 were positively and negatively correlated with plasma cells and T-cell CD4 memory resting cells, respectively; these two DEcircRNA-sponged miRNAs have good diagnostic performance. WGCNA identified six key circRNAs associated with CB progression. The expression patterns of hsa-MROH9_0001 and circRNA_21729 in E and CB groups were confirmed by RT-qPCR. In conclusion, we reported circRNA profiles and the findings demonstrated that hsa-MROH9_0001 and circRNA_21729 may be potential therapeutic targets for LC with COPD.

Key words: Chronic obstructive pulmonary disease — circRNA profile — Emphysema — Chronic bronchitis — Immune regulation

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Introduction

Lung cancer (LC) is a leading cause of cancer-associated mortality worldwide (Bray et al. 2018). Approximately more than one million people die from LC annually. Despite remarkable progress in surgery, radiotherapy, chemotherapy, and targeted therapy in recent years, the 5-year survival rate for late-stage LC is less than 15% (Vyse and Huang 2019). Chronic obstructive pulmonary disease (COPD) is a typical chronic inflammatory pulmonary disease that causes airflow obstruction along with abnormal inflammatory responses (bronchiolitis) and alveolar destruction (emphysema) (Parris et al. 2019). In 2016, 251 million cases of COPD were reported globally; approximately 95 million cases occur annually (Vos et al. 2017). Smoking cigarettes is a major risk factor for LC and COPD (Barreiro et al. 2016; Parris et al. 2019). Furthermore, the interaction between environmental risk factors and individual-related factors may facilitate the progress of LC and COPD. Approximately one-third patients with LC suffer from other lung diseases (i.e., COPD and emphysema), and exhibit much worse pulmonary function (Vogelmeier et al. 2017). Recently, the incidence of COPD has increased the risk of LC, and COPD has even been recognized as an independent risk factor (Ruano-Ravina et al. 2016). In addition, patients with LC who have undergone surgery are more susceptible to suffering from pulmonary complications, such as COPD (Licker et al. 2006). Genome-wide association studies on links between COPD and LC revealed that noncoding RNAs may be involved in the pathogenesis (Booton and Lindsay 2014; Mestdagh et al. 2015). Therefore, understanding the genetic background of LC complicated with COPD is critical for the development of possible therapeutic targets.

Circular RNAs (circRNAs) are a type of endogenous noncoding RNAs with a closed, continuous loop formed by back splicing with covalently joined linking the 3′ and 5′ ends (Chen 2016). Research has addressed the critical role of circRNAs in LC and COPD (Vo et al. 2019; Zeng et al. 2019; Zhou et al. 2019). For example, circ_0078767 is down-regulated in the tumor tissue of non-small cell carcinoma (NSCLC) and promotes RASSF1A expression *via* sponging of miR-330-3p, ultimately leading to the inhibition of NSCLC cell proliferation (Chen et al. 2019). Hsa_circ_0016070 is proved to be related to vascular remodeling in pulmonary arterial hypertension by promoting the proliferation of pulmonary artery smooth muscle cells (Zhou et al. 2019). CircARHGAP10 impedes NSCLC progression by facilitating GLUT1 expression as a miR-150-5p sponge (Jin et al. 2019). The expression profile of circRNAs is regulated by cigarette smoke extract in primary human small airway epithelial cells in COPD (Zeng et al. 2019). These studies have shown that circRNAs are an important participant in the pathological progression of LC and COPD. However, no studies have revealed the circRNA expression profile and the potential function and mechanism of circRNAs in LC complicated with COPD. Although high-throughput sequencing currently has played a leading role in the exploration of potential noncoding RNAs, only approximately 0.1% covered the reverse splicing site in RNA-sequencing results; thus, the efficiency of RNA sequencing to detect circRNA was low (Szabo and Salzman 2016). However, the small number of circRNA detected will limit further research on circRNA in diseases or physiological conditions. A study found that single-sample circRNA microarrays detected at least 2.5 times more circRNAs than transcriptome sequencing, suggesting that circRNA microarrays are faster, more abundant, and more sensitive and provide equally reliable results (Li et al. 2019). Therefore, microarrays are an efficient tool for circRNA profiling.

In the present study, the expression profiles of circRNAs in patients with LC complicated with different COPD subphenotypes, including emphysema and chronic bronchitis, were studied using a circRNA microarray. Candidate differentially expressed circRNAs (DEcircRNAs) were validated by RT-qPCR. The potential functions and mechanisms of DEcircRNAs were analyzed *via* bioinformatics.

Materials and Methods

Study population and lung tissue samples

This study was approved by The Biomedical Ethics Committee [No. 2017 approve (270)] of West China Hospital, Sichuan University, in accordance with the Declaration of Helsinki (as revised in 2013). All patients provided written informed consent prior to enrollment in the study. From 2017 to 2019, 18 patients with LC were hospitalized in the West China Hospital, Sichuan University. However, during the statistical process control using principal component analysis (PCA), four samples were outside the 95% confidence interval. Thus, these four outlying samples were eliminated based on the PCA results. Ultimately, 14 samples were analyzed and divided into three groups: 6 patients with LC but without COPD (Con group), 4 patients with LC complicated with emphysema (E group), and 4 patients with LC complicated with chronic bronchitis (CB group). All patients with COPD, including E and CB subphenotypes, were diagnosed using the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (Singh et al. 2019). The LC was diagnosed based on tissue pathological examination findings, and the tissues were collected during diagnostic bronchoscopy or therapeutic surgery (Reck and Rabe 2017). The inclusion criteria were: Patients aged 35–65 years who did not

receive any treatment, including surgery, chemotherapy, or radiation, were included. The adjacent cancer lung tissue samples were collected strictly prior to starting the treatment. The clinical characteristics of the subjects are shown in Table 1.

RNA isolation and circRNA microarray analysis

Lung tissue samples collected from a region that was 5 cm beside the cancer were immediately frozen in liquid nitrogen until RNA extraction. For RNA isolation, lung tissue samples were processed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, followed by quality evaluation on a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Qualified RNA was subjected to circRNA microarray analysis using the Human circRNA Array V2.0 (8×15K, Aksomics, Shanghai, China) as described previously (Xia et al. 2018). In brief, approximately 1 μg of total RNA was digested using RNase R (RNR0725, Epicentre, USA), and all of these RNAs were then subjected to labeling and hybridization (Aksomics Inc.). Finally, all processed RNA samples were scanned and analyzed to obtain the fluorescence signal using GenePix 4000B (Molecular Devices Corporation, SV, USA).

Data analysis of the microarray assay

Raw data were processed and analyzed using R software (R version 3.6.1). Edge R was utilized to normalize the microarray raw data. DEcircRNAs were identified based on absolute fold change of >2 and $p < 0.05$ using the R package "limma." The heatmaps of DEcircRNAs were visualized using the R package heatmap. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the R package clusterprofiler (https://www.rdocumentation.org/packages/clusterProfiler) (Ma et al. 2022). The raw data have been deposited in the Genome Sequence Archive for Human online database (https://ngdc.cncb.ac.cn/gsa-human/) with reference to accession HRA002550.

Immune cell infiltration analysis

To understand the relationship between DEcircRNAs and immune cell infiltration, all mRNAs were processed in CIBERSORTx software (https://cibersortx.stanford.edu/) on the basis of the expression data of the microarray assay, and the profile of immune cell infiltration was obtained (Yuan et al. 2022). The Pearson correlation method was then used to analyze the correlation between immune cells and

Table 1. Baseline data of LC patients with COPD subphenotypes

Variables	Group		
	Con ($n = 6$)	E ($n = 4$)	CB ($n = 4$)
Age (years)	51.33 ± 7.99	57.00 ± 4.74	54.75 ± 9.80
Gender (male, %)	0 (0%)	4 (100%)	1 (25%)
BMI (kg/m^2)	22.09 ± 2.24	23.23 ± 1.48	22.29 ± 3.41
Smoking history (pack-years)		27.5 ± 14.79	
GOLD classification			
I	NA	NA	1
II	NA	3	2
III	NA	1	NA
FEV1%	2.67 ± 0.27	1.88 ± 0.56	1.96 ± 0.31
FEV1/FVC%	85.32 ± 4.49	57.46 ± 15.82*	60.84 ± 5.11#
Lung cancer histology			
Squamous cell carcinoma r	NA	3	NA
Adenocarcinoma	5	2	3
Other	1	2	1
Hypertension (%)	NA	25%	25%
Diabetes Mellitus (%)	NA	NA	NA

ANOVA followed by Tukey's test. * $p < 0.05$. *vs.* Con group; # $p < 0.05$. *vs.* E group. Except for one LC patient without COPD and one LC patient with chronic bronchitis, who had a smoking history of 0.1 and 0.25 pack-years, respectively, the other patients had no smoking history. LC, lung cancer; COPD, chronic obstructive pulmonary disease; Con group, patients with LC but without COPD; E group, patients with LC complicated with emphysema; CB group, patients with LC complicated with chronic bronchitis; NA, not available.

DEcircRNAs. The R package heatmap was applied to illustrate the immune cell infiltration levels within each sample (Chen et al. 2022). Also, the expression patterns of candidate DEcircRNAs in different tissues were downloaded from the software CircAtlas 2.0 (http://circatlas.biols.ac.cn/).

Network construction

To construct a putative circRNA-centric network based on the competing endogenous RNA (ceRNA) theory, circRNAs targeting miRNAs were predicted using Miranda and RNAhybrid. The miRNA shared in the two software programs were selected as candidates. The network map was visualized using Cytoscape_v3.6.1.

Weighted gene co-expression network analysis (WGCNA) to establish a co-expression network

To perform gene hierarchical clustering analysis, WGCNA was conducted using the WGCNA R package 73. The varFilter function in the R genefilter package was used to remove circRNAs with low expression in all samples and genes with stable constant expression in all samples to improve the accuracy of network construction.

RT-qPCR analysis

Total RNA from three groups (5 samples for the Con group and 4 for the E and CB groups) was extracted using TRI

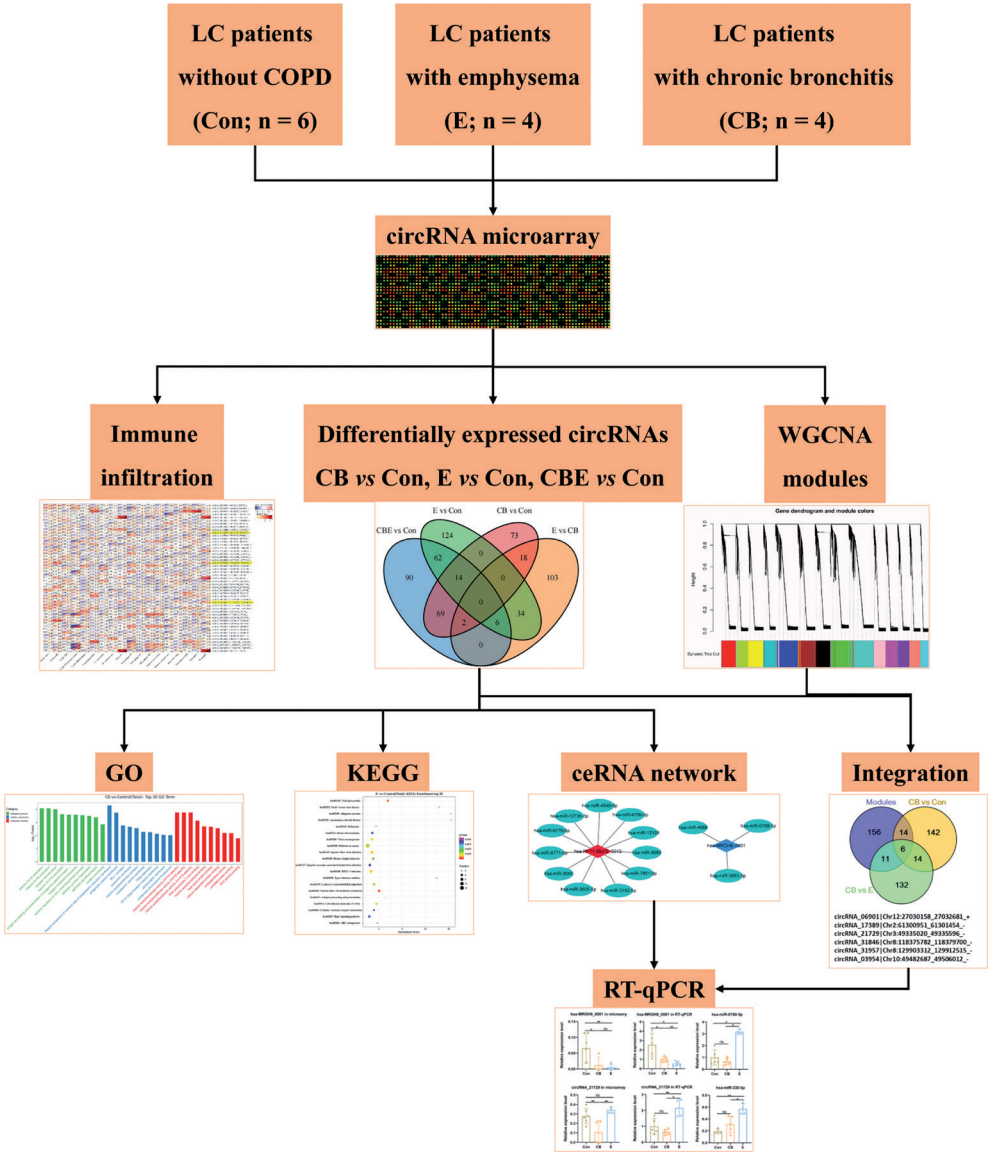

Figure 1. The analysis flowchart of this study. LC, lung cancer; COPD, chronic obstructive pulmonary disease.

Reagent (T9424, Sigma-Aldrich, MO, USA). RNA quality was assessed by agarose gel electrophoresis and NanoDrop® ND-1000. cDNA synthesis was performed on the Gene Amp PCR System 9700 (Applied Biosystems, MA, USA) using reverse-transcription primers. Finally, the 2X PCR master mix (Arraystar) was used to conduct PCR on the QuantStudio5 Real-time PCR System (Applied Biosystems). The expressions of circRNAs and miRNAs were normalized by β-actin and U6, respectively. The gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. The primers used in this study were synthesized by Sangon (Shanghai, China) and are shown in Table S1 in Supplementary material.

Statistical analysis

All statistical analyses of the data were performed using GraphPad Prism 9 (GraphPad Software, San Diego, USA). The comparison between the three groups was analyzed by the one-way ANOVA followed by Tukey's test, and $p < 0.05$ was defined as statistically significant.

Results

Subject demographics

The analysis flowchart is shown in Figure 1. Table 1 provides detailed characteristics of the participants. No differences in age and BMI were observed among LC patients without COPD (Con group), LC patients with emphysema (E group), and LC patients with chronic bronchitis (CB group) (Table 1). Lung function (FEV1% and FEV1/FVC%) of patients in the E group was significantly decreased compared with that of the Con group, and no significant difference was found between the CB and Con groups.

Different expression profiles of circRNAs in patients with LC and COPD subphenotypes

To explore the influence of COPD on the circRNA expression in LC patients, we initially combined CB and E into one (CBE group) and the differential expression profile with the control group was then analyzed. In total, 243 DEcircRNAs (115 upregulated and 128 downregulated circRNAs) with *p*-values <0.05 and |log2fold change| >1 between patients with LC complicated with COPD and patients with LC alone (CBE *vs*. Con) were identified (Fig. 2A,F; Table S2 in Supplementary material). To obtain more information, the DEcircRNAs in the comparisons of CB *vs*. Con and E *vs*. Con were analyzed separately, and 176 (96 upregulated and 80 downregulated) and 240 (130 upregulated and 110 downregulated) DEcircRNAs were discovered, respectively (Fig. 2B,C,F). According to the hierarchical clustering map, a high degree of heterogeneity existed between LC patients with COPD and Con, and similar spectral clustering was observed within the groups. Moreover, the heatmap revealed large expression differences between E and CB subtypes, and 163 DEcircRNAs were identified, including 95 downregulated and 68 upregulated DEcircRNAs, in the comparison between the CB and E groups (Fig. 2D,F). These 163-DEcircRNAs may be the key molecules that distinguished the disease process between the E and CB subtypes. Of note, 14 DEcircRNAs were shared by the CB *vs*. Con, E *vs*. Con, and CBE *vs*. Con groups, as well as by CB *vs*. Con and E *vs*. Con groups (Fig. 2E), suggesting that these 14 DEcircRNAs were simultaneously involved in the pathological process of the E and CB subtypes.

Functional prediction analysis of DEcircRNAs in LC patients with COPD subphenotypes

To predict the potential mechanisms of DEcircRNAs in LC patients with COPD, GO and KEGG analyses were performed. The top 30 enriched GO terms in CB *vs*. Con were shown in Figure 3A with immune and inflammatory associated terms, such as "antigen processing and presentation of exogenous peptide antigen *via* MHC class" in the biological process and "phagocytic vesicle membrane" and "lysosomal membrane" in the cellular component. Furthermore, Figure 3B presents the DEcircRNAs in the top 30 GO terms in E *vs*. Con, which are associated with immune and inflammatory terms, including "interferon-gamma-mediated signaling pathway," "inflammatory response," and "type I interferon signaling pathway" in the biological process, "phagocytic vesicle membrane" in the cellular component, and "peptide antigen binding" in the molecular function.

The KEGG pathway assessment elicited 20 remarkably enriched pathways containing immune and inflammatory pathways, for instance, "Jak–STAT signaling pathway" and "MAPK signaling pathway", "Endocytosis", "Phagosome" and "Lysosome" in CB *vs*. Con (Fig. 4A); "Antigen processing and presentation", "Rap1 signaling pathway", "Cytokine-cytokine receptor interaction", and "Natural killer cell mediated cytotoxicity" in E *vs*. Con (Fig. 4B). Additionally, the "Cell adhesion molecules (CAMs)" existed in CB *vs*. Con and E *vs*. Con comparison groups and were statistically significant. These results suggest that DEcircRNAs in LC patients may participate in COPD progression by regulating tumor immunity.

Moreover, the predictive functions of DEcircRNAs between the E and CB groups were analyzed, and the results revealed that these DEcircRNAs also were enriched in molecular functions and pathways related to immune regulation, including those mentioned above, "antigen processing and presentation of exogenous peptide antigen *via* MHC class" in the biological process, "phagocytic vesicle membrane" and "lysosomal membrane" in the cellular component, and

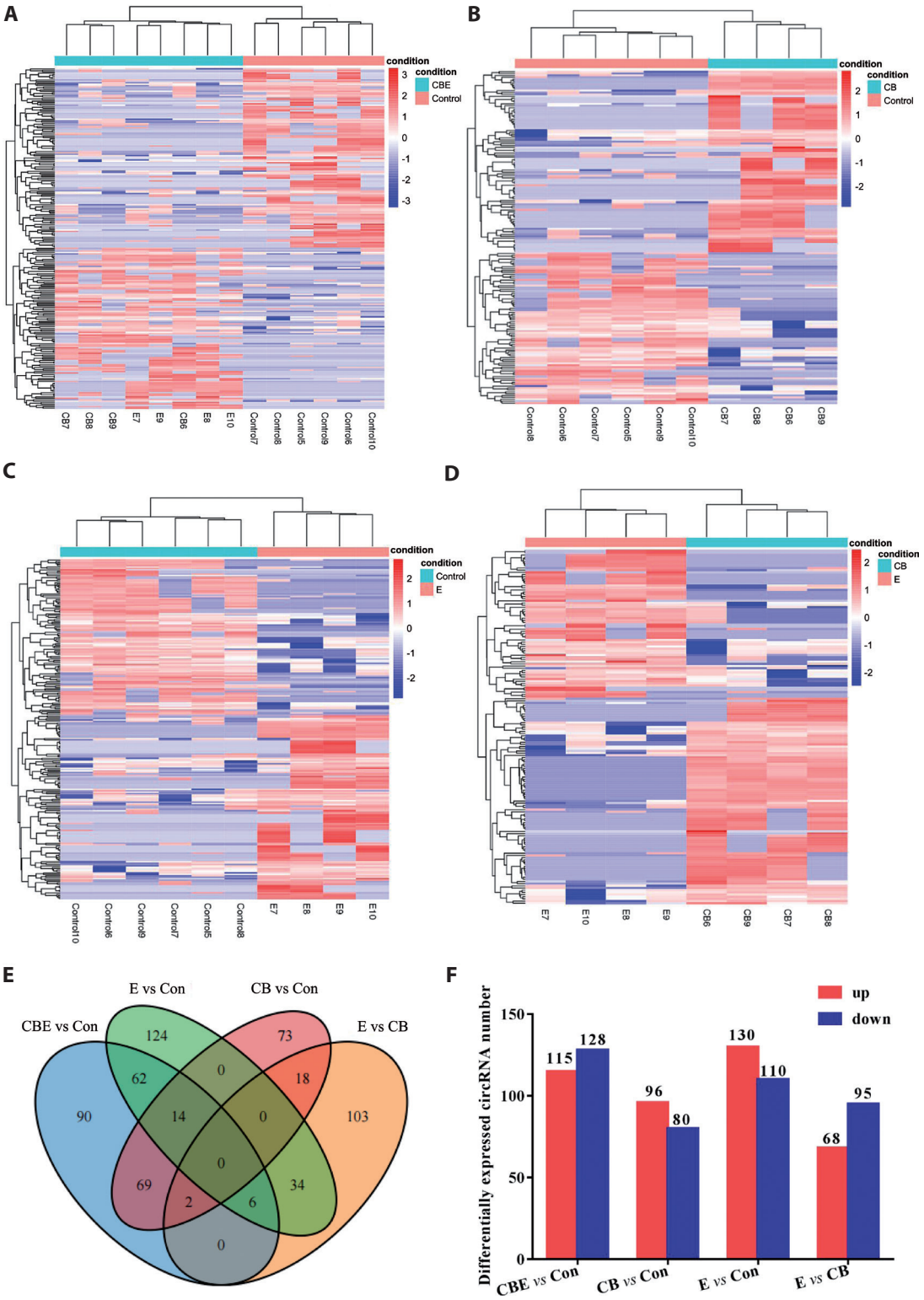

Figure 2. Differential expression of circRNAs in LC complicated with COPD. Cluster heatmaps of DEcircRNAs in LC patients complicated with or without COPD: CBE *vs*. Con (**A**); CB *vs*. Con (**B**); E *vs*. Con (**C**); and E *vs*. CB (**D**). Red and blue indicate high and low expression levels, respectively. **E.** Venn plots of common and specific DEcircRNAs between the comparison groups. **F.** Numbers of DEcircRNAs of lung paracancer tissues in different comparison groups. E and CB are two COPD subtypes. CBE indicates patients with LC complicated with COPD. For color figure see online version of the article.

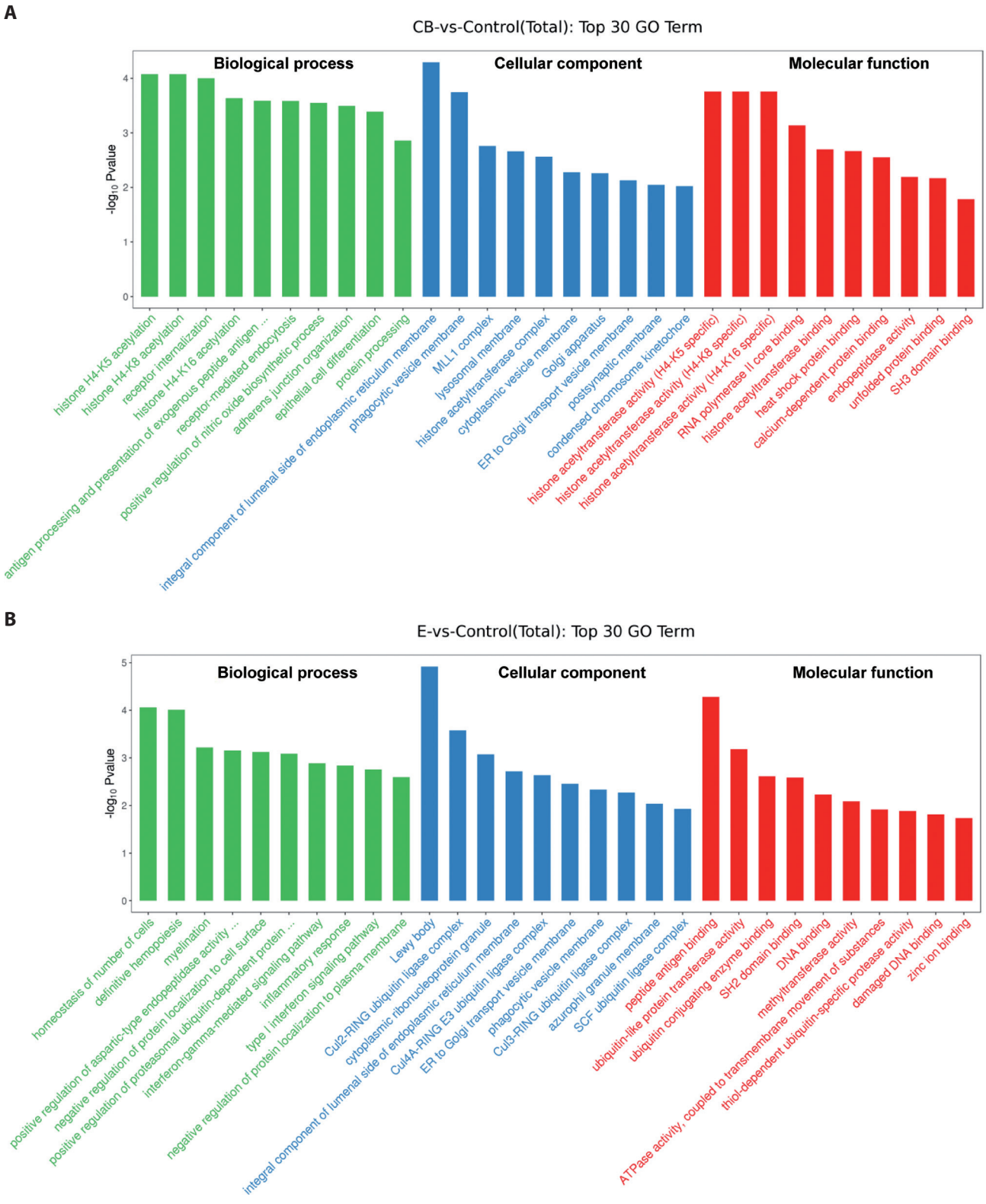

Figure 3. Gene Ontology (GO) analyses of DEcircRNAs in CB *vs*. Con and E *vs*. Con groups. Top 30 enriched GO terms in biological process, cellular component, and molecular function for DEcircRNAs in comparisons between CB *vs*. Con (**A**) and E *vs*. Con (**B**) group. The x- and y-axes show the enriched GO terms and the –log10 (*p*-value), respectively.

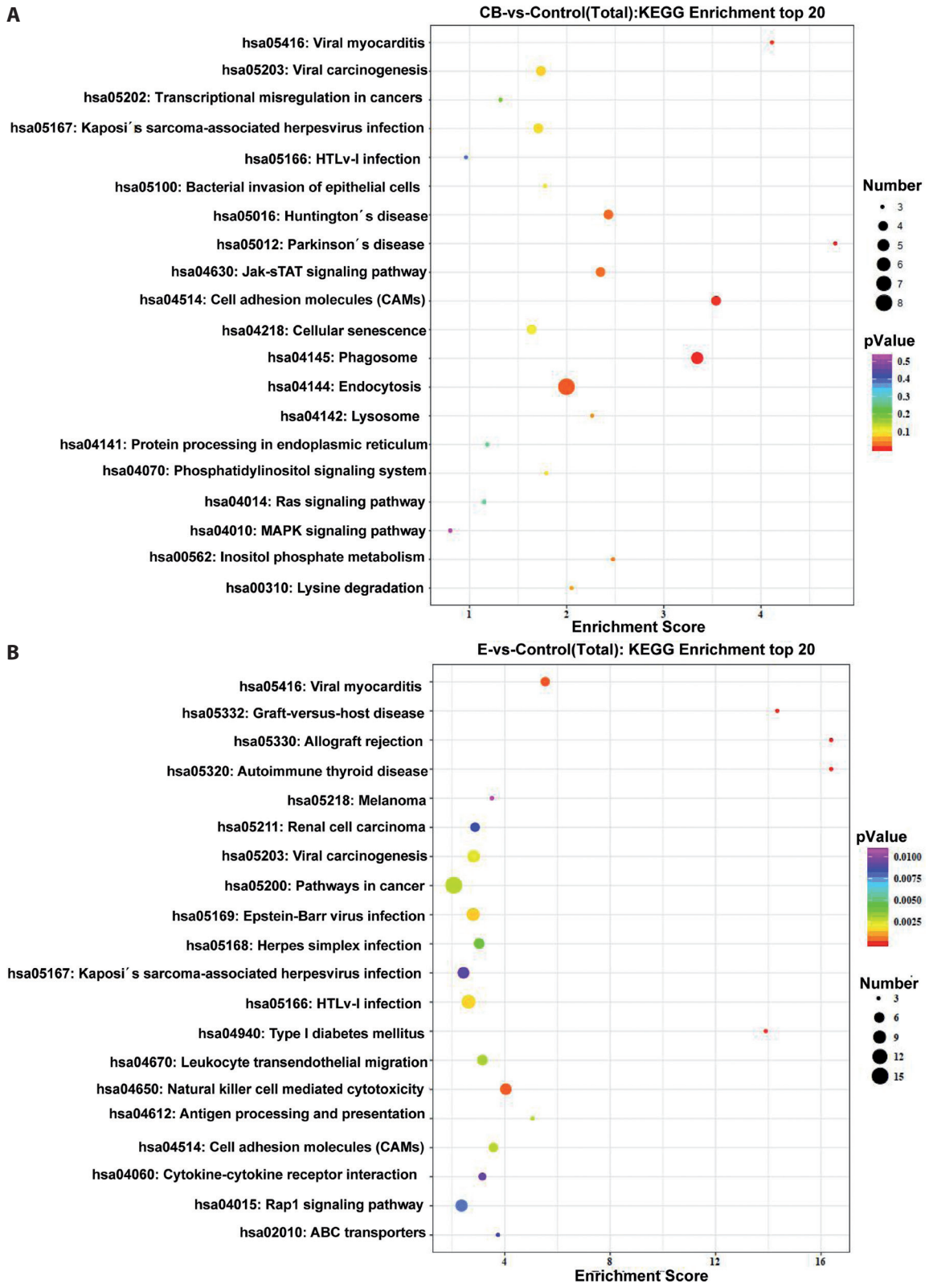

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DEcircRNAs. Top 20 KEGG pathways for DEcircRNAs in comparisons between CB *vs*. Con (**A**) and E *vs*. Con (**B**) groups. The y- and x-axes refer to the KEGG pathway terms and enrichment score, respectively. Node color: *p*-value. Node size: number of genes in the KEGG pathway terms. For color figure see online version of the article.

pathways such as "Natural killer cell mediated cytotoxicity", "Phagosome", "Endocytosis", and "Rap1 signaling pathway" (Fig. 5). These results suggest that DEcircRNAs may mediate the differential differentiation of E and CB subtypes by triggering different immune regulatory pathways.

Immunoinfiltration analysis of DEcircRNAs

The abovementioned findings from the GO and KEGG analyses suggested the possible involvement of DEcircRNAs in immune regulation. Therefore, we used the CIBRESORT database to analyze the association between DEcircRNAs and immune cell infiltration. As shown in Figure 6, 49 DEcircRNAs were correlated to the infiltration of multiple immune cells. For example, circRNA_06682|Chr12:9091201_9164259_- demonstrated a significant positive correlation with neutrophils or monocytes and a negative correlation with macrophages M2. Among the 14 DEcircRNAs shared by the CB *vs*. Con, E *vs*. Con, and CBE *vs*. Con groups, only 3 DEcircRNAs were predicted to be associated with immune cell infiltration, namely, circRNA_01223|Chr1:66871370_66905375_-, that was negatively correlated with macrophages M2; circRNA_02255|Chr1:171024395_171025420_+, that was positively correlated with plasma cells; and circRNA_04710|Chr10:102876954_102890678_+ that was positively correlated with neutrophils and plasma cells. Additionally, T-cell CD4 memory resting cells were significantly negatively correlated with multiple DEcircRNAs, including circRNA_06493|Chr11:128480191_128490576_-, circRNA_06451|Chr11:121045674_121053732_+, circRNA_05712|Chr11:68185781_68190152_-, circRNA_05094|Chr11:7796834_7799295_-, circRNA_03236|Chr1:246726892_246740301_+, and circRNA_01459|Chr1:90937485_90982370_-. On the contrary, neutrophils significantly positively correlated with multiple DEcircRNAs, including circRNA_06682|Chr12:9091201_9164259_-, circRNA_03365|Chr10:7637228_7655675_-, and circRNA_00574|Chr1:32631827_32634110_-. These results suggest that these DEcircRNAs may be the potential key molecules that mediate tumor immune infiltration of LC and participate in the progression of COPD.

CircRNAs-miRNAs-mRNAs interaction network analysis

To further explore candidate circRNAs involved in COPD progression, the expression profiles of the abovementioned immune infiltration-related DEcircRNAs in different tissues were analyzed using the CircAtlas 2.0 database (http://circatlas.biols.ac.cn), which revealed that only circRNA_02255|Chr1:171024395_171025420_+ (circAltas ID: hsa-MROH9_0001) and circRNA_05094|Chr11:7796834_7799295_- (circAltas ID: hsa-RP11-35J10_0013) demonstrated specific expression in lung tissue (Fig. 7A). According to the CircAtlas 2.0 database, hsa-RP11-35J10_0013 is almost exclusively present in the lung and is not expressed in other tissues. circRNAs can sponge with miRNAs to exert their regulatory effects; therefore, the possible interactions of hsa-MROH9_0001 and hsa-RP11-35J10_0013 were assessed. As shown in Figure 7B, hsa-MROH9_0001 and hsa-RP11-35J10_0013 were predicted to bind to the miRNA response elements (MREs) on 3 miRNAs and 11 miRNAs, respectively. The small number of predicted miRNAs may be due to the limited capacity of the database, or it may suggest that they are not dependent on the role of miRNA but participate in the regulation of immune infiltration and COPD progression through an unknown mechanism.

Furthermore, to explore whether miRNAs in the network are expressed differently in different patients, the expression profiles of these miRNAs in lung squamous cell carcinoma (LUSC) were evaluated using the CancerMIRNome database (http://bioinfo.jialab-ucr.org/CancerMIRNome/), which is the most comprehensive tumor miRNA expression profile database (Li et al. 2022). The results revealed that the expression levels of hsa-miR-3663-3p, hsa-miR-4640-5p, and hsa-miR-6789-5p were significantly lower in LUSC tumor tissues than in the normal group, whereas the opposite was observed for hsa-miR-4709-3p (Fig. 8). The expression of the remaining miRNAs in the network was not different between the two groups. Interestingly, the receiver operator characteristic (ROC) curve showed that the areas under the curve (AUC) for all four miRNAs were >0.5, and the AUC of hsa-miR-4709-3p was as high as 0.94 (Fig. 8), suggesting that it has good diagnostic performance. As molecular sponges of these four miRNAs, hsa-MROH9_0001 and hsa-RP11-35J10_0013 have the potential to regulate tumor progression.

WGCNA identification of potential key modules

To establish a circRNA co-expression network, all circRNAs identified by microarray were subjected to WGCNA, and a total of 31 modules were identified based on the average linkage hierarchical clustering (Fig. 9A). Module–trait relationships showed that darkmagenta (cor = 0.66, p = 0.01), darkgrey (cor = 0.65, p = 0.01), and skyblue3 (cor = 0.62, p = 0.02) modules were positively correlated with the CB group (Fig. 9B). Darkmagenta, darkgrey, and skyblue3 modules contained 55, 84, and 48 circRNAs, respectively. Moreover, pink (cor = 0.44, p = 0.1), magenta (cor = 0.44, p = 0.1), and black (cor = 0.44, p = 0.1) modules were closely related to the E group (Fig. 9B) but not significantly. Subjectively, we intersected the DEcircRNAs and circRNAs in modules (darkmagenta, darkgrey, and skyblue3) to try to narrow the scope of circRNAs related to CB progression. As a result, six shared circRNAs were obtained, including circRNA_06901|Chr12:27030158_27032681_+,

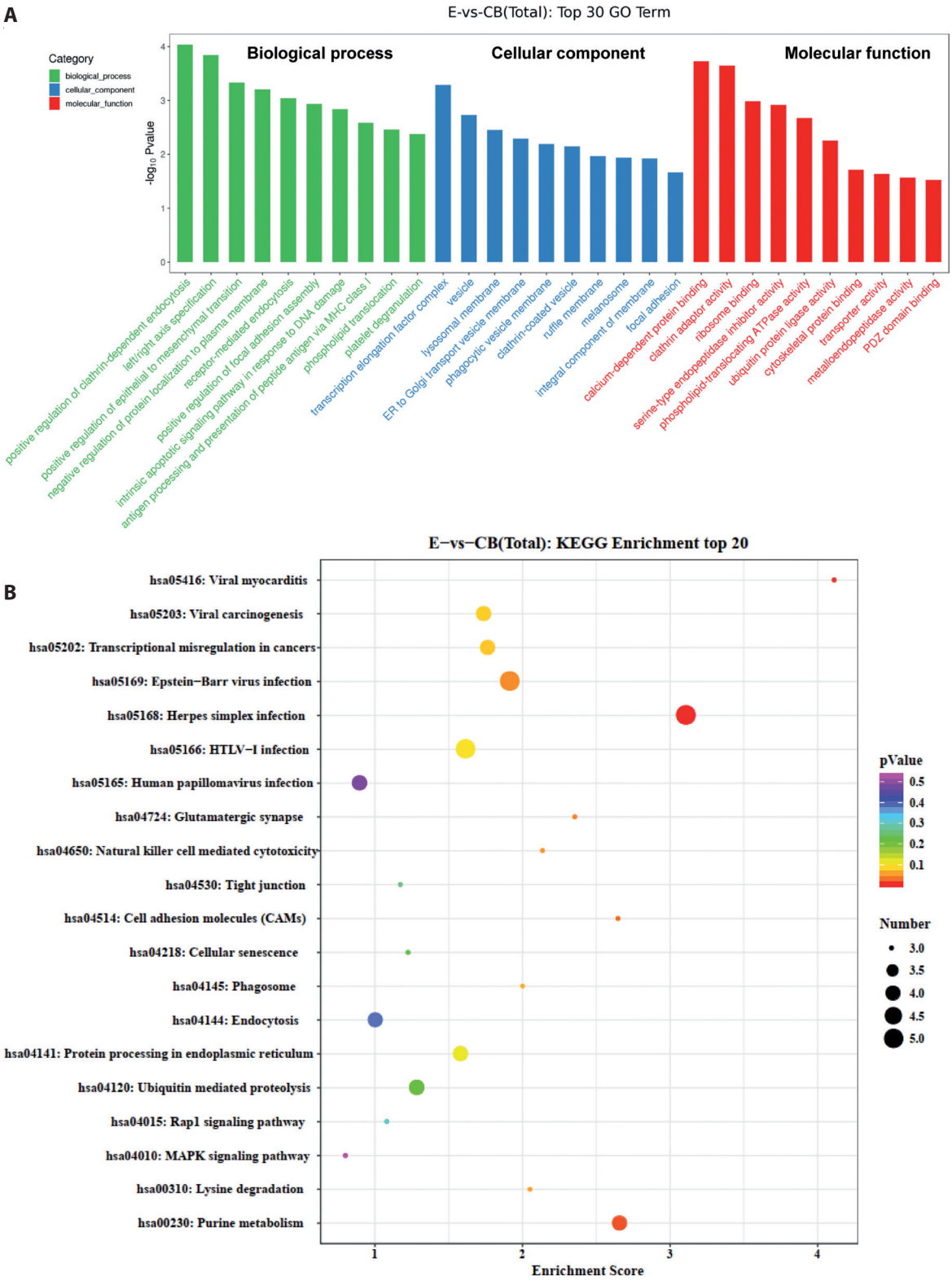

Figure 5. Functional analyses of DEcircRNAs between the E and CB groups. **A.** Top 30 enriched GO terms for DEcircRNAs between the E and CB groups. The x- and y-axes show the enriched GO terms and the –log10 (*p*-value), respectively. **B.** Top 20 KEGG pathways for DEcircRNAs. The y- and x-vertical axes refer to the KEGG pathway terms and enrichment score, respectively. Node color: *p*-value. Node size: number of genes in the KEGG pathway terms. For color figure see online version of the article.

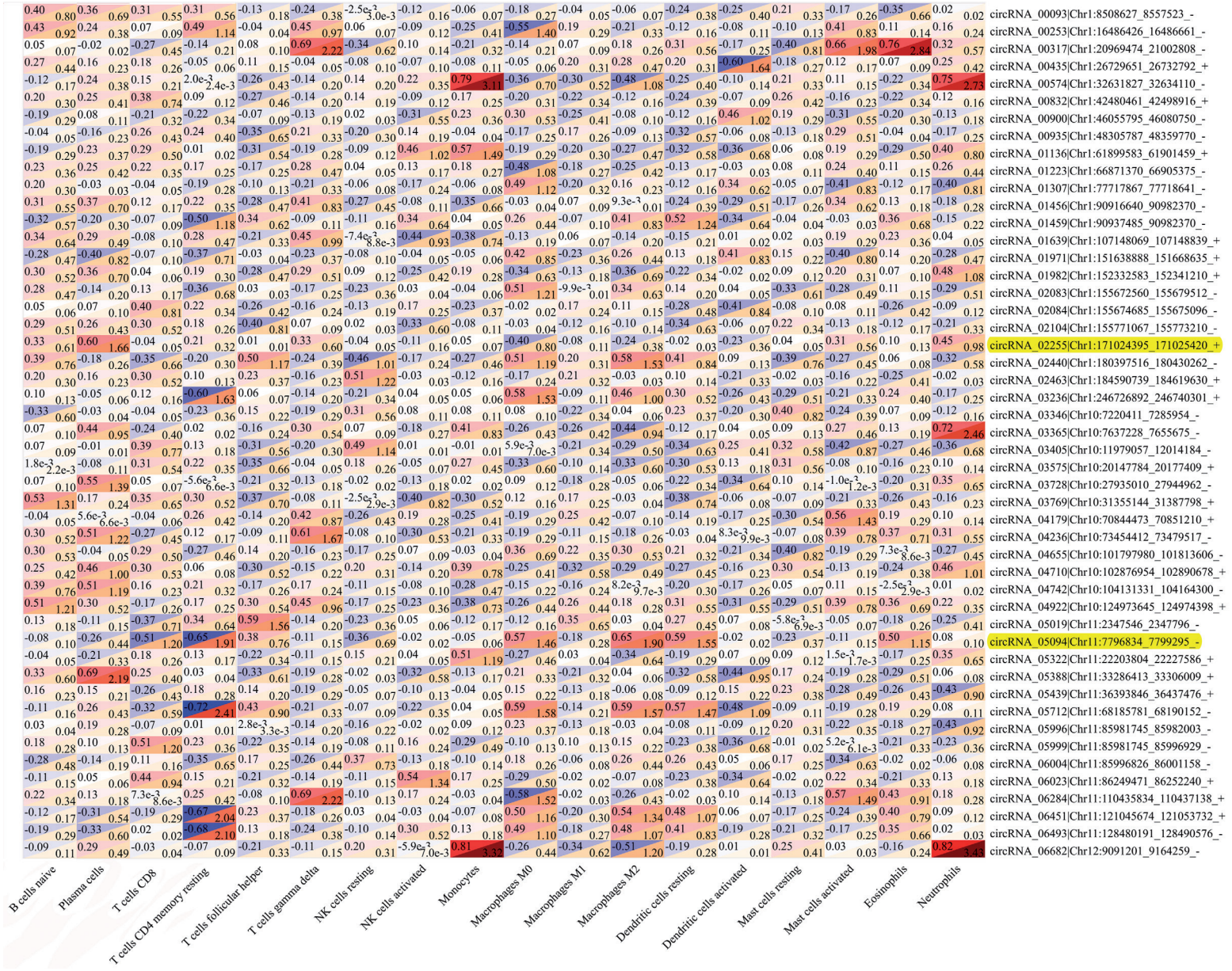

Figure 6. Correlation between immune cell infiltration and DEcircRNAs. DEcircRNAs are represented horizontally, whereas immune cell types are represented vertically. Each square is split in half, with the upper left triangle representing the correlation coefficient and the lower right corner representing log10 (*p*-values). For color figure see online version of the article.

circRNA_17389|Chr2:61300951_61301454_-, circRNA_21729|Chr3:49335020_49335596_-, circRNA_31846|Chr8:118375782_118379700_-, circRNA_31957|Chr8:129903312_129912515_-, and circRNA_03954|Chr10:49482687_49506012_- (Fig. 9C). Therefore, these six circRNAs may be related to CB pathology.

RT-qPCR validation

To further validate the circRNAs identified in clinical samples by microarray analysis, two each from the aforementioned circRNAs and miRNAs were selected for RT-qPCR validation, and the results are shown in Figure 10. The expression patterns of both hsa-MROH9_0001 and circRNA_21729|Chr3:49335020_49335596_- in RT-qPCR were partially consistent with the microarray results, with the difference being that circRNA_21279 expression was not statistically different between the Con and CB groups. Therefore, the RT-qPCR results support the reliability of the microarray analysis results. Furthermore, the expression pattern of hsa-miR-6789-5p (sponged by hsa-MROH9_0001; Fig. 7B) was completely opposite to that of hsa-MROH9_0001, suggesting the possible involvement of hsa-MROH9_0001 in disease progression through hsa-miR-6789-5p adsorption. However, the expression of hsa-miR-330-5p (sponged by circRNA_21279 in the database) appears to be similar to that of circRNA_21279 among the three groups, indicating that circRNA_21279 cannot regulate hsa-miR-330-5p involvement in disease progression through the ceRNA mechanism (refers to a mechanism of interaction between RNAs wherein noncoding RNAs competitively bind miRNAs to regulate the expression of the target genes).

Discussion

COPD and LC are associated diseases, and the presence of COPD raises the incidence and risk of death from LC

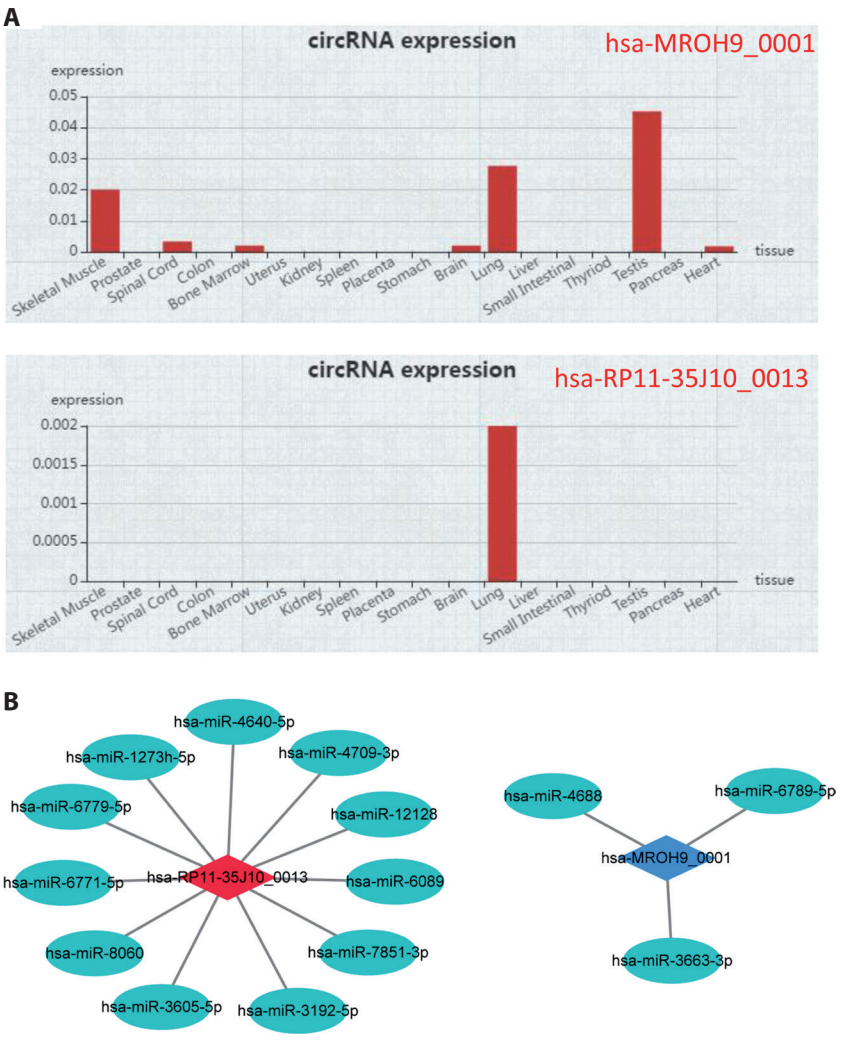

Figure 7. The expression and network of two candidate DEcircRNAs. **A.** Relative expression levels of hsa-MROH9_0001 and hsa-RP11-35J10_0013 in different tissues. **B.** Network of circRNA–miRNA of hsa-MROH9_0001 and hsa-RP11-35J10_0013. The red diamond indicates that hsa-RP11-35J10_0013 is upregulated in the COPD group, and the blue diamond indicates that hsa-MROH9_0001 is downregulated in the COPD group compared with the control group. The ellipses represent miRNAs. For color figure see online version of the article.

(Skillrud et al. 1986; Wilson et al. 2008). CircRNAs are endogenous molecules that are abnormally expressed in numerous human diseases, including LC and COPD. (Nicot 2019; Mei et al. 2020). However, the expression profiles and regulatory network of circRNAs in LC complicated with COPD remain unknown. In the present study, we have revealed for the first time that the circRNAs expression patterns are different in LC complicated by various COPD

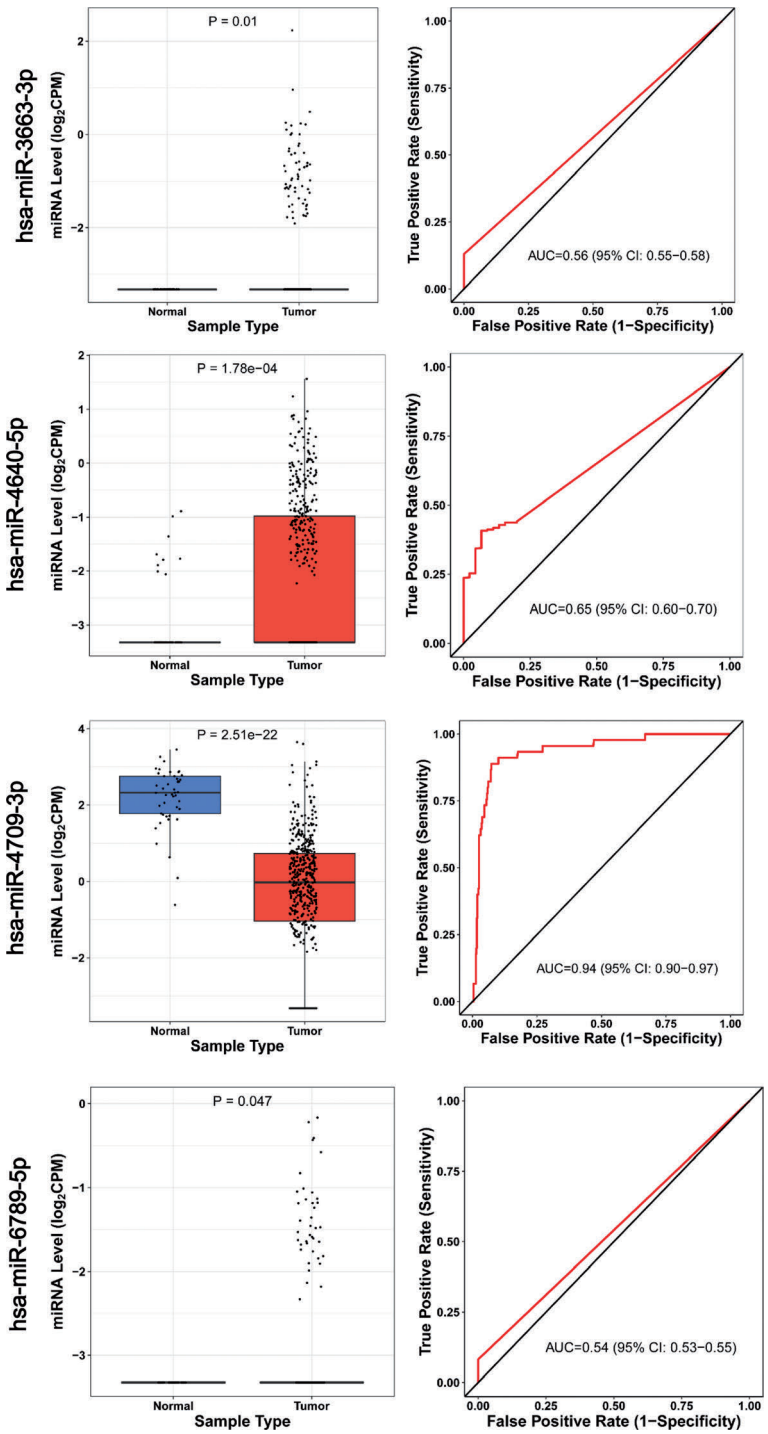

Figure 8. Expression of miRNAs in the network and ROC analysis in LUSC using the CancerMIRNome database. Hsa-miR-3663-3p, hsa-miR-4640-5p, and hsa-miR-4709-3p were sponged by hsa-RP11-35J10_0013, and hsa-miR-6789-5p was sponged by hsa-MROH9_0001.

subphenotypes, such as E and CB. Specifically, GO and KEGG analyses have suggested an intrinsic link between DEcircRNAs and immune regulation. Further correlation analysis confirmed a significant correlation between large amounts of DEcircRNAs and different immune cell infiltration.

In this study, we speculated that hsa-miR-3663-3p, hsa-miR-4640-5p, and hsa-miR-4709-3p sponged by hsa-RP11-35J10_0013, and hsa-miR-6789-5p sponged by hsa-MROH9_0001, hsa-RP11-35J10_0013 and hsa-MROH9_0001 have the potential to regulate tumor progression through these miRNAs. Regrettably, we have not found any literature on hsa-RP11-35J10_0013 and hsa-MROH9_0001. Furthermore, the expression of circRNA_21279 (also named hsa_circUSP4_002 (circBank ID), http://www.circbank.cn/index.html) in CB and E was con-

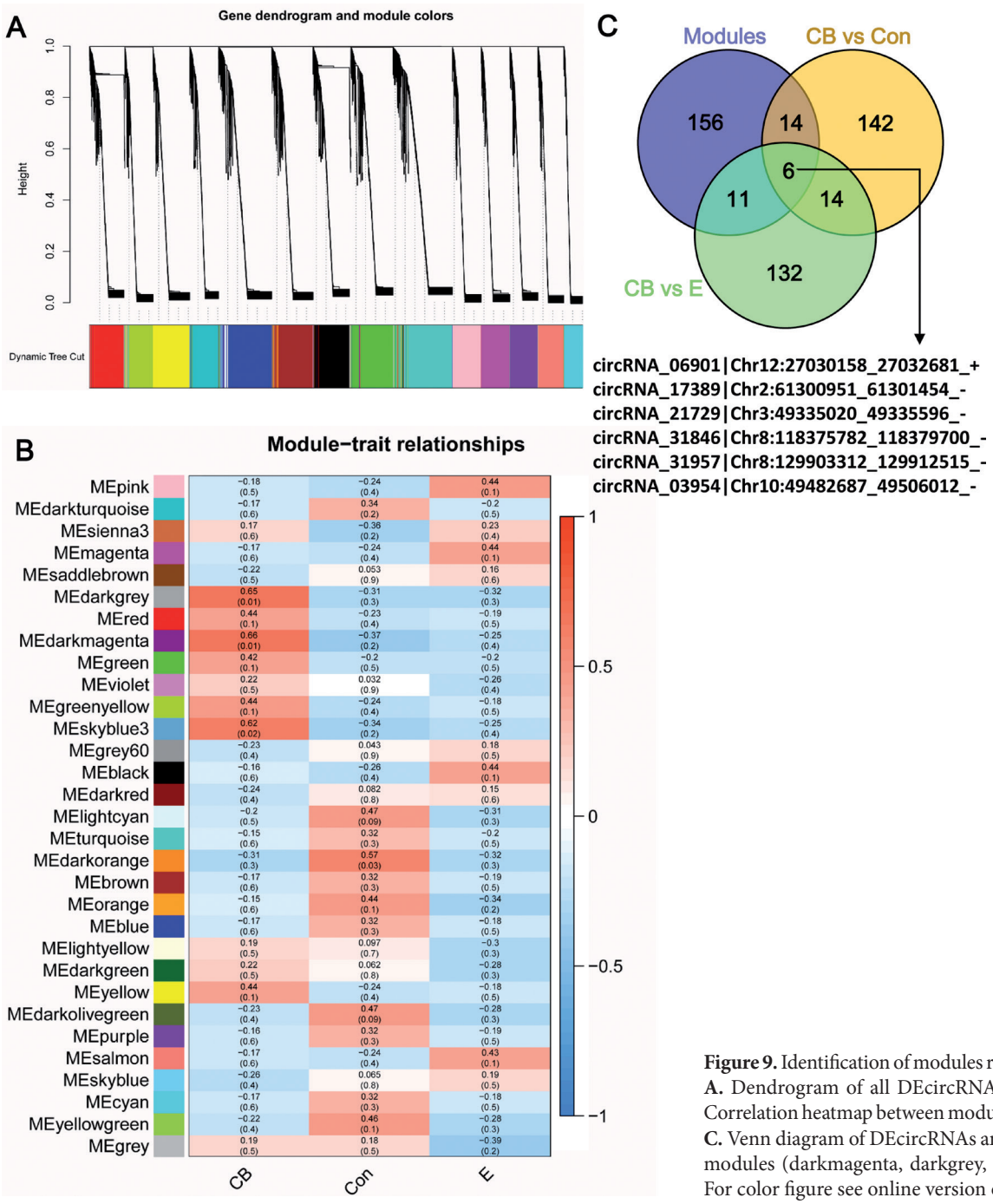

Figure 9. Identification of modules related to group. **A.** Dendrogram of all DEcircRNAs clustered. **B.** Correlation heatmap between modules and groups. **C.** Venn diagram of DEcircRNAs and circRNAs in modules (darkmagenta, darkgrey, and skyblue3). For color figure see online version of the article.

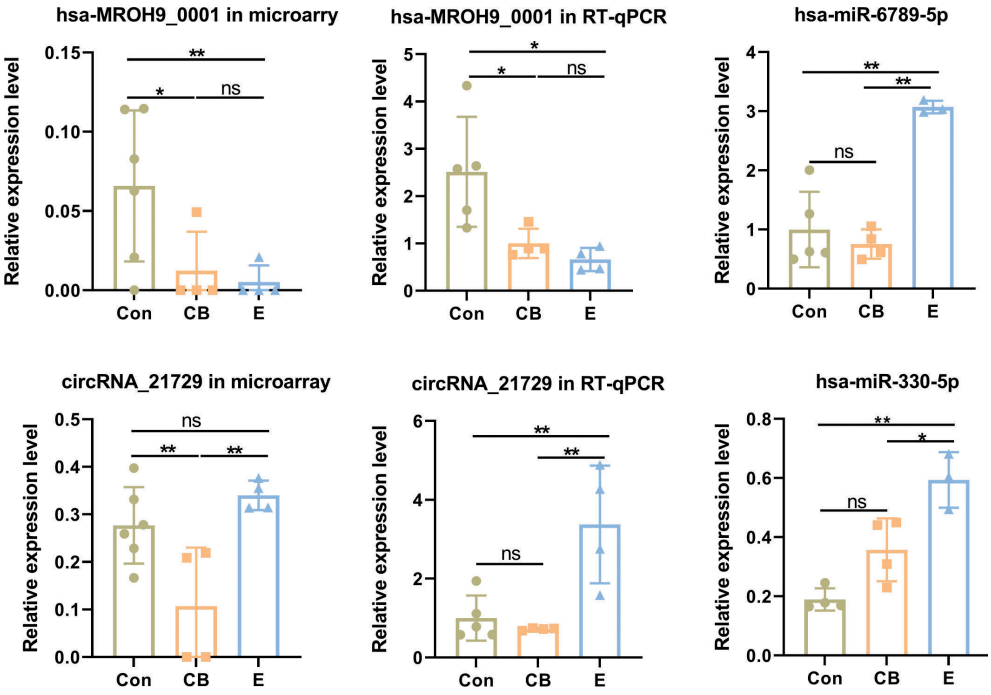

Figure 10. RT-qPCR validation. The circRNA of hsa-MROH9_0001 was selected from the network, and circRNA_21729|Chr3:49335020_49335596_- was selected from WGCNA. Hsa-miR-6789-5p sponged by hsa-MROH9_0001, and hsa-miR-330-5p sponged by circRNA_21279. The expression of circRNAs and miRNAs were normalized by β-actin and U6, respectively. The gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. *p<0.05, **p<0.01, ns means no significance.

firmed by RT-qPCR; however, literature on circRNA_21279 was not retrieved from Google Scholar. These unconfirmed circRNAs indicate that the known_circRNAs remain in the minority compared with the huge number of circRNAs, and further exploration and research on circRNAs still has great value.

In our study, we found that the dysregulated circRNAs in LC patients complicating with COPD were associated with immune and inflammatory pathways such as "Th1 and Th2 cell differentiation", "Natural killer cell mediated cytotoxicity", "Jak–STAT signaling pathway" and "Wnt signaling pathway". All these pathways have been previously implicated in LC or COPD pathogenesis. For example, the cytotoxic function of these NK T-like cells was compromised in these patients in some cancer cases (Wang et al. 2008). Activating Wnt/β-catenin by lithium chloride could have mitigated the decline in lung function and airspace enlargement in mice with emphysema (Kneidinger et al. 2011). Similarly, phosphorylating and activating the JAK-STAT signaling pathway by PART1 contributed to promoting NSCLC cell progression (Zhu et al. 2019). Therefore, we speculated that the change in DEcircRNAs is the consequence of changes in the immune environment.

Moreover, as a chronic inflammatory disease, cellular immune environments in COPD serve as promising diagnostic and therapeutic targets (Henrot et al. 2019; Norman et al. 2019). This implies that molecules that induce changes in the cellular immune environment may be predisposing factors for COPD. As shown in Figure 6, this study predicted a significant association between 49 DEcircRNAs and the infiltration of 18 types of immune cells. For example, neutrophils were positively and negatively correlated with multiple DEcircRNAs. Neutrophils have been proven to be involved in COPD pathogenesis as important infiltrating innate immune cells in the lung (Trivedi et al. 2021). Neutrophilic inflammation is the most predominant inflammatory feature of COPD, and studies have shown that neutrophil infiltration is regulated by circRNA. For instance, abnormal neutrophil infiltration in asymptomatic cases (Ma et al. 2019) and symptomatic cases (Zhao et al. 2017) of moyamoya disease regulated by circRNA has been partially revealed. However, the analysis of neutrophilic inflammation regulated by circRNA in COPD patients is less explored. For the first time, this study predicts that multiple potential circRNAs are involved in neutrophil infiltration in COPD. In future studies, we will further verify if DEcircRNAs are involved in the pathological progression of COPD by regulating neutrophil infiltration *via* the abovementioned pathways.

Finally, we would like to acknowledge several limitations of this study. First, the three groups included a small number of samples, which may have biased the results. Second, the sex distribution was uneven in each group; thus, given the heterogeneity of the sexes of the samples between the groups, the influence of sex on the results could not be excluded. Third, the number of candidate DEcircRNAs validated by RT-qPCR was very small, which may have biased the results.

In summary, this study elucidates the DEcircRNA profiles and possible regulating pathways as well as the relationship between DEcircRNAs and immune cell infiltration in LC patients with different COPD subphenotypes, such as CB or E. Our study demonstrated that circRNAs such as hsa-MROH9_0001 and hsa-RP11-35J10_0013 might participate in the development of LC patients with COPD by regulating inflammation and immune-related pathways. This study provides evidence to guide immunotherapy for LC complicated with COPD and potential targets for immunotherapy in LC complicated with COPD.

Acknowledgement. A pre-print version of this study was deposited on 17 August 2020 (Link: https://www.researchsquare.com/article/rs-47904/v1). However, the pre-print is completely different from the current manuscript. The preprint only identified DEcircRNAs between the Con and CBE groups, and GO, KEGG and network analyses were done on these DEcircRNAs. The pre-print version did not identify the DEcircRNAs between Con and CB groups, Con and E groups, nor did it undergo immune infiltration analysis, miRNA prediction, ROC analysis, WGCNA, RT-qPCR validation. Therefore, the current manuscript is a more in-depth study of the subject and more informative than the pre-print version.

Conflict of interest. The authors have no conflicts of interest to declare.

Author contributions. (I) Conception and design: Lunxu Liu and Fuqiang Wen; (II) Administrative support: Lunxu Liu and Fuqiang Wen; (III) Provision of study materials or patients: Xiaoou Li, Jiahan Cheng, Zhicheng Yuan, and Jun Chen; (IV) Collection and assembly of data: Xiaoou Li, Yongchun Shen, Lei Chen, Jiahan Cheng; (V) Data analysis and interpretation: Xiaoou Li, Jun Chen, Zhicheng Yuan, Tao Wang and Lei Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Data availability statement. The raw data of circRNA microarray has been deposited to the Genome Sequence Archive for Human online database (https://ngdc.cncb.ac.cn/gsa-human/) referenced accession HRA002550.

Statement of ethics. Our study was approved by The Biomedical Ethics Committee [approval NO. 2017 approve (270)] of West China Hospital, Sichuan University. All patients provided written informed consent prior to enrollment in the study.

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Supplementary Material

Differential expression profiles and functional prediction of circular RNAs in lung cancer patients with chronic obstructive pulmonary disease: a pilot study

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Supplementary Tables

Table S1. The primers used in this study

Gene	Primer
RT-U6	5'CGCTTCACGAATTTGCGTGTCAT3'
RT-hsa-miR-330-5p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCCTAAG3'
RT-hsa-miR-4709-3p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCTACAG3'
RT-hsa-miR-6789-5p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCCCGCG3'
U6	F:5'GCTTCGGCAGCACATATACTAAAAT3' R:5'CGCTTCACGAATTTGCGTGTCAT3'
hsa-miR-330-5p	GSP:5'GGGTCTCTGGGCCTGTGTC3' R:5'GTGCGTGTCGTGGAGTCG3'
hsa-miR-4709-3p	GSP:5'GGGGTTGAAGAGGAGGTGCT3' R:5'CAGTGCGTGTCGTGGAGT3'
hsa-miR-6789-5p	GSP:5'GTAGGGGCGTCCCGGGC3' R:5'GTGCGTGTCGTGGAGTCG3'
β-actin	F:5'GTGGCCGAGGACTTTGATTG3' R:5'CCTGTAACAACGCATCTCATATT3'
hsa-MROH9_0001	F:5'TGATACCTTACGATTCCTGTGG3' R:5'GCTACTCTTTTATCATCATCCTCC3'
circRNA_21729	F:5'ATAACTAGTGTGAGGAGAGGTTGA3' R:5'ACTGCCCAGGTGAATGTTTAG3'

F, forward primers; R, reverse primers; RT, reverse transcription.

Table S2. The 243 differentially expressed circRNAs in adjacent tissues from LC patients with COPD compared with Con

circRNA_id	log2FoldChange	pval	up_down	circbase_id
circRNA_00093|Chr1:8508627_8557523_-	-1,960773406	0,025001729	Down	hsa_circ_0005829
circRNA_00253|Chr1:16486426_16486661_-	-1,963736452	0,017855579	Down	-
circRNA_00317|Chr1:20969474_21002808_-	-2,882471559	0,043239889	Down	hsa_circ_0005604
circRNA_00435|Chr1:26729651_26732792_+	-1,714035672	0,019996766	Down	hsa_circ_0008494
circRNA_00574|Chr1:32631827_32634110_-	#NÁZOV?	0,029524145	Down	-
circRNA_00832|Chr1:42480461_42498916_+	-3,012814091	0,033324893	Down	-
circRNA_00900|Chr1:46055795_46080750_-	1,543267436	0,011961295	Up	hsa_circ_0012300
circRNA_00935|Chr1:48305787_48359770_-	-2,270126464	0,045211903	Down	-
circRNA_01136|Chr1:61899583_61901459_+	-3,029985192	0,044634034	Down	hsa_circ_0012782
circRNA_01223|Chr1:66871370_66905375_-	#NÁZOV?	0,003218062	Down	-
circRNA_01307|Chr1:77717867_77718641_-	Inf	0,016212355	Up	hsa_circ_0012992
circRNA_01456|Chr1:90916640_90982370_-	-2,692255002	0,039583771	Down	-
circRNA_01459|Chr1:90937485_90982370_-	1,487105799	0,020557279	Up	-
circRNA_01639|Chr1:107148069_107148839_+	-3,650720858	0,013089866	Down	hsa_circ_0013364
circRNA_01971|Chr1:151638888_151668635_+	3,31853154	0,029701628	Up	hsa_circ_0000131
circRNA_01982|Chr1:152332583_152341210_+	#NÁZOV?	0,012620523	Down	-
circRNA_02083|Chr1:155672560_155679512_-	Inf	0,030826788	Up	hsa_circ_0014604
circRNA_02084|Chr1:155674685_155675096_-	#NÁZOV?	0,027536876	Down	-
circRNA_02104|Chr1:155771067_155773210_-	-1,520502701	0,033199265	Down	-
circRNA_02255|Chr1:171024395_171025420_+	-2,80096897	0,001917412	Down	-
circRNA_02440|Chr1:180397516_180430262_-	2,629919369	0,049952705	Up	hsa_circ_0000164
circRNA_02463|Chr1:184590739_184619630_+	Inf	0,025401438	Up	-
circRNA_03236|Chr1:246726892_246740301_+	Inf	0,034280048	Up	-
circRNA_03346|Chr10:7220411_7285954_-	Inf	0,018452023	Up	hsa_circ_0017628
circRNA_03365|Chr10:7637228_7655675_-	#NÁZOV?	0,037123583	Down	-
circRNA_03405|Chr10:11979057_12014184_-	Inf	0,031255351	Up	hsa_circ_0017713
circRNA_03575|Chr10:20147784_20177409_+	-1,946248271	0,046891975	Down	hsa_circ_0000224
circRNA_03728|Chr10:27935010_27944962_-	#NÁZOV?	0,028103562	Down	-
circRNA_03769|Chr10:31355144_31387798_+	-1,988455217	0,030751292	Down	hsa_circ_0007045
circRNA_04179|Chr10:70844473_70851210_+	#NÁZOV?	0,034251314	Down	-
circRNA_04236|Chr10:73454412_73479517_-	#NÁZOV?	0,012686803	Down	hsa_circ_0018814
circRNA_04655|Chr10:101797980_101813606_-	Inf	0,027156256	Up	hsa_circ_0019610
circRNA_04710|Chr10:102876954_102890678_+	#NÁZOV?	0,004832468	Down	-
circRNA_04742|Chr10:104131331_104164300_-	-3,680917051	0,033445852	Down	-
circRNA_04922|Chr10:124973645_124974398_+	-1,535060934	0,04020426	Down	hsa_circ_0020340
circRNA_05019|Chr11:2347546_2347796_-	Inf	0,02203345	Up	-
circRNA_05094|Chr11:7796834_7799295_-	Inf	0,030676203	Up	-
circRNA_05322|Chr11:22203804_22227586_+	#NÁZOV?	0,042591624	Down	hsa_circ_0021506
circRNA_05388|Chr11:33286413_33306009_+	-3,102825178	0,025486393	Down	-
circRNA_05439|Chr11:36393846_36437476_+	Inf	0,034828922	Up	-
circRNA_05712|Chr11:68185781_68190152_-	3,105979398	0,026677164	Up	-
circRNA_05996|Chr11:85981745_85982003_-	Inf	0,03883106	Up	hsa_circ_0023904
circRNA_05999|Chr11:85981745_85996929_-	#NÁZOV?	0,006666901	Down	hsa_circ_0002264
circRNA_06004|Chr11:85996826_86001158_-	3,307049834	0,039213413	Up	hsa_circ_0002513
circRNA_06023|Chr11:86249471_86252240_+	#NÁZOV?	0,024760002	Down	-
circRNA_06284|Chr11:110435834_110437138_+	#NÁZOV?	0,042014276	Down	-
circRNA_06451|Chr11:121045674_121053732_+	Inf	0,00617914	Up	hsa_circ_0000365
circRNA_06493|Chr11:128480191_128490576_-	1,87162364	0,018582489	Up	hsa_circ_0002083
circRNA_06682|Chr12:9091201_9164259_-	#NÁZOV?	0,038188191	Down	-
circRNA_06831|Chr12:22643763_22673660_+	-1,447984272	0,024003047	Down	hsa_circ_0008664

(continued)

Table S2. (continued)

circRNA_id	log2FoldChange	pval	up_down	circbase_id
circRNA_07042|Chr12:40028301_40048210_-	-3,649303529	0,015809573	Down	hsa_circ_0007918
circRNA_07392|Chr12:66203711_66217235_+	1,069209157	0,025931538	Up	hsa_circ_0008381
circRNA_07409|Chr12:68650400_68654252_+	Inf	0,008794398	Up	hsa_circ_0027464
circRNA_07931|Chr12:109938380_109939247_-	4,064736058	0,007631496	Up	-
circRNA_08023|Chr12:111865250_111871180_+	Inf	0,027851256	Up	hsa_circ_0004466
circRNA_08107|Chr12:116964697_116988869_+	#NÁZOV?	0,016266984	Down	hsa_circ_0005916
circRNA_08169|Chr12:120782655_120791557_-	2,26151642	0,021419069	Up	hsa_circ_0004479
circRNA_08178|Chr12:121221913_121223043_+	Inf	0,049073925	Up	hsa_circ_0028945
circRNA_08378|Chr12:133191489_133192003_+	-2,943461334	0,032011499	Down	hsa_circ_0029596
circRNA_08473|Chr13:24294739_24297735_+	Inf	0,023763711	Up	-
circRNA_08482|Chr13:24484653_24503777_-	Inf	0,034071815	Up	hsa_circ_0029767
circRNA_08625|Chr13:37040405_37051583_-	1,563331361	0,024052189	Up	hsa_circ_0000475
circRNA_08748|Chr13:45151687_45207505_+	1,566249759	0,042234707	Up	hsa_circ_0007259
circRNA_08945|Chr13:72773684_72778380_-	Inf	0,04698362	Up	-
circRNA_08963|Chr13:75326197_75349302_-	Inf	0,034913195	Up	hsa_circ_0002917
circRNA_09051|Chr13:95161189_95170628_-	1,823471637	0,023116305	Up	hsa_circ_0006659
circRNA_09117|Chr13:99842996_99843523_-	#NÁZOV?	0,037561956	Down	-
circRNA_09273|Chr14:23033368_23033674_-	2,583319589	0,018085722	Up	hsa_circ_0008758
circRNA_09639|Chr14:49831299_49834449_-	Inf	0,040802042	Up	hsa_circ_0008076
circRNA_09773|Chr14:55350381_55355222_+	Inf	0,04575966	Up	-
circRNA_09932|Chr14:65561337_65629606_+	3,797437619	0,027587813	Up	-
circRNA_09940|Chr14:66965191_67058786_+	#NÁZOV?	0,010316561	Down	hsa_circ_0032254
circRNA_09998|Chr14:71045133_71057724_+	1,628797066	0,04319544	Up	hsa_circ_0001997
circRNA_10027|Chr14:73077369_73083551_+	#NÁZOV?	0,015044789	Down	hsa_circ_0007053
circRNA_10042|Chr14:73147795_73198129_+	#NÁZOV?	0,045583665	Down	hsa_circ_0002289
circRNA_10490|Chr14:105742341_105769986_-	3,182713016	0,020582636	Up	hsa_circ_0000578
circRNA_10794|Chr15:42871445_42878684_-	#NÁZOV?	0,026397293	Down	-
circRNA_10870|Chr15:44550876_44551522_+	Inf	0,009599372	Up	-
circRNA_10925|Chr15:49235851_49283718_+	2,244443365	0,040301477	Up	hsa_circ_0035188
circRNA_11311|Chr15:64859894_64865228_+	Inf	0,019089747	Up	hsa_circ_0007152
circRNA_11724|Chr15:80978766_80982182_-	-2,339498828	0,026933691	Down	hsa_circ_0002255
circRNA_12099|Chr16:3791981_3793626_-	#NÁZOV?	0,037409204	Down	-
circRNA_12288|Chr16:16014491_16016621_+	-1,615315773	0,0131411	Down	hsa_circ_0038085
circRNA_12355|Chr16:19642396_19652090_+	Inf	0,042102303	Up	hsa_circ_0006730
circRNA_12527|Chr16:30496097_30496566_+	-1,114725756	0,020428473	Down	-
circRNA_12535|Chr16:30704064_30704315_+	3,08411494	0,046484334	Up	hsa_circ_0039076
circRNA_12641|Chr16:53255600_53274302_+	-1,801828662	0,02922592	Down	hsa_circ_0039366
circRNA_12746|Chr16:65810040_65812776_-	2,682171292	0,039584499	Up	-
circRNA_12796|Chr16:68121987_68158068_+	Inf	0,034109051	Up	hsa_circ_0039927
circRNA_12915|Chr16:71115696_71186918_-	#NÁZOV?	0,04091397	Down	-
circRNA_12968|Chr16:74915870_74916259_-	-1,373488345	0,023499147	Down	hsa_circ_0004910
circRNA_13199|Chr17:3705098_3705645_-	Inf	0,00476046	Up	-
circRNA_13514|Chr17:28122603_28172618_+	1,294907691	0,040354585	Up	hsa_circ_0002103
circRNA_13624|Chr17:31155983_31169997_+	-1,457916749	0,041528127	Down	hsa_circ_0007542
circRNA_13664|Chr17:32171043_32176213_+	#NÁZOV?	0,007611394	Down	hsa_circ_0008604
circRNA_13721|Chr17:36182524_36195912_-	4,605760742	0,036430527	Up	-
circRNA_14234|Chr17:61015751_61040892_+	#NÁZOV?	0,043877297	Down	hsa_circ_0044999
circRNA_14522|Chr17:76007060_76010543_-	2,618298581	0,046217602	Up	-
circRNA_14540|Chr17:76767386_76776541_+	-3,250398461	0,04089262	Down	hsa_circ_0045842
circRNA_14785|Chr18:9367488_9388140_+	#NÁZOV?	0,03133147	Down	-

(continued)

Table S2. (continued)

circRNA_id	log2FoldChange	pval	up_down	circbase_id
circRNA_14931|Chr18:21778103_21779685_+	1,595238154	0,009880624	Up	hsa_circ_0008190
circRNA_15009|Chr18:31846716_31849739_-	#NÁZOV?	0,034639057	Down	-
circRNA_15082|Chr18:42015476_42029441_+	-3,475459521	0,00177534	Down	hsa_circ_0002560
circRNA_15154|Chr18:49363161_49379758_-	1,196033473	0,008013407	Up	hsa_circ_0000849
circRNA_15208|Chr18:55350359_55351003_-	3,376499703	0,026452698	Up	hsa_circ_0004223
circRNA_15423|Chr19:1271329_1272051_+	-1,583674129	0,036235364	Down	hsa_circ_0007715
circRNA_15545|Chr19:9337796_9339319_+	#NÁZOV?	0,047894085	Down	hsa_circ_0000888
circRNA_15610|Chr19:10795372_10798572_+	2,282135118	0,004719393	Up	hsa_circ_0049335
circRNA_15679|Chr19:12244581_12250368_-	#NÁZOV?	0,045404668	Down	-
circRNA_15782|Chr19:17259289_17260022_-	Inf	0,006939103	Up	hsa_circ_0049965
circRNA_15841|Chr19:20117781_20121346_+	-3,810779721	0,015571693	Down	-
circRNA_15877|Chr19:22072809_22073564_+	1,863901554	0,015570228	Up	hsa_circ_0050301
circRNA_16009|Chr19:36145806_36146312_+	1,880824535	0,033896031	Up	hsa_circ_0002356
circRNA_16173|Chr19:46320443_46329478_+	#NÁZOV?	0,049871919	Down	-
circRNA_16722|Chr2:29131258_29135666_+	Inf	0,011772919	Up	hsa_circ_0006348
circRNA_16772|Chr2:32174063_32184338_+	-2,114359209	0,006226684	Down	hsa_circ_0005695
circRNA_16855|Chr2:33300451_33301644_+	#NÁZOV?	0,024826787	Down	hsa_circ_0002247
circRNA_17531|Chr2:66464109_66548019_+	-2,610388928	0,044039873	Down	hsa_circ_0008158
circRNA_17608|Chr2:70160625_70181997_-	-2,051608006	0,045335988	Down	hsa_circ_0004975
circRNA_17660|Chr2:72718103_72731245_-	-2,371957277	0,046649548	Down	-
circRNA_17878|Chr2:99194751_99195756_+	Inf	0,014911486	Up	hsa_circ_0008609
circRNA_17914|Chr2:101257791_101266879_+	#NÁZOV?	0,031662264	Down	-
circRNA_17965|Chr2:109329224_109347949_+	Inf	0,006688989	Up	hsa_circ_0003523
circRNA_18316|Chr2:152147162_152148300_-	-2,804808815	0,042269543	Down	-
circRNA_18475|Chr2:168138088_168182090_-	#NÁZOV?	0,040922941	Down	hsa_circ_0002029
circRNA_18504|Chr2:169872236_169875949_+	#NÁZOV?	0,012874042	Down	hsa_circ_0056971
circRNA_18668|Chr2:181539092_181549708_-	#NÁZOV?	0,016588874	Down	-
circRNA_18950|Chr2:201565729_201575326_-	-2,066473376	0,038745872	Down	hsa_circ_0057768
circRNA_19114|Chr2:206149020_206153682_-	Inf	0,042548117	Up	-
circRNA_19486|Chr2:241325993_241343093_+	Inf	0,045393383	Up	hsa_circ_0059053
circRNA_19552|Chr20:2947982_2975241_+	Inf	0,02488485	Up	-
circRNA_19587|Chr20:3912458_3918796_+	Inf	0,038872955	Up	-
circRNA_19684|Chr20:17956933_17961326_-	-2,96492895	0,028785176	Down	-
circRNA_19784|Chr20:33619517_33628435_+	3,136916902	0,043609311	Up	hsa_circ_0059862
circRNA_19887|Chr20:35858302_35871829_+	-1,597038901	0,010319576	Down	hsa_circ_0003322
circRNA_19920|Chr20:36935029_36941111_-	Inf	0,043996352	Up	-
circRNA_19939|Chr20:38040435_38066256_+	2,543710406	0,042366939	Up	hsa_circ_0008006
circRNA_20074|Chr20:49090742_49094963_+	-3,377446163	0,021124717	Down	hsa_circ_0060762
circRNA_20229|Chr21:15777904_15833548_+	#NÁZOV?	0,032006879	Down	-
circRNA_20320|Chr21:33421480_33432871_+	Inf	0,020134989	Up	hsa_circ_0002113
circRNA_20412|Chr21:37420299_37428908_+	Inf	0,002585597	Up	-
circRNA_20493|Chr21:45118564_45134832_+	#NÁZOV?	0,002410345	Down	hsa_circ_0061997
circRNA_20701|Chr22:28694032_28695873_-	2,868927358	0,043082717	Up	hsa_circ_0004811
circRNA_20828|Chr22:37687910_37688992_+	Inf	0,045637252	Up	hsa_circ_0004543
circRNA_21068|Chr3:3068983_3070311_-	-2,532893106	0,046246624	Down	-
circRNA_21071|Chr3:3137260_3144710_+	1,300114314	0,016463564	Up	-
circRNA_21185|Chr3:12496518_12503784_+	#NÁZOV?	0,043283843	Down	hsa_circ_0006673
circRNA_21407|Chr3:32733423_32737490_+	3,974655814	0,003735025	Up	-
circRNA_21422|Chr3:33516023_33538942_-	-3,674153353	0,015378814	Down	-
circRNA_21858|Chr3:56669700_56673725_-	1,110003054	0,043908582	Up	hsa_circ_0001316

(continued)

Table S2. (continued)

circRNA_id	log2FoldChange	pval	up_down	circbase_id
circRNA_21996|Chr3:67400731_67409034_-	-3,365353491	0,043259257	Down	hsa_circ_0005688
circRNA_22115|Chr3:89340916_89342090_+	Inf	0,044130508	Up	hsa_circ_0066598
circRNA_22136|Chr3:98768430_98773983_+	-3,813709765	0,038696107	Down	hsa_circ_0066608
circRNA_22158|Chr3:100732508_100736716_+	3,300346193	0,041725268	Up	hsa_circ_0008839
circRNA_22170|Chr3:100885536_100902686_-	Inf	0,028469559	Up	-
circRNA_22228|Chr3:108647943_108672656_+	Inf	0,014935972	Up	-
circRNA_22247|Chr3:111945268_111962312_+	#NÁZOV?	0,024311433	Down	hsa_circ_0005128
circRNA_22254|Chr3:112537735_112550262_-	#NÁZOV?	0,014814306	Down	hsa_circ_0066801
circRNA_22656|Chr3:138570318_138571356_-	-1,142328229	0,015957344	Down	hsa_circ_0002468
circRNA_22833|Chr3:151581539_151600951_-	-2,240357893	0,001949546	Down	-
circRNA_22839|Chr3:152414941_152432920_+	1,076688193	0,035202152	Up	hsa_circ_0001349
circRNA_22952|Chr3:167554650_167633532_-	-3,804976112	0,030536275	Down	-
circRNA_23011|Chr3:170145423_170178938_-	Inf	0,02205356	Up	hsa_circ_0005228
circRNA_23193|Chr3:183866625_183868060_-	#NÁZOV?	0,036764446	Down	-
circRNA_23350|Chr3:195708134_195708841_+	-3,035160365	0,047250255	Down	-
circRNA_23540|Chr4:3154293_3157199_+	3,618138845	0,048509023	Up	-
circRNA_23905|Chr4:42485496_42524847_-	#NÁZOV?	0,047238096	Down	hsa_circ_0069608
circRNA_23910|Chr4:42503450_42543986_-	-1,613334117	0,036358705	Down	hsa_circ_0069613
circRNA_24076|Chr4:61497121_61497348_+	#NÁZOV?	0,014964177	Down	hsa_circ_0069859
circRNA_24171|Chr4:76996425_77030970_+	-1,807760029	0,029021346	Down	hsa_circ_0070057
circRNA_24374|Chr4:88061906_88065879_+	-2,2771479	0,013825928	Down	hsa_circ_0004577
circRNA_24947|Chr4:150467673_150491035_-	-1,817440564	0,006771479	Down	hsa_circ_0006867
circRNA_25346|Chr5:21491321_21495182_+	#NÁZOV?	0,010648408	Down	hsa_circ_0072023
circRNA_25534|Chr5:43161249_43161931_+	-1,399417052	0,033426566	Down	hsa_circ_0072380
circRNA_25535|Chr5:43292474_43297166_-	Inf	0,005153199	Up	hsa_circ_0008621
circRNA_25556|Chr5:50399107_50428227_-	#NÁZOV?	0,037182404	Down	-
circRNA_25596|Chr5:55322327_55328811_+	#NÁZOV?	0,011500357	Down	-
circRNA_25638|Chr5:56856600_56865977_+	Inf	0,017886413	Up	-
circRNA_25847|Chr5:73840480_73840760_+	-1,017526954	0,012936535	Down	hsa_circ_0005777
circRNA_25909|Chr5:77463095_77464809_-	-1,587785595	0,004512621	Down	hsa_circ_0001498
circRNA_25915|Chr5:78089393_78129307_-	-2,60065613	0,031627233	Down	hsa_circ_0008164
circRNA_25940|Chr5:79619612_79620083_+	#NÁZOV?	0,007347862	Down	-
circRNA_25987|Chr5:80775694_80813741_+	-3,925952626	0,038421818	Down	-
circRNA_26090|Chr5:94868333_94912976_-	Inf	0,043644803	Up	hsa_circ_0005540
circRNA_26217|Chr5:110605109_110627880_-	#NÁZOV?	0,003533146	Down	-
circRNA_26418|Chr5:132927121_132934941_-	-1,747419978	0,035359818	Down	hsa_circ_0073904
circRNA_26430|Chr5:133959494_133960020_-	-3,627729044	0,001120215	Down	-
circRNA_26524|Chr5:138377720_138381590_+	#NÁZOV?	0,022865376	Down	hsa_circ_0074111
circRNA_26544|Chr5:138810038_138827718_+	-1,204204068	0,023551975	Down	hsa_circ_0007440
circRNA_26570|Chr5:140167365_140167802_+	#NÁZOV?	0,002823481	Down	hsa_circ_0002423
circRNA_26704|Chr5:154026399_154034967_-	-2,911825293	0,024556703	Down	-
circRNA_26714|Chr5:154790325_154795319_+	#NÁZOV?	0,011664502	Down	hsa_circ_0007069
circRNA_27065|Chr6:10935058_10956242_+	#NÁZOV?	0,014988384	Down	hsa_circ_0075625
circRNA_27279|Chr6:29945234_30009177_+	Inf	0,018024156	Up	-
circRNA_27326|Chr6:32521905_32581838_-	Inf	2,42E-05	Up	-
circRNA_27537|Chr6:47283938_47286595_-	1,21914664	0,034323971	Up	hsa_circ_0001610
circRNA_27661|Chr6:61975887_62047994_-	2,039028466	0,002614826	Up	-
circRNA_27743|Chr6:73635382_73638499_-	Inf	0,04030096	Up	-
circRNA_27795|Chr6:75828540_75835956_+	#NÁZOV?	0,048035194	Down	-
circRNA_28124|Chr6:109419187_109436199_-	-1,487068169	0,036622145	Down	-

(continued)

Table S2. (continued)

circRNA_id	log2FoldChange	pval	up_down	circbase_id
circRNA_28318|Chr6:129049918_129059896_+	#NÁZOV?	0,047768461	Down	-
circRNA_28476|Chr6:138473306_138496371_-	3,714613589	0,036786744	Up	hsa_circ_0006835
circRNA_28553|Chr6:144513909_144516948_+	Inf	0,01159945	Up	-
circRNA_28594|Chr6:145893215_145894977_-	2,827312093	0,04531995	Up	hsa_circ_0078139
circRNA_28792|Chr6:158429736_158431663_+	-2,551606984	0,039315701	Down	hsa_circ_0001660
circRNA_28842|Chr6:163455279_163535125_+	-1,381725842	0,037151898	Down	-
circRNA_28868|Chr6:167870383_167902386_+	Inf	0,04578509	Up	-
circRNA_28964|Chr7:5901657_5910116_+	-1,407497125	0,04377317	Down	hsa_circ_0004703
circRNA_29241|Chr7:30778432_30782212_+	-2,531909881	0,047664603	Down	hsa_circ_0079733
circRNA_29440|Chr7:44674411_44675268_+	1,481228758	0,029005512	Up	hsa_circ_0005004
circRNA_29441|Chr7:44674411_44676149_+	Inf	0,018222552	Up	hsa_circ_0001701
circRNA_29482|Chr7:50703821_50705323_-	Inf	0,016724169	Up	hsa_circ_0080212
circRNA_29705|Chr7:77274482_77280653_+	-2,558098581	0,015388506	Down	-
circRNA_29770|Chr7:77749424_77750064_+	#NÁZOV?	0,04151907	Down	-
circRNA_29789|Chr7:80646088_80674147_+	Inf	0,016658779	Up	-
circRNA_29893|Chr7:92294889_92333720_+	-3,85358711	0,028250761	Down	hsa_circ_0002497
circRNA_30300|Chr7:128641203_128648982_+	1,970022879	0,013419207	Up	hsa_circ_0005921
circRNA_30483|Chr7:140334429_140337349_-	#NÁZOV?	0,016348076	Down	hsa_circ_0001757
circRNA_31142|Chr8:42861745_42887871_-	Inf	0,023969269	Up	hsa_circ_0084135
circRNA_31404|Chr8:67266006_67277457_-	-1,758556462	0,041987284	Down	-
circRNA_31715|Chr8:99575658_99585517_+	1,87563842	0,03145296	Up	-
circRNA_31797|Chr8:107284682_107303367_-	#NÁZOV?	0,035504748	Down	-
circRNA_32011|Chr8:132836540_132844318_+	-1,678322995	0,034681777	Down	hsa_circ_0085644
circRNA_32310|Chr9:17226203_17236588_+	-2,492403242	0,046723086	Down	-
circRNA_32395|Chr9:20923660_20933132_+	2,825249515	0,005734514	Up	hsa_circ_0005646
circRNA_32416|Chr9:27009020_27029104_+	Inf	0,029465537	Up	-
circRNA_32493|Chr9:33960826_33963791_-	-1,440603725	0,046908485	Down	hsa_circ_0001849
circRNA_33158|Chr9:112574057_112575251_+	Inf	0,012012797	Up	hsa_circ_0002925
circRNA_33275|Chr9:124301936_124313985_+	2,624241778	0,037873433	Up	hsa_circ_0088485
circRNA_33468|Chr9:132296887_132311856_-	3,619978336	0,006106579	Up	hsa_circ_0089282
circRNA_33753|ChrX:43683513_43712796_+	#NÁZOV?	0,032588593	Down	-
circRNA_33775|ChrX:47846105_47895940_+	1,262367835	0,019141302	Up	hsa_circ_0006364
circRNA_33896|ChrX:75098942_75114834_-	Inf	0,043115623	Up	hsa_circ_0007675
circRNA_34065|ChrX:118542725_118546116_+	-1,841516161	0,023797682	Down	-
circRNA_34256|ChrY:2953909_2961646_+	Inf	4,28E-07	Up	hsa_circ_0001953
circRNA_34258|ChrY:2961074_2961646_+	Inf	0,014913628	Up	hsa_circ_0007907
circRNA_34260|ChrY:3079284_3102194_+	Inf	0,000624758	Up	-
circRNA_34286|ChrY:12909360_12913062_+	Inf	0,000438336	Up	hsa_circ_0008297
circRNA_34288|ChrY:12912963_12914649_+	Inf	0,041128167	Up	hsa_circ_0005757
circRNA_34305|ChrY:18927076_18933475_-	Inf	0,035387346	Up	-
circRNA_34310|ChrY:19041370_19045288_-	Inf	0,017491661	Up	hsa_circ_0009023
circRNA_34315|ChrY:19587210_19587507_+	Inf	2,11E-06	Up	hsa_circ_0009024
circRNA_34319|ChrY:20507352_20521300_-	Inf	0,008026565	Up	-