CircRNA Expression Profile in Lung Cancer Complicated with Chronic Obstructive Pulmonary Disease Patients

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Abstract

Background: Lung cancer complicated with chronic obstructive pulmonary disease (COPD) are major causes of mortality worldwide, and the incidence of lung cancer and COPD increasing significantly. Circular RNAs (circRNAs), have been reported to participate in various biological processes, whereas the role of circRNAs in lung cancer complicated with COPD remains unclear. We aims to identify differentially expressed circRNAs (DEcircRNAs) between lung cancer complicated with COPD and lung cancer without COPD.

Method: The circRNAs expression profiles were identified using a high-throughput circRNA microarray in cancer adjacent tissues from 6 lung cancer without COPD patients and 8 lung cancer complicated with COPD patients. Bioinformatic analyses were conducted to identify the functions of DEcircRNAs.

Result: A total of 115 up- and 128 down-regulated circRNAs were screened in lung cancer complicated with COPD patients compared with lung cancer without COPD patients. The myD88-dependent toll-like receptor signaling pathway and positive regulation of nitric oxide biosynthetic process ranked the top 2 enriched biological processes in Gene Ontology analysis. Signaling transduction and infectious diseases were the most significantly enriched Kyoto Encyclopedia of Genes and Genomes pathways in both up- and down-regulated circRNAs. Compared with lung cancer without COPD, circRNAs are dysregulated in the adjacent tissues of lung cancer with COPD.

Conclusion: The DEcircRNAs might act as potential targets for the diagnosis of lung cancer with COPD.

Introduction

Lung cancer is a main cause of cancer-related mortality worldwide [1]. Latest cancer statistics reported that, lung cancer and bronchus cancer have a proportion of 13% in estimated new cancer cases and 24% in estimated deaths in 2019 [2]. Moreover, the 5-year relative survival rate is 19% in the United States (2008–2014) [2]. In particular, chronic obstructive pulmonary disease (COPD) is significantly associated with increasing morbidity and mortality after surgical resection for early-stage non-small cell lung cancer (NSCLC) [3–5]. COPD is a chronic inflammatory lung disease that causes airflow obstruction, which is also a major cause of morbidity and mortality [6]. Some common pathogenic factors and pathways contribute to lung cancer and COPD. Tobacco smoking is the most common risk for lung cancer and COPD [6, 7]. Besides, occupational exposure, physical activity, radiation, and air pollution are relevant pathogenic factors of lung cancer and COPD [7]. It is reported that a large proportion of lung cancer patients suffer from COPD and COPD promotes the incidence of lung cancer [8]. However, COPD differs from lung cancer in phenotype and presents as dissimilar diseases [2, 7]. Chronic lung damage such as irreversible airflow limitation, inflammation of small airways, and emphysema are the key features of COPD. In contrast, lung cancer character with the uncontrollable proliferation of cells. The effect of COPD on the prognosis of NSCLC patients has no definite conclusion [6, 9]. In clinical, treatment prefers chemotherapy to surgery in lung cancer patients complicated with COPD and COPD and some researchers considered that improved treatment should be adopted in lung cancer complicated with COPD compared with lung cancer patients [6]. However, the diagnostic biomarker for lung cancer complicated with COPD remain lacking.

Circular RNAs (circRNAs) is an endogenous non-coding RNA, forms a closed continuous loop by back splicing with covalently joined between 3' and 5' ends [10]. Accumulating researchers reveal the critical role of circRNAs in multiple biological processes [11] and diseases [12, 13], including cancer and COPD [14–16]. The anomalously expressed circRNAs might provide new diagnose biomarker and therapeutic targets in diseases. Circ_0078767 is a down-regulated circRNA, promoted the expression of RASSF1A via acting as a sponge of miR-330-3p in NSCLC. Overexpression of Circ_0078767 significantly repressed the proliferation of NSCLC cells [17]. CircRNA hsa_circ_0016070 was reported to promote the proliferation of pulmonary artery smooth muscle cells [15]. CircARHGAP10 suppresses NSCLC progression by promoting GLUT1 expression via sponging miR-150-5p [18]. The expression profile of circRNAs in primary human small airway epithelial cells with or without cigarette smoke extract reveals that circRNAs might participate in the regulation of COPD [16]. However, the expression profile and the underlying functions of circRNAs in lung cancer complicated with COPD remain unclear.

In present research, we investigated the expression profiles of circRNAs in lung cancer patients and lung cancer complicated with COPD patients. This might provide new circRNA targets in lung cancer complicated with COPD diagnose and therapy.

Materials And Methods

Ethics

This work was conducted in accordance with the China Human Genome/Gene Research guidelines and was approved by the West China Hospital Research Ethics Committee, Sichuan University. The informed materials were signed.

Patients

All of the 14 lung cancer patients were recruited from 2017–2019 in the West China Hospital. Patients were divided into two groups: the control group was without COPD, and the disease group was diagnosed as COPD. COPD was diagnosed according to the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The case inclusion criteria: emphysema (E) and chronic bronchitis (CB). The clinical characteristics were shown in Table 1.

Table 1
Clinical characteristic of the patients.

Patient number	Age	Gender	Smoking (cigarette/per day, smoked)	TNM stage	FEV1	FEV1%PRE	FEV1/FVC	GOLD Categorization
Con5	36	female	0	none	2.77	104.6	85.88	none
Con6	50	female	0	IVb	3.05	124	81.34	none
Con7	53	female	0	Ila	2.46	112.7	88.21	none
Con8	63	female	1, 2 years	IIIb	2.79	119	79.81	none
Con9	54	female	0	IIb	2.77	149.1	83.47	none
Con10	52	female	0	Ia	2.19	114.8	93.24	none
E7	56	male	20, 30 years	IIIa	1.93	66.4	64.47	COPD II
E8	50	male	20, 10 years	none	2.63	80.8	70.94	COPD II (emphysematous bullae)
E9	59	male	10, 40 years	none	1.06	31.7	30.46	COPDIII (emphysematous bullae)
E10	63	male	20, 50 years	Ib	1.93	78	63.96	COPD II
CB6	63	male	10, 0.5 years	Ib	2.31	78.1	60.67	COPD II
CB7	50	female	0	IIIb	2.03	80.6	54.67	COPD II
CB8	41	female	0	none	-	-	-	Chronic bronchitis (no pulmonary function)
CB9	65	female	0	Ila	1.55	105.8	67.19	COPD I
COPD was diagnosed according to the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease as follow: 1) non-COPD was defined as FEV1/FVC $\geq$ 70%, 2) mild COPD was defined as FEV1/FVC < 70% and FEV1 $\geq$ 80% of the predicted, 3) moderate COPD was defined as FEV1/FVC < 70% and 50% $\leq$ FEV1 < 80% of the predicted, 4) severe COPD was defined as FEV1/FVC < 70% and 30% $\leq$ FEV1 < 50% of the predicted, and 5) very severe COPD was defined as an FEV1/FVC < 70% and FEV1 < 30%. "-" indicates incomplete clinical data due to patient transfer.								

RNA Extraction and circRNA microarray

The adjacent cancer lung tissues at 5 cm were collected and frozen in liquid nitrogen immediately. Total RNA was extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The quality of RNA was detected using Nanodrop spectrophotometer (Thermo Fisher, Waltham, MA, USA). The circRNA microarray was performed with the Human circRNA Array V2.0 (8 × 15K, Aksomics, Shanghai, China) as the previous description [19]. In brief, 1 µg total RNA was digested with RNase R (RNR0725, Epicentre, Madison, WI, USA). The samples were labeled and hybridized in Aksomics Inc. The fluorescence signal was scanned and analyzed using GenePix 4000B (Molecular Devices Corporation, SV, USA).

Data analysis

The raw data were processed using R software (R version 3.6.1). DEcircRNAs were based on absolute fold change > 2, and P < 0.05. The GeneOntology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using R package clusterprofile (<https://www.rdocumentation.org/packages/clusterProfiler>). To construct the putative circRNA-mediated competing endogenous RNA (ceRNA) network, the circRNA targeting miRNA was predicted using Miranda and RNAhybird. The common miRNA targets were selected in both two software. The network was constructed using Cytoscape_v3.6.1.

Quantitative reverse transcription PCR validation

Validation of candidate circRNAs was performed using quantitative reverse transcription PCR (qRT-PCR). A total of 500 ng RNA were reverse-transcribed into cDNA using the cDNA Synthesis kit (#K1622, Thermo Fisher, Waltham, MA, USA) following manufacturer's instructions. qRT-PCR was carried out on an ABI Q6 (Applied Biosystems Inc., USA) using SYBR Green Real-Time PCR Master Mix (Toyobo, number QPK-201). The qRT-PCR procedure had the following stages: 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 60 °C for 60 s and a final dissociation stage. The relative expression levels were quantified by the $2^{-\Delta\Delta Ct}$ method. Primers used for this study are listed below:

hsa_circ_0002560-F 5'-ATTTGTAGATCGGTTGGTG-3'

hsa_circ_0002560-R 5'-CAGGAGGAGGTGAAGAGAC-3'

hsa_circ_0005646-F 5'-CCAAGAGATGAACCTTCTTCTG-3'

hsa_circ_0005646-R 5'-TTGCCTTTCACCTACCGGAC-3'

β-actin-F 5'-CATTCCAAATATGAGATGCGTT-3'

β-actin-R 5'-TACACGAAAGCAATGCTATCAC-3'.

Statistical analysis

Statistical analysis were performed using the SPSS package 17.0 (IBM, Armonk, NY, USA). The comparisons of means between two groups were analyzed by the two-tailed student’s *t*-test. The results are presented as the means ± standard deviation (SD). A value of *p* < 0.05 was considered statistically significant.

Results

Overview of circRNA expression profiles

To reveal the circRNA expression profiles in lung cancer complicated with COPD, the microarray assay was used to analyze the circRNA signature in lung cancer complicated with COPD compared with lung cancer alone patients. The clinical characteristics of patients were listed in Table 1. A total of 243 differentially expressed circRNAs (DEcircRNAs) were obtained from 13,617 human circRNAs detected using microarray assay. Among them, 115 circRNAs were up-regulated and 128 circRNAs were down-regulated in lung cancer complicated with COPD patients compared with lung cancer without COPD patients. The volcano plot were shown in Fig. 1A. The clustering result of 14 samples with 243 DEcircRNAs was presented with a heatmap in Fig. 1B. All samples were assigned to two groups: the control group and lung cancer complicated with COPD group. Furthermore, all chronic bronchitis patients besides CB6 were distinguished with emphysema patients. The top 10 up- and down-regulated circRNAs were listed in Table 2.

Table 2
Top 10 dysregulated circRNAs.

Circbase_id	CircRNA_id	FoldChange	up_down	circRNA_length	Type	Gene
-	circRNA_13721 Chr17:36182524_36195912_-	24.3	Up	12968	sense-overlapping	CCL3L3
-	circRNA_07931 Chr12:109938380_109939247_-	16.7	Up	272	sense-overlapping	GIT2
-	circRNA_21407 Chr3:32733423_32737490_+	15.7	Up	380	sense-overlapping	CNOT10
-	circRNA_09932 Chr14:65561337_65629606_+	13.9	Up	926	sense-overlapping	FUT8
hsa_circ_0006835	circRNA_28476 Chr6:138473306_138496371_-	13.1	Up	335	sense-overlapping	NHSL1
hsa_circ_0089282	circRNA_33468 Chr9:132296887_132311856_-	12.2	Up	675	sense-overlapping	SETX
-	circRNA_23540 Chr4:3154293_3157199_+	12.2	Up	255	sense-overlapping	HTT
hsa_circ_0004223	circRNA_15208 Chr18:55350359_55351003_-	10.3	Up	180	sense-overlapping	TCF4
hsa_circ_0000131	circRNA_01971 Chr1:151638888_151668635_+	9.97	Up	838	sense-overlapping	SNX27
hsa_circ_0002513	circRNA_06004 Chr11:85996826_86001158_-	9.89	Up	365	sense-overlapping	PICALM
-	circRNA_25987 Chr5:80775694_80813741_+	0.06	Down	560	sense-overlapping	MSH3
hsa_circ_0002497	circRNA_29893 Chr7:92294889_92333720_+	0.06	Down	6697	sense-overlapping	ANKIB1
hsa_circ_0066608	circRNA_22136 Chr3:98768430_98773983_+	0.07	Down	416	sense-overlapping	ST3GAL6
-	circRNA_15841 Chr19:20117781_20121346_+	0.07	Down	3566	sense-overlapping	ZNF90
-	circRNA_22952 Chr3:167554650_167633532_-	0.07	Down	29929	sense-overlapping	WDR49
-	circRNA_04742 Chr10:104131331_104164300_-	0.07	Down	1792	sense-overlapping	CFAP43
-	circRNA_21422 Chr3:33516023_33538942_-	0.07	Down	706	sense-overlapping	CLASP2
hsa_circ_0013364	circRNA_01639 Chr1:107148069_107148839_+	0.07	Down	771	exonic	NTNG1
hsa_circ_0007918	circRNA_07042 Chr12:40028301_40048210_-	0.07	Down	369	sense-overlapping	SLC2A13
-	circRNA_26430 Chr5:133959494_133960020_-	0.08	Down	527	exonic	C5orf15

GO and KEGG pathway analyses

To reveal the functions of these DEcircRNAs, the GO and KEGG pathway analyses were performed using R package clusterprofile. The assessed gene list was obtained by predicting target gene of DEcircRNAs' sponge miRNAs. GO enrichment analysis showed that the most significant biological process in up-regulated DEcircRNAs was the MyD88-dependent toll-like receptor signaling pathway (Fig. 2A), while hemopoiesis and spinal cord development in down-regulated DEcircRNAs (Fig. 2B). The integral component of luminal side of endoplasmic reticulum membrane and endocytic vesicle membrane were the top cellular component involved with up-regulated DEcircRNAs (Fig. 2A). The basal cortex was the top cellular component involved with down-regulated DEcircRNAs (Fig. 2B). The heat shock protein binding was the top molecular function involved with up-regulated DEcircRNAs (Fig. 2A). In the down-regulated circRNAs, phospholipid-translocation ATPase activity is the most significantly enriched molecular function (Fig. 2B).

KEGG enrichment analysis showed that the signal transduction, infectious disease, immune system were the most enriched pathways in up-regulated DEcircRNAs (Fig. 3A). And the signal transduction, infectious disease, and cancers were the most enriched pathways in down-regulated

DEcircRNAs (Fig. 3B). The results show that signal transduction and infectious diseases in both the up-regulated and down-regulated DEcircRNAs are the most significant enrichment of pathways.

CircRNAs-miRNAs interreaction network

To explore the function of circRNAs as miRNA sponges, the network of top 300 significant circRNAs-miRNAs interreaction predicted by Miranda and RNAhybrid. A total of 700 miRNAs targeted with DEcircRNAs were obtained. And the network was constructed using Cytoscape_v3.6.1 (Fig. 4). CircRNA_10490, CircRNA_27279, CircRNA_17965, and CircRNA_27326 were the top 4 circRNAs interacted with miRNAs. Hsa-miR-6754-5p and hsa-miR-661 ranked the top 2 miRNAs which were predicted to be regulated by 12 and 10 DEcircRNAs, respectively. For example, the up-regulated circRNA_27279 might regulate the expression of the hsa-miR-575 targeted genes by sponging hsa-miR-575.

Validation of circRNAs expression

To confirm the microarray data, we selected 2 down-regulated (hsa_circ_0002560, hsa_circ_0005646) circRNAs. The expression of these circRNAs significantly down-regulating in the patient's paracancer tissues of lung cancer complicated with COPD compared with the patient's paracancer tissues of lung cancer complicated without COPD. The qRT-PCR results of hsa_circ_0002560 and hsa_circ_0005646 were shown in supplemental Fig. 1, which was consistent with the dysregulation of microarray result. This result indicates that the majority of circRNAs identified by microarray are reliable.

Discussion

Recently, the incidence of lung cancer complicated with COPD patients is increasing, and the mortality of lung cancer complicated with COPD patients is significantly higher than the patients of lung cancer complicated without COPD. The circRNAs microarray assays results revealed that the different circRNAs expression patterns in lung cancer complicated with or without COPD. Uncovered 115 up-regulated and 128 down-regulated circRNAs in lung cancer with COPD patients. Among these, circRNA_0002560 and circRNA_0005646 were down-regulated with top magnitudes. Furthermore, we identified that the inflammation status might distinguish lung cancer with COPD from lung cancer without COPD.

Accumulating researches have revealed the significance roles of circRNA in lung cancer and COPD. For example, overexpressed circFGFR1 can promote the migration, invasion, and proliferation of NSCLC cells via sponging miR-381-3p [20]. Weimei Huang et.al performed circRNA microarray in NCI-H69 and NCI-H69AR cells and identified CircRNA cESRP1 as a potential prognostic biomarker in small cell lung cancer [21]. Ni Zeng et.al reported the expression profiles of circRNAs and mRNAs in cigarette smoke extract treated human small airway epithelial cells [16]. Our study reported DEcircRNAs between lung cancer with and without COPD patients.

CircRNAs have a crucial role to acting as the competitive endogenous RNA (ceRNA) via sponging miRNA [22]. To predict the functions of DEcircRNAs, we performed the ceRNA mechanism. MyD88-dependent toll-like receptor signaling pathway is the most significantly enriched pathway in up-regulated DEcircRNAs. Inflammation is the key signature of COPD and positively correlates with disease severity [23]. The downstream of myD88-dependent toll-like receptor signaling pathway activates NF- κ B and transcription of cytokines [24]. It was reported that several interleukins and cyclooxygenase-2 activity may contribute to the formation of lung tumors and COPD [7, 25]. Although the common role of cytokines in lung cancer and COPD, the identification of unique inflammatory molecules is considered to be a key point in developing novel therapeutic strategies against lung cancer or lung cancer with COPD. Our results showed that the up-regulated circRNAs enriched in myD88-dependent toll-like receptor signaling pathway may provide novel potential targets that uniquely expressed in lung cancer with COPD. Reactive nitrogen and oxygen species (RNOS) is also considered as a common causative agent in lung cancer and COPD [26]. The positive regulation of nitric oxide biosynthetic process ranks the second in up-regulated circRNAs related biological process in our study. The cellular components impacted focus on endocytic vesicle membrane, specific granule membrane, and lysosomal membrane. The result in accord with previous study that signaling pathways and cell membrane functions are significantly involved in cystic fibrosis lung airway and parenchyma tissues [27].

KEGG analysis showed that signal transduction, infectious diseases, immune system are the most significantly enriched pathways, which are closely related to inflammatory. Cellular immune environments and chemokines were considered as promising diagnostic and therapeutic targets in COPD [28, 29]. Besides surgery, the inflammatory condition of COPD might limit the use of the programmed death 1 immune checkpoint inhibitor in lung cancer [30]. Then, the immune disorder clues would provide standard for the use of immune checkpoint inhibitor in treating lung cancer with COPD. Meanwhile, pathway in cancers is enriched in both up- and down-regulated circRNAs, which suggests the promoting cancer role of COPD.

Given that circRNAs could serve as miRNA sponges and completely bind miRNA against mRNA, we predicted the miRNAs interacted with DEcircRNAs. Some DEcircRNAs could influence many miRNAs. However, the functions of these DEcircRNAs remain further studied. Hsa-miR-661 regulated by most DEcircRNAs, was reported to be regulated by Circ_RUSC2 and regulated the proliferation, apoptosis, phenotypic modulation, and migration of vascular smooth muscle cells [31]. Guang-Hua Zhou et.al reported that hsa-miR-661 was up-regulated in NSCLC patients serum and positively correlated with poor overall survival [32]. These results suggested that DEcircRNAs regulating hsa-miR-661 such as circRNA_03769, circRNA_05439, circRNA_06682, circRNA_10490, and circRNA_13721 might be potential diagnostic biomarker in lung cancer with COPD. However, some limitations remain investigated in this study. The functions of DEcircRNAs candidates need further investigate.

In conclusion, we identified the circRNA expression profiles of adjacent tissue from lung cancer complicated with COPD patients by comparing with lung cancer without COPD patients, and 243 DEcircRNAs (115 up-regulated and 128 down-regulated) were identified. GO analysis revealed that myD88-dependent toll-like receptor signaling pathway was the most significantly enriched term of up-regulated DEcircRNAs. This study provided new circRNAs clues for the diagnose and therapy of lung cancer complicated with COPD.

Declarations

Ethics approval and consent to participate

This work was conducted in accordance with the China Human Genome/Gene Research guidelines and was approved by the West China Hospital Research Ethics Committee, Sichuan University. The informed materials were signed.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization and Funding acquisition: LL and FW. Data curation: XL, JC and LC. Formal analysis: XL, YS, JHC and TW. Experimental studies: XL, YS, JHC, JC, ZY, TW and LC. Software: YS and ZY. Writing – original draft & review & editing: All authors. All authors read and approved the final manuscript.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34.
3. Papi A, Casoni G, Caramori G, Guzzinati I, Boschetto P, Ravenna F, et al. COPD increases the risk of squamous histological subtype in smokers who develop non-small cell lung carcinoma. *Thorax*. 2004;59(8):679-81.
4. Zhai R, Yu X, Shafer A, Wain JC, Christiani DC. The impact of coexisting COPD on survival of patients with early-stage non-small cell lung cancer undergoing surgical resection. *Chest*. 2014;145(2):346-53.
5. Kim H, Yoo H, Pyo H, Ahn YC, Noh JM, Ju SG, et al. Impact Of Underlying Pulmonary Diseases On Treatment Outcomes In Early-Stage Non-Small Cell Lung Cancer Treated With Definitive Radiotherapy. *Int J Chron Obstruct Pulmon Dis*. 2019;14:2273-81.
6. Parris BA, O'Farrell HE, Fong KM, Yang IA. Chronic obstructive pulmonary disease (COPD) and lung cancer: common pathways for pathogenesis. *J Thorac Dis*. 2019;11:S2155-S72.
7. Barreiro E, Bustamante V, Curull V, Gea J, López-Campos JL, Muñoz X. Relationships between chronic obstructive pulmonary disease and lung cancer: biological insights. *J Thorac Dis*. 2016;8(10):E1122-E35.
8. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. *Am J Respir Crit Care Med*. 2017;195(5):557-82.
9. Sin DD, Man SFP. Chronic Obstructive Pulmonary Disease as a Risk Factor for Cardiovascular Morbidity and Mortality. *Proc Am Thorac Soc*. 2005;2(1):8-11.

10. LL C. The biogenesis and emerging roles of circular RNAs. *Nat Rev Mol Cell Biol.* 2016;17(4):205-11.
11. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet.* 2019;20(11):675-91.
12. Zhang SB, Lin SY, Liu M, Liu CC, Ding HH, Sun Y, et al. CircAnks1a in the spinal cord regulates hypersensitivity in a rodent model of neuropathic pain. *Nat Commun.* 2019;10(1):4119.
13. Garikipati VNS, Verma SK, Cheng Z, Liang D, Truongcao MM, Cimini M, et al. Circular RNA CircFndc3b modulates cardiac repair after myocardial infarction via FUS/VEGF-A axis. *Nat Commun.* 2019;10(1):4317.
14. Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Zhang Y, et al. The Landscape of Circular RNA in Cancer. *Cell.* 2019;176(4):869-81.e13.
15. Zhou S, Jiang H, Li M, Wu P, Sun L, Liu Y, et al. Circular RNA hsa_circ_0016070 Is Associated with Pulmonary Arterial Hypertension by Promoting PASM C Proliferation. *Mol Ther Nucleic Acids.* 2019;18:275-84.
16. Zeng N, Wang T, Chen M, Yuan Z, Qin J, Wu Y, et al. Cigarette smoke extract alters genome-wide profiles of circular RNAs and mRNAs in primary human small airway epithelial cells. *J Cell Mol Med.* 2019;23(8):5532-41.
17. Chen T, Yang Z, Liu C, Wang L, Yang J, Chen L, et al. Circ_0078767 suppresses non-small-cell lung cancer by protecting RASSF1A expression via sponging miR-330-3p. *Cell Prolif.* 2019;52(2):e12548.
18. Jin M, Shi C, Yang C, Liu J, Huang G. Upregulated circRNA ARHGAP10 Predicts an Unfavorable Prognosis in NSCLC through Regulation of the miR-150-5p/GLUT-1 Axis. *Mol Ther Nucleic Acids.* 2019;18:219-31.
19. Xia P, Wang S, Ye B, Du Y, Li C, Xiong Z, et al. A Circular RNA Protects Dormant Hematopoietic Stem Cells from DNA Sensor cGAS-Mediated Exhaustion. *Immunity.* 2018;48(4):688-701.e7.
20. Zhang PF, Pei X, Li KS, Jin LN, Wang F, Wu J, et al. Circular RNA circFGFR1 promotes progression and anti-PD-1 resistance by sponging miR-381-3p in non-small cell lung cancer cells. *Mol Cancer.* 2019;18(1):179.
21. Huang W, Yang Y, Wu J, Niu Y, Yao Y, Zhang J, et al. Circular RNA cESRP1 sensitises small cell lung cancer cells to chemotherapy by sponging miR-93-5p to inhibit TGF- β signalling. *Cell Death Differ.* 2019.
22. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. *Nature.* 2013;495(7441):384-8.
23. Shaykhiev R, Crystal RG. Innate immunity and chronic obstructive pulmonary disease: a mini-review. *Gerontology.* 2013;59(6):481-9.
24. Wang L, Yu K, Zhang X, Yu S. Dual functional roles of the MyD88 signaling in colorectal cancer development. *Biomed Pharmacother.* 2018;107:177-84.
25. Bozinovski S, Vlahos R, Anthony D, McQualter J, Anderson G, Irving L, et al. COPD and squamous cell lung cancer: aberrant inflammation and immunity is the common link. *Br J Pharmacol.* 2016;173(4):635-48.
26. Durham AL, Adcock IM. The relationship between COPD and lung cancer. *Lung Cancer.* 2015;90(2):121-7.
27. Kumar P, Sen C, Peters K, Frizzell RA, Biswas R. Comparative analyses of long non-coding RNA profiles in vivo in cystic fibrosis lung airway and parenchyma tissues. *Respir Res.* 2019;20(1):284.
28. Norman KC, Freeman CM, Bidthanapally NS, Han MK, Martinez FJ, Curtis JL, et al. Inference of Cellular Immune Environments in Sputum and Peripheral Blood Associated with Acute Exacerbations of COPD. *Cell Mol Bioeng.* 2019;12(2):165-77.
29. Henrot P, Prevel R, Berger P, Dupin I. Chemokines in COPD: From Implication to Therapeutic Use. *Int J Mol Sci.* 2019;20(11):pii: E2785..
30. Suzuki Y, Inui N, Karayama M, Imokawa S, Yamada T, Yokomura K, et al. Effect of PD-1 inhibitor on exhaled nitric oxide and pulmonary function in non-small cell lung cancer patients with and without COPD. *Int J Chron Obstruct Pulmon Dis.* 2019;14:1867-77.
31. Sun J, Zhang Z, Yang S. SYK/Circ_RUSC2 upregulates the expression of miR-661 target gene and regulates the function of vascular smooth muscle cells. *Biochem Cell Biol.* 2019;97(6):709-14.
32. Zhou GH, Yang WH, Sun B. Clinical impact of serum miR-661 in diagnosis and prognosis of non-small cell lung cancer. *Eur Rev Med Pharmacol Sci.* 2017;21(24):5696-701.

Figures

Figure 1

CircRNAs expression profile in lung cancer complicated with COPD and lung cancer patients. A. Volcano plot of circRNAs in lung cancer complicated with COPD compared with that of lung cancer patients. Red dots, up-regulated circRNAs; Green dots, down-regulated circRNAs. P-values < 0.05. B. Cluster heatmaps of DEcircRNAs in lung cancer patients complicated with or without COPD. Each column corresponds to the expression profiles of one patient sample, and each row represents a DEcircRNA. Red, white, blue indicated high, unchanged, low relative expression, respectively. Con, lung cancer alone patients. E_CB, lung cancer complicated with COPD patients. COPD, chronic obstructive pulmonary disease.

Figure 2

The Gene Ontology (GO) analysis of up- and down-regulated circRNAs based on DEmiRNAs target genes. A. Enriched GO terms of up-regulated circRNAs. B. Enriched GO terms of down-regulated circRNAs. The y-axis represents the P value. Each bar represents a GO term which is classified in biological process, cellular component, and molecular function.

Figure 3

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for dysregulated circRNA. A. Enriched KEGG pathways of up-regulated circRNAs. B. Enriched KEGG pathways of down-regulated circRNAs. The left side represents the GO term, the right represents the number of circRNAs.