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Comprehensive Analysis of Circular RNA Expression Profiles in Gefitinib-Resistant Lung Adenocarcinoma Patients

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Abstract

Introduction: Gefitinib is a selective epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) widely used in lung adenocarcinoma (LUAD) patients harboring sensitive EGFR mutations. Although it has a good initial efficacy, acquired resistance to gefitinib is eventually inevitable. Studies have shown that circular RNA (circRNA) is involved in the development of acquired resistance to different anti-cancer drugs, but the comprehensive analysis of its expression profile and functions on acquired gefitinib resistance remains poor. **Methods:** To explore the aberrant circRNAs expression profiles, we collected peripheral plasma samples from 4 gefitinib-sensitive and 4 gefitinib-resistant patients for performing microarray analysis. Candidates of differentially expressed circRNAs were used and analyzed by bioinformatics modalities including gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and a constructed circRNA-microRNA RNA network. The differential expression of selected circRNAs was verified by quantitative real-time PCR (qRT-PCR). **Results:** A total of 2571 circRNAs with significantly different expression between the groups were identified by microarray analysis. GO, KEGG, and pathway enrichment analyses reveal that these differentially expressed circRNAs (DECs) were complicated in many biological pathways that may be related to EGFR-TKI resistance such as ABC transporter and PI3K-Akt pathways. A circRNA-microRNA network was constructed by 10 circRNAs potentially involved in EGFR-TKI resistance togethering with their corresponding microRNAs (miRNAs). Consistent with the results of microarray assay, hsa_circ_0030591 and hsa_circ_0040348 were validated to be upregulated in gefitinib-resistant patients by qRT-PCR. **Conclusions:** Our study provides valuable data on circRNAs expression profiles detected in liquid biopsy for LUAD patients with acquired gefitinib resistance, and we validate that upregulations of hsa_circ_0030591 and hsa_circ_0040348 may play key roles in EGFR-TKI resistance and thus serving as candidates for biomarker.

Keywords

circRNAs, gefitinib, lung adenocarcinoma, resistance, microarray

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Introduction

Lung cancer is one of the leading causes of cancer-related mortality among both men and women worldwide,[1] which is classified as 2 main types: small cell lung cancer and nonsmall cell lung cancer (NSCLC), accounting for about 15% and 85%, respectively. Among NSCLC, lung adenocarcinoma (LUAD) is the most common subtype accounting for more than 50% and has an increasing frequency.[2] Over the past decades, the discoveries of oncogenic driver genes and mutations for lung cancer allows the development of personalized targeted therapy, especially EGFR-TKIs. Gefitinib has been widely used as front-line EGFR-TKIs therapy in LUAD patients harboring sensitive EGFR mutations and confers favorable disease control. However, acquired resistance is eventually inevitable after an average of 9 to 11 months of gefitinib use, and the underlying mechanism remains a biological challenge.[3] Although it is believed that second mutations of EGFR, genetic amplifications or mutations of other driver genes, and histological transformation involving in EGFR-TKIs resistance may serve as predictive factors, most of these signatures need tumor biopsy for detection which largely limits its clinical practice. Therefore, the discovery of predictive biomarkers for acquired resistance to EGFR-TKIs resistance by different molecular signatures on different types of biopsies is of great clinical significance. The circulating RNAs, such as miRNAs, long noncoding RNAs (lncRNAs), and circRNAs, detected in plasma are emerging as a novel class of candidate biomarkers for molecular monitoring during targeted therapy.

Circular RNAs (circRNAs) are a newly defined class of noncoding RNAs originated from back-splicing events.[4] Based on its closed circular structure, circRNA specifically exhibits extraordinary stability, due to the lack of accessible ends exposed to nucleolytic degradation.[4] Recently, a line of studies has shown that circRNAs contain target sites for miRNAs and act as competitive endogenous RNA (ceRNA) to regulate miRNAs and other noncoding RNAs-related signaling axis, thus exerting various biological functions in cancer development.[5] With the development of circRNAs microarray and high-throughput second-generation sequencing, the identification of circRNAs expressed in various cancers is gradually coming to light. Studies have shown that circRNAs play an indispensable role in the occurrence, development, metastasis, and drug resistance of cancer. For example, hsa_circ_0004015 is reported to regulate the proliferation, invasion, and TKI resistance of NSCLC[6]; Hsa_circ_0007798 was shown to enhance the gefitinib sensitivity of LUAD cells via activating ASK1-dependent apoptosis.[7] In supporting of its intrinsic features and based on these research evidences, circRNA is emerging as a novel potential biomarker for cancer prognosis and treatment.

In this present study, we did a comprehensive analysis of circRNAs expression profiles in gefitinib-treated lung adenocarcinoma patients, and the analysis strategy of this study is shown in a flow chart (Figure 1). A total of 8 peripheral plasma samples from 2 groups of LUAD patients (4 gefitinib-sensitive and 4 gefitinib-resistant) harboring EGFR-sensitive mutations (L858R or 19Del) were collected to perform circRNAs microarray assay, The results showed that there were 2571 differentially expressed circRNAs (DECs) between 2 groups. Based on the bioinformatics analysis for these DECs involving in diverse biological processes, we constructed a circRNA-miRNA network including 10 circRNAs and their corresponding miRNAs. And we validated that hsa_circ_0030591 and hsa_circ_0040348 were indeed upregulated in gefitinib-resistant patients by qRT-PCR. Our study provides data on plasma circRNAs expression profiles for NSCLC patients with acquired gefitinib resistance, and we anticipate that hsa_circ_0030591 and hsa_circ_0040348 may serve as a potential biomarker for NSCLC patients treated with gefitinib.

Methods

Patients and Samples Collection

A total of 16 LUAD patients with EGFR Exon 19 deletion or Exon 21 L858R mutations treated with gefitinib in Ningbo Hwamei Hospital of the University of Chinese Academy of Sciences between March 2017 and May 2020 were included in this study. In the beginning, each patient is sensitive to gefitinib. After 5 to 24 months of treatment, 8 patients remained sensitive to gefitinib and were designated as the gefitinib-sensitive group, while 8 patients developed resistance and were designated as the gefitinib-resistant group. 8 patients' peripheral plasma samples (4 gefitinib-resistant and 4 gefitinib-sensitive patients) were used for microarray assay, and all 16 patients' plasma samples were used for validation by RT-PCR. 2.5 mL of peripheral plasma was collected from each patient and was stored in the PAXgene plasma RNA tubes (Becton, Dickinson and Company, American). The plasma samples were collected at a room temperature of 18–25° C and stored in −80°C refrigerator. This study was approved by the Medical Ethics Committee of Ningbo Hwamei Hospital, and all patients signed the informed consent before they were enrolled.

RNA Extraction, Amplification and Labeling

We used Trizol reagent (Invitrogen) to extract total RNA containing small RNA from whole plasma samples and purified it with miVana miRNA isolation kit (Ambion, Austin, TX, USA) according to manufacturer's protocol. The OD260/280 reading was measured using a spectrophotometer (Nanodrop ND-1000) to determine the purity and concentration of RNA. RNA integrity was detected by 1% formaldehyde denaturing gel electrophoresis. RNA was digested, amplified, and labeled by the cRNA Amplification and Labeling Kit (CapitalBio, Beijing, China) according to the manufacturer's product instruction.

CircRNA Microarray Assay

The purified RNA was hybridized with the CapitalBio Technology Human CircRNA Array v2 which was designed with 4 identical arrays per slide (4 × 180 K format) and each array containing probes interrogating about 170,340 human circRNAs.

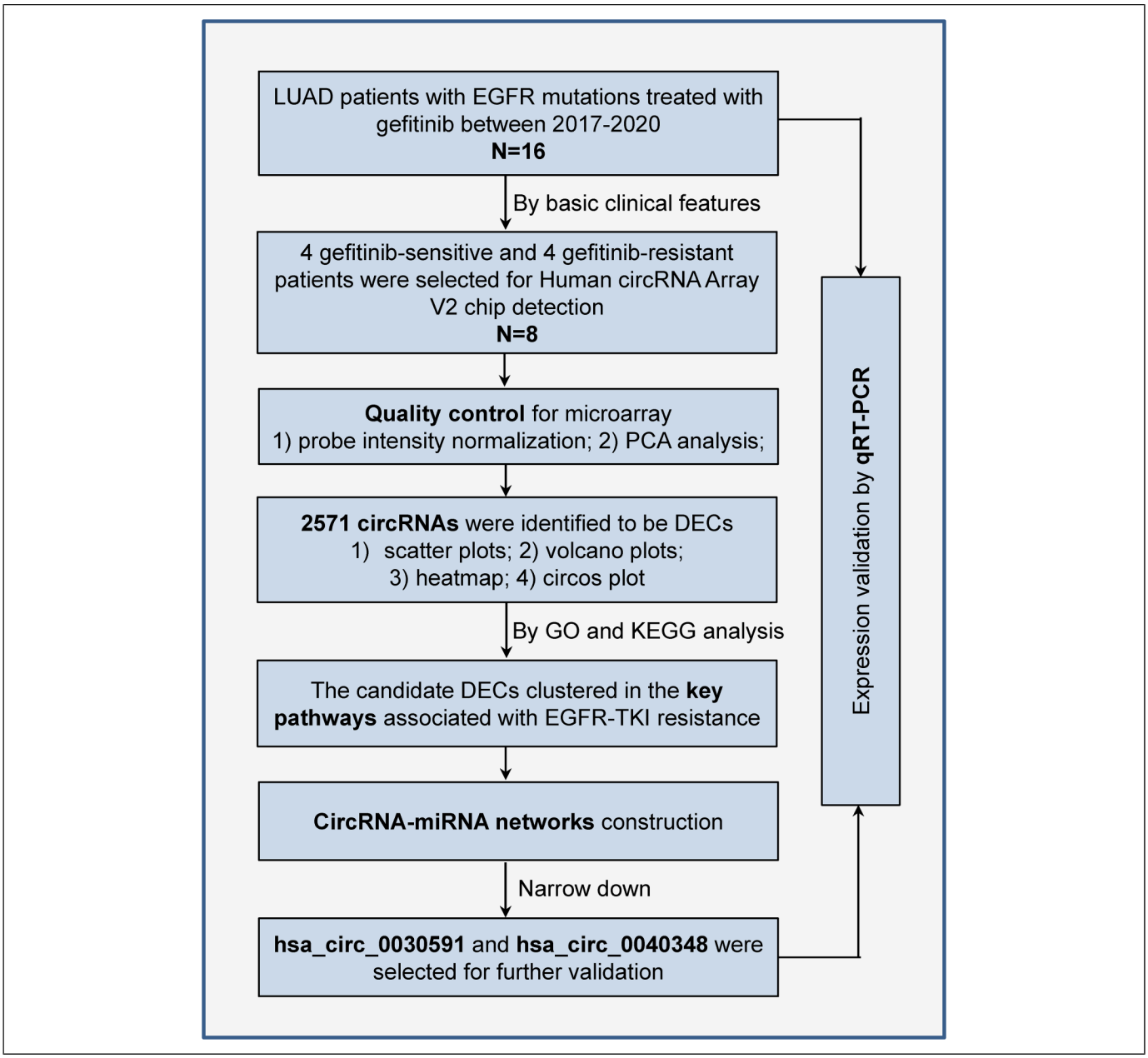

Figure 1. Flow chart of this study design.

Table 1. Information of Patients Subjected to circRNAs Microarray Assay.

Label	Group	Gender	Age (years)	Mutation type*	TNM stage	Duration of medication (months)
T1	Sensitive	Female	68	19Del	IV	23
T2	Sensitive	Female	57	L858R	IV	5
T3	Sensitive	Female	63	19Del	IV	18
T8	Sensitive	Male	70	L858R	IV	7
T4	Resistant	Male	66	19Del	IV	24
T5	Resistant	Female	72	L858R	IV	22
T6	Resistant	Male	59	19Del	IV	12
T7	Resistant	Male	67	L858R	IV	6

*19Del: exon 19 deletion; L858R: L858R mutation in exon 21

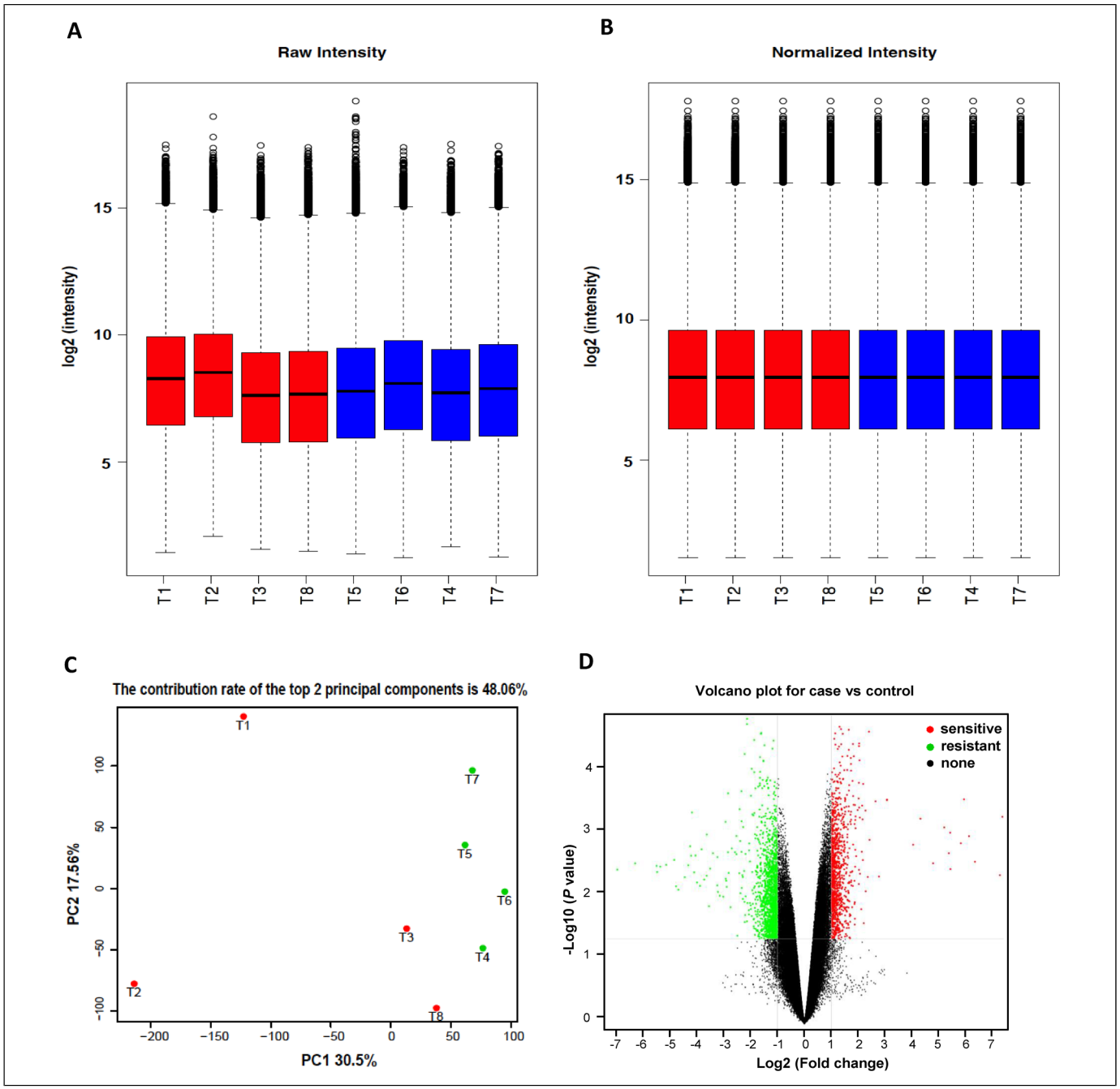

Figure 2. Expression profiles of circRNAs in gefitinib-sensitive and gefitinib-resistant groups. (A-B) Box plots are showing the distribution of circRNA expression in the 8 samples (red box: the sensitive group, blue box: the resistant group). (A) Is showing the raw probe signal, and (B) is showing the normalized probe signal. (C) PCA analysis (2D) of circRNA expression profiles. (D) The volcano plot. The abscissa value is −log10(*P*-values) and the ordinate value is log2(fold change). Both in (B) and (C), the upregulated genes are marked in red, the downregulated genes are marked in green, and the nonsignificantly different genes are marked in black.

The circRNA array data were analyzed by the GeneSpring software V13.0 (Agilent) and R software package (R version 3.1.2). Box plot was used to visualize the distribution of the intensities of all circRNAs in the samples before and after normalization. Principal component analysis (PCA) of circRNA expression profiles was completed by the Stats package. Scatter plot was used to assess the differences in circRNA expression between the 2 groups. Volcano plot was used to display the DECs. Heatmap was used to analyze the DECs in different samples completed by pheatmap package. Threshold values of fold change (FC) ≥ 2 or ≤ 0.5 with $P < 0.05$ were considered significantly differentially expressed.

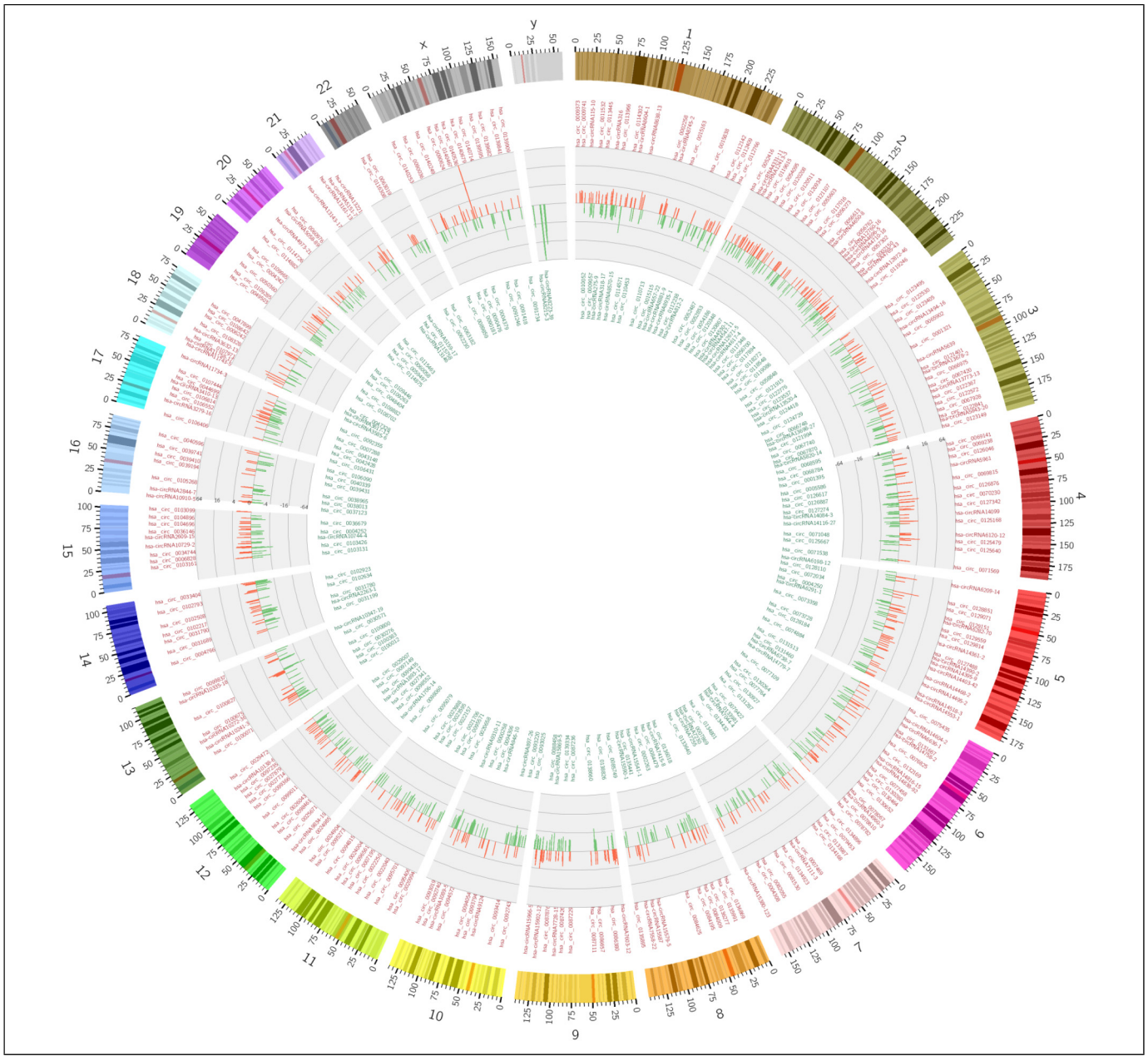

Figure 3. The circos plots. The outer colored bands indicate different chromosomes, and the inner circles indicate the degree of difference of different genes in the chromosomal position. Red represents upregulated differential genes, green represents downregulated differential genes, and the length of the column represents the fold change of the differential genes.

Construction of the circRNA-miRNA Network

The miRanda-3.3 software was used to select the DECs and predict their corresponding target miRNA. Narrow down by fold change > 1.5, $P < 0.05$ and each original signal value > 100, 5 circRNAs associated with the ATP-binding cassette (ABC) transporter pathway, and 5 circRNAs associated with the PI3K-Akt signaling pathway were selected togethering with their predictive target miRNAs to construct the circRNA-miRNA RNA network.

Validation of circRNAs Expression by qRT-PCR

Quantitative real-time PCR (qRT-PCR) was performed by SYBR-Green Premix Ex Taq Kit (Takara Bio, Japan), and the remix was applied by ABI PRISM 7500 sequence detection system (Life Technologies, USA) at 95 °C for 5 min and amplified by 40 cycles of denaturing at 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 40 s. The CT value was the fractional cycle number at which the fluorescence exceeded the given threshold. GAPDH was used to normalize the RNA preparation. The relative expression levels of

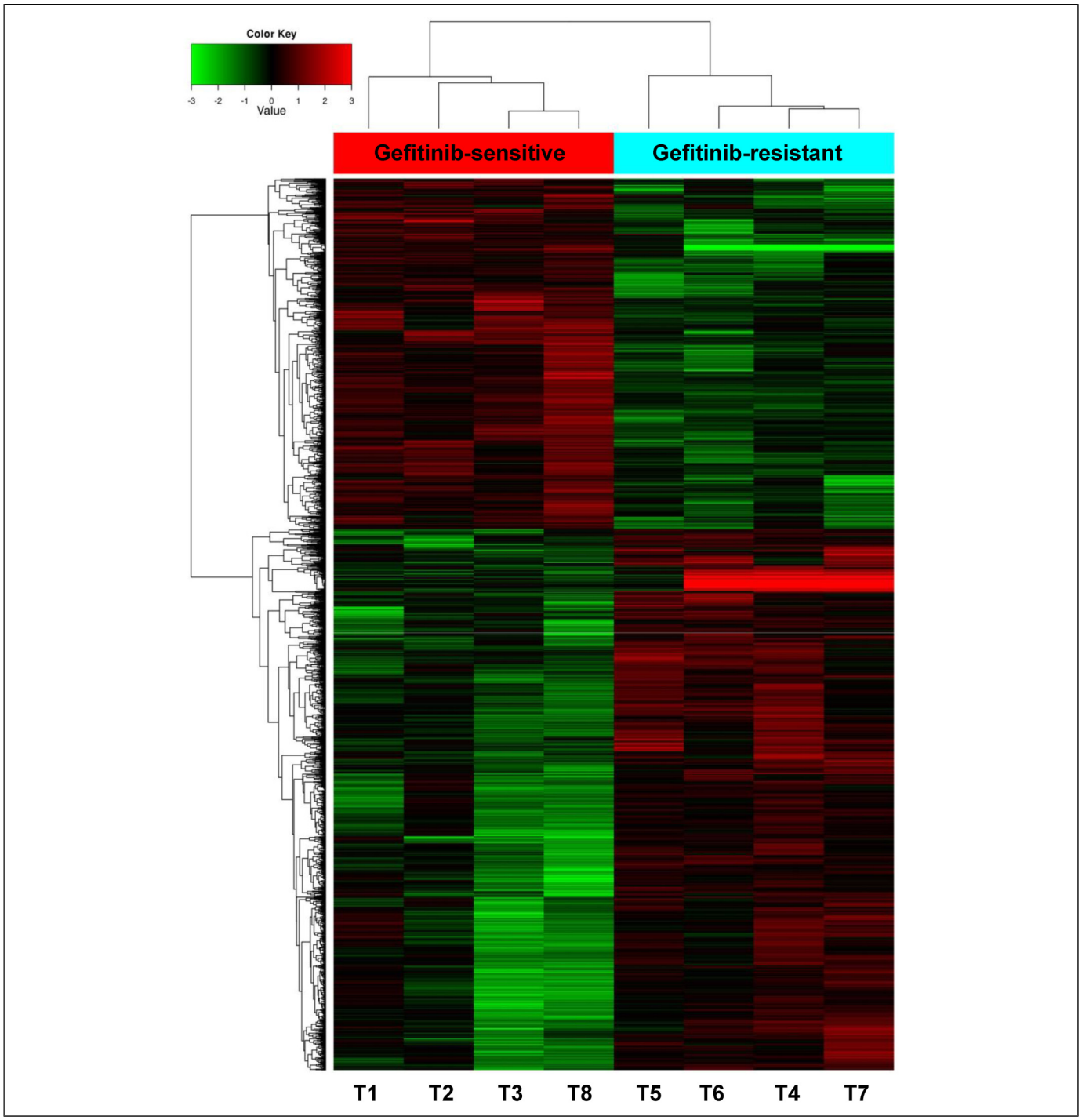

Figure 4. The heatmaps. Cluster analysis diagram of the sample. T1, T2, T3, and T8 are samples from patients in the sensitive group, while T4, T5, T6, and T7 are samples from patients in the resistant group.

circRNAs were calculated using $2^{-\triangle\triangle Ct}$ method. The primers were designed using Primer 6.0. The primers sequences of hsa_circ_0030591 were as follows: 5′-GTGCTCACTGGATTGTCTTCAT-3′ (forward) and 5′-GACTTTTCCCAGGCGTACATTT-3′ (reverse); The primers sequences of hsa_circ_0040348 were: 5′-CATGTGCAAAACCTTCCAACAC-3′ (forward) and 5′-TAACACGAGAGATCCACGGTAC-3′ (reverse). The primers sequences of GAPDH were: 5′-CAAATTCCATGGCACCGTCA-3′(forward) and 5′-AGCATCGCCCCACTTGATTT-3′ (reverse).

Statistical Analysis

SPSS v19.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses, and data were expressed as the mean ± SD. Student's two-tailed unpaired *t*-test was used to compare

Table 2. Top 20 Most Significant Aberrant Expressed circRNAs Based on Fold Change.

CircRNAs name	*P* value	Fold change	Trend
hsa_circ_0140537	.000666	159.90	up
hsa_circ_0091073	.005263	149.94	up
hsa_circ_0140759	.004314	120.95	down
hsa_circ_0140538	.003263	79.28	up
hsa_circ_0140760	.003450	76.38	down
hsa_circ_0140552	.001324	68.00	up
hsa_circ_0140536	.000359	59.80	up
hsa_circ_0140549	.001700	55.12	up
hsa_circ_0140758	.003777	44.20	down
hsa_circ_0140736	.004693	43.51	down
hsa_circ_0140539	.004238	42.24	up
hsa_circ_0140553	.001170	42.00	up
hsa-circRNA16166-3	.002420	40.58	up
hsa_circ_0003368	.003870	40.30	down
hsa_circ_0140547	.000971	36.04	up
hsa-circRNA16316-11	.003632	34.58	down
hsa_circ_0008297	.003070	28.14	down
hsa-circRNA16166-1	.003451	27.09	up
hsa_circ_0140746	.007790	26.87	down
hsa-circRNA16316-4	.008787	25.26	down

the circRNAs expression levels between 2 groups. $P < 0.05$ was considered statistically significant.

Results

Expression Profiles of circRNAs in Gefitinib-Sensitive and Gefitinib-Resistant Groups

To explore the potential circRNAs related to EGFR-TKI resistance, the Human circRNA Array V2 chip detection was performed in 8 peripheral plasma samples from 4 gefitinib-sensitive and 4 gefitinib-resistant patients; the clinical features of all 8 patients are listed in Table 1. Quality control for microarray was performed on the raw and normalized data. The box plots showed the raw probe intensity and the normalized intensity after log2 RNA signal transformation (Figure 2A and B). PCA analysis showed a clear segregation between 2 groups, indicating distinguished circRNA expression patterns (Figures 2C and S1A). In total, 2571 circRNAs were identified to be differentially expressed between these 2 groups, including 1011 upregulated and 1560 downregulated circRNAs ($P < 0.05$; FC$\geq$2 or $\leq$0.5), as shown in the scatter plots (Figure S1B) and the volcano plots (Figure 2D). The distribution of the parent genes of these candidate DECs was completed as circos plot, showing a wide distribution almost on all human chromosomes (Figure 3). Then the DECs were included in the hierarchical cluster heatmaps among gefitinib-sensitive and gefitinib-resistant groups and were also shown good discrimination between these 2 groups (Figure 4). The top 20 DECs with the rank of significant fold changes and their detailed information are listed in Table 2.

Functional Annotation of DECs

Next, we performed GO and KEGG pathway enrichment analyses to further explore the functions of these DECs. GO-enrichment items contained 6645 biological processes, 891 cellular components, and 1396 molecular functions, and the top 30 terms are shown in Figure 5A and B, and their hierarchies are shown in Figure S2A to C. The most enriched 3 GO terms of the biological process were chromosome segregation, cell cycle process, and single-organism organelle organization. KEGG pathway enrichment items included 100 BioCyc, 270 KEGG pathway, 91 Panther, and 1130 Reactome, and the top 30 terms are shown in Figure 5C and D, and the KEGG classification is shown in Figure S3. Specifically, DECs were most enriched in the ECM–receptor interaction, the cell cycle, and the Ribosome biogenesis pathways. More importantly, we found that ABC transporters ($P = 0.01$), which had been well established to involve in multidrug resistance,[8,9] and PI3K-Akt signaling pathway ($P = 0.02$), which are associated with EGFR-TKI resistance,[10,11] were both ranked in top 10 pathways enriched.

CircRNA-miRNA Networks

As mentioned earlier, ABC transporters and PI3K-Akt signaling were 2 enriched pathways related to drug resistance; we then narrowed down the top 5 circRNAs (hsa_circ_0030591, hsa_circ_0030574, hsa_circ_0030589, hsa_circ_0030598, and hsa_circ_0030600) associated with ABC transporters pathway and top 5 (hsa_circ_0040355, hsa_circ_0040341, hsa_circ_0105896, hsa_circ_0040348, and hsa_circ_00403681and) associated with PI3K-Akt pathway by significant fold change, respectively. All these 10 circRNAs were subject to miRanda-3.3 and predicted to have 83 target miRNAs (more than one binding site). The RNA networks of circRNA-miRNA are presented in Figure 6. In particular, we found that hsa_circ_0030591 (associated with the ABC transporters pathway) has 12 predictive target miRNAs related to lung cancer with one or more than one binding site, including hsa-let-7,[12] hsa-miR-103b,[13] hsa-miR-125a-5p,[14] hsa-miR-23,[15] and hsa-miR-449c-5p.[16] Similarly, hsa_circ_0040348 (associated with the PI3K-Akt signaling pathway) has 5 corresponding miRNAs with more than one binding site, and was predicted to bind to many lung cancer-related miRNAs with binding site, such as hsa-let-7,[12] hsa-miR-27a-3p,[17] hsa-miR-432-5p,[18] hsa-miR-513c-5p,[19] hsa-miR-622,[20] and hsa-miR-548.[21] More importantly, both of these 2 circRNAs met the selection criteria: (1) original processed signal of the microarray>100; (2) fold change>2; (3) $P < .05$; (4) upregulated in the gefitinib-resistant group; (5) predicted with more than 5 target miRNAs involving in lung cancer development or drug resistance signals. Based on this, hsa_circ_0030591 and hsa_circ_0040348 were 2 circRNAs selected for further validation, which may potentially involve in resistance of gefitinib treatment of LUAD patients.

Validation of the Selected DECs by qRT-PCR

To validate the narrow downed DECs from circRNA microarray assay, we used qRT-qPCR to detect the expressions

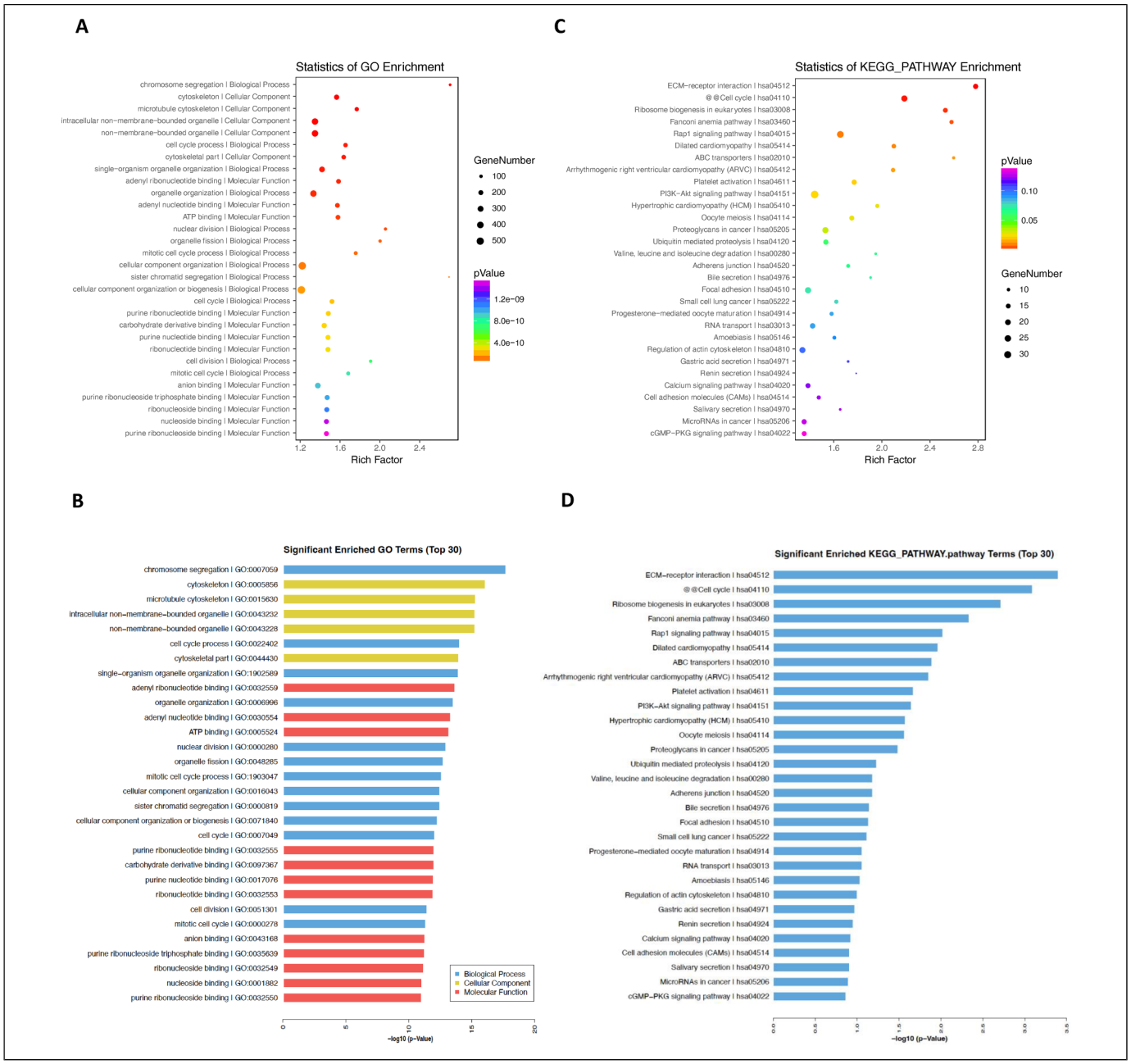

Figure 5. GO and KEGG pathway enrichment plot. (A-B) Statistics of GO-enrichment plot and bar graph (molecular function, biological process, and cellular component. (C-D) Statistics of pathway enrichment plot and bar graph. Enrichment items are obtained from databases of KEGG, Reactome, Panther, and BioCyc.

of hsa_circ_0030591 and hsa_circ_0040348 in peripheral plasma of all 16 LUAD patients harboring sensitive EGFR mutation, including 8 gefitinib-sensitive and 8 gefitinib-resistant patients. As shown in Figure 6, both hsa_circ_0030591 and hsa_circ_0040348 were indeed upregulated in the gefitinib-resistant group, compared with the gefitinib-sensitive group. The FCs of hsa_circ_0030591 and hsa_circ_0040348 were 3.41 ($P = .011$) and 2.54 ($P = .039$), respectively (Figure 7), which were basically consistent with the results obtained from microarray assay (FC = 2.14 and FC = 2.27).

Discussion

Gefitinib, as a widely used first-generation of EGFR-TKI, has been successfully applied in the treatment of patients with advanced NSCLC, especially LUAD harboring sensitive EGFR mutations. However, acquired resistance to EGFR-TKI is almost inevitable and most patients suffer disease recurrence after 1 to 2 years. CircRNAs, as an intriguing class of RNA with high stability and complicated functions in gene regulation, have been found to be involved in both cancer onset and progression through diverse biological mechanisms of action.[5,22]

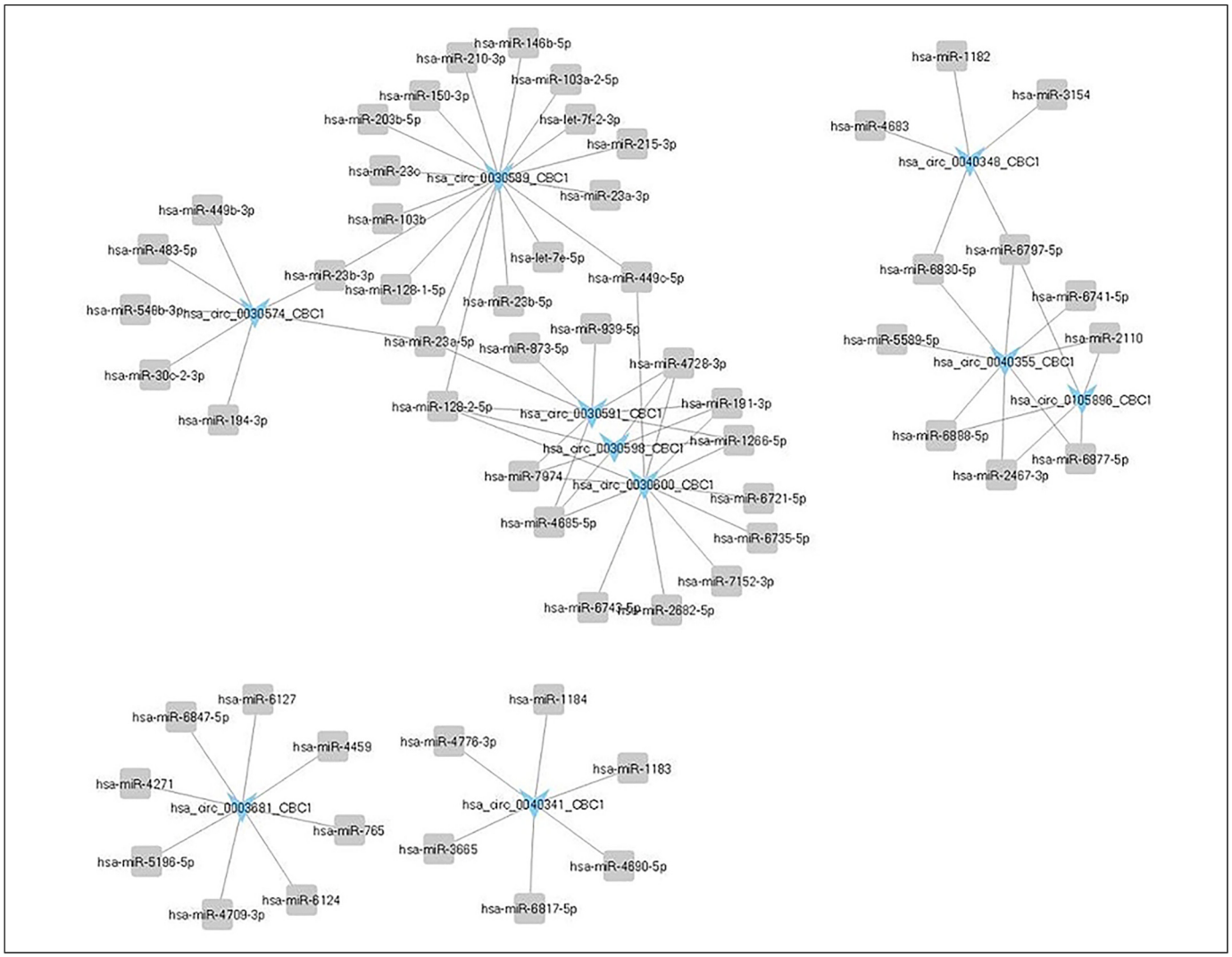

Figure 6. CircRNA/miRNA network. Top 10 circRNAs associated with ABC transporters and PI3K-Akt signaling pathways togethering with their 83 target miRNAs for construction of the RNA networks of circRNA-miRNA.

More importantly, recent studies showed that circRNAs may also play critical roles in drug resistance.[23] Further, circRNA expression can be tissue specific and detected in exosomes, cell-free saliva, and plasma.[4,24] Therefore, emerging interests focus on identifying and developing circRNAs as potential biomarkers for diagnostic and therapeutic utilities.[23]

In the present study, we collected a total of 16 peripheral plasma samples from LUAD patients harboring EGFR-sensitive mutations (L858R or 19Del) who were treated with gefitinib, including 8 cases of them were considered as gefitinib-sensitive and 8 were gefitinib-resistant. 4 gefitinib-sensitive and 4 gefitinib-resistant samples were subject to performing circRNAs microarray assay. It was found that there were 2571 DECs in the patients with acquired resistance to gefitinib compared with that of sensitive patients. The identified DECs were used for bioinformatics analysis including GO and KEGG analyses, the results showed that a line of items of biological process, cellular component, and molecular function were enriched, and chromosome segregation, cell cycle process, and single-organism organelle organization were the top 3 most significant biological processes. Furthermore, the hierarchy of enriched biological processes suggested mitotic cell cycle process, sister chromatid segregation, and nuclear division, which have been proven to be related with drug resistance in tumors,[25,26] might be involved in the mechanism of EGFR-TKI resistance in LUAD. Importantly, we found that ABC transporters and PI3K-Akt signaling pathway were ranked in the top 10 items with the most significant differences in the KEGG pathway. As a well-established multidrug resistance-related signaling and one of the classic pathways of EGFR-TKI in lung cancer, of which dysregulation may lead to rational resistance events consequently. Therefore, we selected the top 10 circRNAs in ABC transporters and PI3K-Akt signaling pathway and their corresponding miRNAs for constructing of circRNA-miRNA RNA network. In particular, many miRNAs associated with lung cancer growth, such as hsa-miR-128, let-7, and hsa-miR-27a,[6,12] were predicted to have binding sites within hsa_circ_0030591 and hsa_circ_0040348.

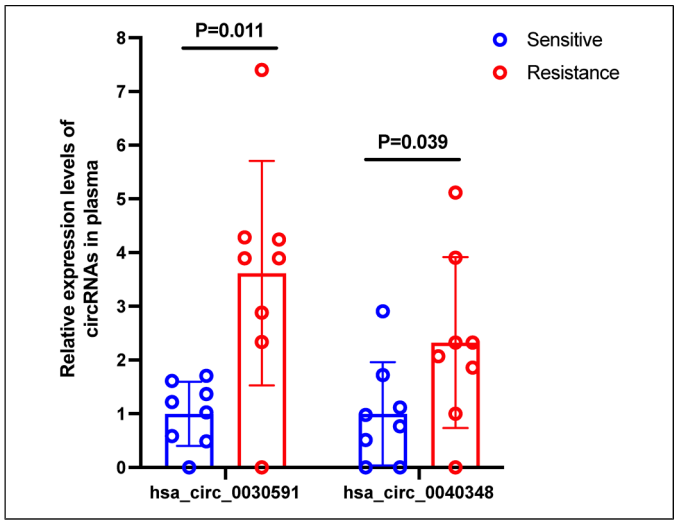

Figure 7. qRT-PCR validation. The relative expression levels of hsa_circ_0030591 and hsa_circ_0040348 in peripheral plasma of all 16 LUAD patients harboring sensitive EGFR mutation.

We then validated that hsa_circ_0030591 and hsa_circ_0040348 were indeed upregulated in gefitinib-resistant patients by qRT-PCR, which is consistent with the results of microarray.

For the limitations of our study, due to the nature of this study, we did not strictly calculate the sample size for performing circRNAs microarray assay (a small sample size with 4 gefitinib-sensitive and 4 gefitinib-resistant samples were included), which may result in selection bias. And for the biological validation step, the sample size was also limited to 8 gefitinib-sensitive and 8 gefitinib-resistant blood samples for qRT-PCR. A study with a larger sample size is warranted in the future to further confirm these results. Besides, we respectively collected the samples from matched gefitinib-sensitive and gefitinib-resistant patients; it may be unable to exhibit the dynamic change before and after gefitinib treatment; therefore, it would be a different good angle in the future studies to prospectively collect plasma samples of the same case before and after gefitinib treatment, which may specifically exhibit the dynamic change during the development acquired gefitinib resistance.

In summary, our study provides data and comprehensive analysis on plasma circRNAs expression profiles for NSCLC patients with acquired gefitinib resistance and showed that circRNAs may play key roles in gefitinib resistance. In particular, hsa_circ_0030591 and hsa_circ_0040348 may be candidates for further validation as potential biomarkers for therapeutic monitoring EGFR-TKIs resistance in NSCLC patients.

Author Contributions

CHW and HYL had the idea for the article and provided the final approval of the version to be published. JYZ performed the literature search and data analysis and drafted the manuscript. WL, SSX, ZKT, and XLS were involved in revising the manuscript critically for the important scientific content. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplemental material. Further inquiries can be directed to the corresponding authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Ningbo Hwamei Hospital of the University of Chinese Academy of Sciences (Approval number: PJ-NBEY-KY-2020-095-01; Approval date: 04/02/2020; Address of the review board: Room 402, Building 3, No. 41, Northwest Street, Haishu District, Ningbo City, Zhejiang, ZIP Code 315010). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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Supplemental Material

Supplemental material for this article is available online.

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. Succony L, Rassl DM, Barker AP, McCaughan FM, Rintoul RC. Adenocarcinoma spectrum lesions of the lung: Detection, pathology and treatment strategies. *Cancer Treat Rev*. 2021;99:102237.
3. Westover D, Zugazagoitia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. *Ann Oncol*. 2018;29(suppl_1):i10-i19.
4. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: Background, mechanisms, and therapeutic approaches. *Physiol Rev*. 2016;96(4):1297-1325.
5. Patop IL, Wust S, Kadener S. Past, present, and future of circRNAs. *EMBO J*. 2019;38(16):e100836.

6. Zhou Y, Zheng X, Xu B, et al. Circular RNA hsa_circ_0004015 regulates the proliferation, invasion, and TKI drug resistance of non-small cell lung cancer by miR-1183/PDPK1 signaling pathway. *Biochem Biophys Res Commun*. 2019;508(2):527-535.
7. Wang T, Liu Z, She Y, et al. A novel protein encoded by circASK1 ameliorates gefitinib resistance in lung adenocarcinoma by competitively activating ASK1-dependent apoptosis. *Cancer Lett*. 2021;520:321-331.
8. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer*. 2018;18(7):452-464.
9. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov*. 2006;5(3):219-234.
10. Morgillo F, Della Corte CM, Fasano M, Ciardiello F. Mechanisms of resistance to EGFR-targeted drugs: Lung cancer. *ESMO Open*. 2016;1(3):e000060.
11. Tian X, Gu T, Lee MH, Dong Z. Challenge and countermeasures for EGFR targeted therapy in non-small cell lung cancer. *Biochim Biophys Acta Rev Cancer*. 2022;1877(1):188645.
12. Hong W, Xue M, Jiang J, Zhang Y, Gao X. Circular RNA circ-CPA4/ let-7 miRNA/PD-L1 axis regulates cell growth, stemness, drug resistance and immune evasion in non-small cell lung cancer (NSCLC). *J Exp Clin Cancer Res*. 2020;39(1):149.
13. Wu J, Feng Z, Wang R, et al. Integration of bioinformatics analysis and experimental validation identifies plasma exosomal miR-103b/877-5p/29c-5p as diagnostic biomarkers for early lung adenocarcinoma. *Cancer Med*. 2022:1–11.
14. Naidu S, Shi L, Magee P, et al. PDGFR-modulated miR-23b cluster and miR-125a-5p suppress lung tumorigenesis by targeting multiple components of KRAS and NF-kB pathways. *Sci Rep*. 2017;7(1):15441.
15. Bersimbaev R, Pulliero A, Bulgakova O, Asia K, Aripova A, Izzotti A. Radon biomonitoring and microRNA in lung cancer. *Int J Mol Sci*. 2020;21(6):2154.
16. Zhou H, Manthey J, Lioutikova E, et al. The up-regulation of myb may help mediate EGCG inhibition effect on mouse lung adenocarcinoma. *Hum Genomics*. 2016;10(Suppl 2):19.
17. Lu X, Kang N, Ling X, Pan M, Du W, Gao S. MiR-27a-3p promotes non-small cell lung cancer through SLC7A11-mediated-ferroptosis. *Front Oncol*. 2021;11:759346.
18. Pal AS, Bains M, Agredo A, Kasinski AL. Identification of microRNAs that promote erlotinib resistance in non-small cell lung cancer. *Biochem Pharmacol*. 2021;189(SI):114154.
19. Tong C, Wang C, Wang Y, Xiao X. TNRC6C-AS1 Promotes thyroid cancer progression by upregulating LPAR5 via miR-513c-5p. *Cancer Manag Res*. 2021;13:6141-6155.
20. Liu S, Hu C, Li M, et al. Estrogen receptor beta promotes lung cancer invasion via increasing CXCR4 expression. *Cell Death Dis*. 2022;13(1):70.
21. Liu XW, Zhang CC, Zhang T. MiR-376b-3p functions as a tumor suppressor by targeting KLF15 in non-small cell lung cancer. *Eur Rev Med Pharmacol Sci*. 2020;24(18):9480-9486.
22. Guarnerio J, Bezzi M, Jeong JC, et al. Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell*. 2016;165(2):289-302.
23. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. *Nat Rev Clin Oncol*. 2022;19(3):188-206.
24. Vo JN, Cieslik M, Zhang Y, et al. The landscape of circular RNA in cancer. *Cell*. 2019;176(4):869-881. e813.
25. Sapkota H, Wasiak E, Daum JR, Gorbsky GJ. Multiple determinants and consequences of cohesion fatigue in mammalian cells. *Mol Biol Cell*. 2018;29(15):1811-1824.
26. Sinha D, Duijf PHG, Khanna KK. Mitotic slippage: An old tale with a new twist. *Cell Cycle*. 2019;18(1):7-15.