Analysis of circular RNA expression profiles of lung cancer in Xuanwei, China

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Abstract

Background: Mounting evidence indicates that circular RNAs (circRNAs) could play a pivotal role in cancers. However, due to the lack of sensitive biomarkers, most lung cancer in Xuanwei (LCXW) patients are still diagnosed at an advanced stage accompanied with distant metastasis.

Methods: According to the stage of LCXW patients and tissue sources, circRNAs microarray detection was carried out in six groups. Considering fold change, raw intensity, the length of circRNAs, and P-value, we selected eight circRNAs for further study. A total of 50 paired LCXW tissues were carried out real-time quantitative polymerase chain reaction (RT-qPCR) in order to extended sample size to verify the expression of these circRNAs.

Results: We designed 13 617 human circRNA probes for the human circular RNA microarray, detected 10 819 circRNA in six groups of samples; 537 circRNAs were differentially expressed consistently in every stage. Through RT-qPCR, we selected 8 circRNAs, three of which were upregulated (hsa_circ_0005927, hsa_circ_0069397 and hsa_circ_0000937) and five were downregulated (hsa_circ_0001936, hsa_circ_0005255, hsa_circRNA_406010, hsa_circ_0007064, hsa_circ_0000907) in tumor tissues, only hsa_circ_0001936 showed the opposite expression between microarray and RT-qPCR, others were consistent. Additionally, hsa_circ_0005927 and hsa_circ_0001936 were significantly correlated with tumor size, and hsa_circRNA_406010 was related to the prognosis of LCXW patients.

Conclusion: Together, these results suggest that hsa_circ_0005927, hsa_circ_0001936, and hsa_circRNA_406010 may serve as the novel potential biomarkers for LCXW. Moreover, these results may provide a new insight for the pathogenesis of LCXW.

Keywords
biomarker, circRNA microarray, circular RNA, LCXW, microRNA

Yan Wang and Ling-Jiao Lu contributed equally to this study.

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1 | INTRODUCTION

Lung cancer is the most frequent cause of cancer-related death worldwide, while lung cancer in Xuanwei (LCXW) is one of the most deadly types of lung cancers in China, and the prognosis of LCXW remains poor. 5,6 Even though current advances in the chemotherapy and molecular targeting therapy for LCXW, the overall 5-year survival rate for the disease is less than 15% due to the limited therapeutic options, tumor metastasis, and recurrence. 2,3 Undoubtedly, a better understanding of the carcinogenesis is critical for the advance of diagnostic markers and aid novel effective therapies for LCXW patients.

In Xuanwei, located on the southwestern border of China, the morbidity and mortality of lung cancer are higher than the other regions of China. According to a survey in 2018, the male mortality rate in LCXW patients was 98.10/105, and the female mortality rate was 83.28/105. 4 Besides, LCXW possesses five unique epidemiological characteristics compared to lung cancer in other regions: related to burning coal rather than smoking; the main histological type is adenocarcinoma; the female is more likely suffered; farmers are more susceptible to lung cancer than urban residents; family aggregation phenomenon between the lung cancer patients. However, the researches were mainly on its epidemiology at present; the explanation for the molecular pathogenesis of LCXW patients.

Institutional Ethics Review Board of Kunming Medical University (Kunming, Yunnan, PR China). Informed consent was obtained from all patients.

2.1 | Patients and tissues

The LCXW patients enrolled met the following criteria: (a) the patient came from Xuanwei District of Yunnan and lived in this area for more than 15 years; (b) pathological diagnosis belongs to lung cancer; (c) excluding patient who suffered radiotherapy, chemotherapy or immunotherapy before operation; (d) excluding patient with immune system diseases, chronic consumptive diseases or infectious diseases; (e) excluding patient with other malignant tumors. A total of 26 patients (stage I: 10/26; stage II: 9/26; stage III: 7/26) were available for the circRNA microarray. The detection of circRNA microarray was carried out in six groups according to the stage of LCXW patients and tissue sources. For each group of samples, a sample labeled A which instead of LCXW tissues was mixed to cross a dot matrix; a sample labeled P which instead of adjacent normal lung tissues from LCXW patients was mixed to hybridize a dot matrix. Also, 50 paired tissues from LCXW patients were carried out RT-qPCR to extended sample size to verify the expression of circRNAs. All of the patients’ clinical information was recorded in detail. All samples including lung cancer tissues and their paired adjacent normal lung tissues were collected from patients who had received surgical treatment at the Thoracic Surgery Department of the First Affiliated Hospital of Kunming Medical University or Xuanwei Yunfeng hospital between September 2011 and September 2017. The pathological diagnosis and the stage of LCXW are obtained by professional pathologists. All the tissue samples were collected, immediately snap-frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

The use of all tissue blocks for this study was approved by the Institutional Ethics Review Board of Kunming Medical University (Kunming, Yunnan, PR China). Informed consent was obtained from all patients.

2.2 | Total RNA isolation and quality control

The total RNA was extracted from tissues with TRizol reagent (Invitrogen), according to the manufacturers’ protocol. The purity and concentration of RNA were determined by OD260 and OD280 using a NanoDrop ND-1000 (Thermo Fisher Scientific). The integrity of RNA was assessed by electrophoresis on a denaturing agarose gel.
2.3 | CircRNAs labeling and hybridization

Sample labeling and array hybridization were performed according to the manufacturer's protocol (Arraystar Inc). Briefly, total RNAs were digested with Rnase R (Epicentre, Inc) to remove linear RNAs and enrich circular RNAs (circRNAs). Then, the enriched circRNAs were amplified and transcribed into fluorescent cRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; Arraystar). The labeled cRNAs were purified by RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured by NanoDrop ND-1000. 1 μg of each labeled cRNA was fragmented by adding 5 μL 10× Blocking Agent and 1 μL of 25× Fragmentation Buffer and then heated the mixture at 60°C for 30 minutes. Finally, 25 μL 2× Hybridization buffer was added to dilute the labeled cRNA. 50 μL of hybridization solution was dispensed into the gasket slide and assembled to the circRNA expression microarray slide. The slides were incubated for 17 hours at 65°C in an Agilent Hybridization Oven. The hybridized arrays were washed, fixed, and scanned using the Agilent Scanner G2505C.

2.4 | Data collection and analysis of circRNA microarray

Scanned images were imported into Agilent Feature Extraction software for raw data extraction. Quantile normalization of raw data and subsequent data processing were performed using the R software limma package (Kangcheng Bio-tech). After quantile normalization of the raw data, low-intensity filtering was performed, and the circRNAs that at least three out of six samples have flags in “P” or “M” (“All Targets Value”) were retained for further analyses. When comparing two groups of profile differences (such as disease vs control), the “fold change” (ie, the ratio of the group averages) between the groups for each circRNA is computed. The statistical significance of the difference may be conveniently estimated by t test. circRNAs were having fold changes ≥2 and P-values < .05 are selected as the significantly differentially expressed.

2.5 | Annotation for circRNA/miRNA interaction

The circRNA/miRNA interaction was predicted with Arraystar’s homemade miRNA target prediction software based on TargetScan and miRanda. The five putative target miRNAs were identified from the above analysis. The putative target genes of these miRNAs were identified by Targetscan. Moreover, the differentially expressed circRNAs within all the comparisons were annotated in detail with the circRNA/miRNA interaction information.

2.6 | Filter valuable circRNA that potentially related to LCXW

To find valuable circRNAs for further study, we conducted five steps to filter circRNAs, which differentially expressed between tumor tissues and adjacent normal tissues at different stage: (a) Sort from the largest to smallest according to the fold change (FC ≥ 2); (b) The group raw intensity of all tested samples were rather than 200 (including 200); (c) CircRNAs length at 200-2000 bp; (d) P-value less than .05.

2.7 | Synthesis of cDNA and RT-qPCR

Total RNA of tissues was extracted using TRizol (Invitrogen) and RNA/DNA mini Kit (QIAGEN) according to the manufacturer’s protocol. Reverse transcription was carried out with PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa) according to the

| TABLE 1 | The PCR primers for related gene |
|----------|----------------------------------|
| Gene name | Primer sequences | Size (bp) |
| hsa_circRNA_104600 | Forward 5′-TGAATTTGGAGGTTCTATCTACCAG-3′ | 162 |
|   | Reverse 5′-CCTTAAATTTCCCCACTTCTTT-3′ | |
| hsa_circRNA_069397 | Forward 5′-TATCTCCCCCTATGCGCTTCTTTA-3′ | 165 |
|   | Reverse 5′-TCTACTGGGTTTCTCTCTATCA-3′ | |
| hsa_circRNA_105013 | Forward 5′-TTCTTCAGGAGTGTGAGGT-3′ | 60 |
|   | Reverse 5′-CTTGGTCAGTTGTTTGTGC-3′ | |
| hsa_circRNA_406010 | Forward 5′-TGCTTTTGAAGGTGATCTGC-3′ | 150 |
|   | Reverse 5′-AACGTCTGCTATCTGAGG-3′ | |
| HECW2 | Forward 5′-CCTTCTGGAGAACGGAGCCT-3′ | 193 |
|   | Reverse 5′-GCTGATCTACCTCTTGGAGCC-3′ | |
| β-actin (H) | Forward 5′-GTTGGCGAGAGGACTTGGATG-3′ | 73 |
|   | Reverse 5′-CTCTGAAACAACGGCATTCTATATT-3′ | |
manufacturer’s instructions. The cDNA was amplified by All-in-One™ qPCR Mix (GeneCopoeia, America) according to the manufacturer’s instructions in a SLAN-96P system. The primers were designed by Primer 3.0 and primer sequence of each circRNA and β-actin in this study were listed (Table 1). The conditions of amplification for hsa_circ_0005927 and hsa_circ_0069397: one cycle at 95°C for 10 minutes, 40 cycles at 95°C for 10 seconds followed by 61°C for 60 seconds; for hsa_circ_0001936: one cycle at 95°C for 10 minutes, 35 cycles at 95°C for 10 seconds followed by 64°C for 55 seconds; for hsa_circRNA_406010: one cycle at 95°C for 10 minutes, 40 cycles at 95°C for 10 seconds followed by 60°C for 45 seconds; for HECW2: one cycle at 95°C for 10 minutes, 40 cycles at 95°C for 10 seconds followed by 67°C for 45 seconds. Sequencing was applied to validate whether the products of qPCR were our target products. β-actin served as internal control.

2.8 | Statistical analysis

RT-qPCR results were analyzed and visualized by GraphPad Prism 5.0 (GraphPad Software). Wilcoxon signal rank test was applied to compare the expression level of selected circRNAs between XWLC tissue group and adjacent normal tissue group, and a P-value < .05 was considered statistically significant. Chi-square test was also applied to determine correlations between expression levels of circRNAs and various clinicopathological parameters of XWLC. Kaplan-Meier survival analysis was used to evaluate the relationship between circRNA expression and prognosis of LCXW.

3 | RESULTS

3.1 | Circular RNA microarray profile of lung cancer in Xuanwei

Total RNA was extracted from LCXW patients as previously described. First, microarray was performed to design 13 617 human circRNA probes, and 10 819 circRNA were detected in six groups of samples. As shown in Figure 1A, the results of six groups of samples have good uniformity. Next, we screened differentially expressed circRNA and drew the volcanic map, the red region represents circRNA, and the difference ratio is more than two times (Figure 1B, P ≤ .05).
3.2 | The differential expression of circRNAs

The fold changes of all differentially expressed circRNAs were more than 2.0. The differentially expressed identified circRNAs of upregulated and downregulated from I to III stages showed a gradually decreasing trend (Table 2). At each stage, the numbers of downregulated circRNAs were more than the upregulated circRNAs. 537 circRNAs were expressed consistently in all stages (Figure 2), including 143 upregulated circRNAs and 394 downregulated circRNAs.

3.3 | Analyses of the candidate circRNA

In order to find valuable circRNAs for further study, we conducted five steps to filter circRNAs which differentially expressed. Common circRNAs: differentially expressed in every stage, we selected hsa_circ_0005927, hsa_circ_0069397, hsa_circ_0001936, hsa_circ_0005255, and hsa_circRNA_406010 for further verification. The circRNAs of up and down from I to III stage gradually decreased: for specifically differentially expressed circRNAs at I stage, we selected hsa_circ_0000937, hsa_circ_0007064 for further study; For III stage, only hsa_circ_0000907 satisfied the criteria as previously described. The information of these circRNAs was listed in Table 3.

3.4 | Verification of 8 circRNAs expression

Initially, eight circRNAs were selected for validation, including three upregulated circRNAs (hsa_circ_0005927, hsa_circ_0069397, hsa_circRNA_0000937) and five downregulated circRNAs (hsa_circ_0001936, hsa_circ_0005255, hsa_circ_0007064, hsa_circ_0000907, hsa_circRNA_406010). However, after trying various primers, hsa_circ_0005255, hsa_circRNA_0000937, hsa_circ_0007064, and hsa_circ_0000907 cannot be amplified. Moreover, hsa_circ_0005927, hsa_circ_0001936, and hsa_circRNA_406010 showed statistically significant (Figure 3A-E), and only hsa_circ_0001936 showed the opposite expression between microarray and qRT-PCR. The characteristics of successfully validated circRNAs were shown in Table 4. Because some studies had shown intronic circRNA might regulate their parental gene, we selected intronic circRNA—hsa_circRNA_406010 for investigating its corresponding linear mRNA HECW2 in 37 pairs of XWLC tissues (Figure 3F), the qRT-PCR result showed there was no correlation between the expression of hsa_circ_406010 and HECW2 (Figure 3G), but HECW2 was significantly downregulated which shared the same tendency with hsa_circ_406010 in LCXW.

TABLE 2 The differentially expressed circRNAs from I to III stage in XWLC

| IA_vs_IP | IIA_vs_IIP | IIIA_vs_IIIP |
|----------|------------|--------------|
| Up       | Down       | Up           |
| 717      | 1055       | 612          |
| 183      | 40         | 537          |
| 45       | 350        | 680          |

FIGURE 2 Venn diagram of differentially expressed circRNAs at different stage in LCXW. The green cycle showed the differential expressed circRNAs at stage I, the red cycle showed the differential expressed circRNAs at stage II, and the blue cycle showed the differential expressed circRNAs in stage III.
HOXA10.28 MiR-619-5p was upregulated in prostatic cancer.29 Thus, we may believe hsa_circ_0005927, hsa_circ_0001936, and hsa_circcRNA_406010 might have a certain function in the development of LCXW via absorbing their predicted miRNAs.

4 | DISCUSSION

In this study, we discovered 537 differentially expressed circRNAs in every stage of LCXW, including 143 upregulated and 394 downregulated. Moreover, it indicated that differentially expressed circRNAs might be related to pathogenesis and development of LCXW. Through RT-qPCR, we identified hsa_circ_0005927 and hsa_circ_0001936 were significantly upregulated and hsa_circRNA_406010 was statistically downregulated in tumor tissues. Besides, upregulated hsa_circ_0005927 and hsa_circ_0001936 were significantly associated with tumor size, and downregulated hsa_circRNA_406010 was related to the prognosis of LCXW patients. Recently, Huang et al.30 analyzed three paired gastric cancer tissues and paired adjacent normal tissues by utilizing circRNA microarray and RT-qPCR, and they found that hsa_circ_0005927 is significantly downregulated in gastric cancer tissues. However, in this study, both microarray and RT-qPCR result showed hsa_circ_0005927 was upregulated in LCXW, and it is illustrated

**TABLE 3**  Eight candidate circular RNA

| CircRNA name              | Alias          | Regulation | Fold change | Length (bp) | Type | P-value            | Differentially expressed at |
|---------------------------|----------------|------------|-------------|-------------|------|--------------------|-----------------------------|
| hsa_circRNA_104600       | hsa_circ_0005927 | Up         | 9.6217641   | 379         | exonic | .01109507271      | Every stage                 |
| hsa_circRNA_069397       | hsa_circ_0069397 | Up         | 7.342784    | 1646        | exonic | .01439756458      | Every stage                 |
| hsa_circRNA_105013       | hsa_circ_0001936 | Down       | -10.4451322 | 676         | exonic | .02822017592     | Every stage                 |
| hsa_circRNA_005255       | hsa_circ_0005255 | Down       | -8.3910993  | 311         | exonic | .01946425299      | Every stage                 |
| hsa_circRNA_406010       |                | Down       | -7.0959405  | 1242        | intronic |                  | Every stage                 |
| hsa_circRNA_000937       | hsa_circ_0000937 | Up         | 7.5472908   | 1059        | exonic |                  | I stage                     |
| hsa_circRNA_102151       | hsa_circ_0007064 | Down       | -4.05287    | 356         | exonic |                  | I stage                     |
| hsa_circRNA_102471       | hsa_circ_0000907 | Down       | -2.5308133  | 898         | exonic |                  | III stage                   |

**FIGURE 3**  A–D, The relative expression levels of hsa_circRNA_104600, hsa_circRNA_069397, hsa_circRNA_105013, and hsa_circRNA_406010 were examined in 50 paired tissue samples by RT-qPCR; E, Comparison of circRNA expression levels between RT-qPCR results and microarray. Candidate circRNAs of differential expression in microarray were validated by RT-qPCR in LCXW samples. The heights of the columns represent the mean fold changes of expression level between LCXW and normal group. Asterisks indicate significant differences (* means $P \leq .05$; ** means $P \leq .01$; *** means $P \leq .005$; **** means $P \leq .001$). F, The relative expression levels of mRNA HECW2 were examined in 37 paired tissue samples by RT-qPCR; mRNA HECW2 was significantly downregulated in LCXW tissues. G, The relative expression levels of mRNA HECW2 and hsa_circRNA_406010 in 37 paired LCXW samples have no correlation.
dysregulation of hsa_circ_0005927 happened in the progression of tumor. Besides, this study also found that dysregulated hsa_circ_0005927 was related to the tumor size, suggesting that the expression of hsa_circ_0005927 may be related to the growth and proliferation of LCXW. What is more, the downregulated of predicted miRNA of hsa_circ_0005927 is also associated with progression of some tumors.22-25,31 It is worth noting that predicted miR-592 function as a tumor suppressor in NSCLC by targeting SOX9,25 SOX9 is significantly upregulated in NSCLC and NSCLC cell lines32 and promote the proliferation of lung cancer cells,33 and SOX9 also mediates Notch1-induced epithelial mesenchymal transformations (EMT) in lung adenocarcinoma.34 Therefore, all above suggested hsa_circ_0005927 may serve as a potential biomarker for LCXW.

RT-qPCR showed that hsa_circ_0001936 was significantly downregulated in LCXW, which was not consistent with the microarray result. The possible reason was that this study employed a method of mix samples when hybridized microarray. However, this method may contribute to losing personality characteristics of the individual sample; it was a benefit for acquiring a general trend. However, RT-qPCR was a further verification for microarray, and the result from RT-qPCR was more credible. There is no report about hsa_circ_0001936 with disease so far. However, this study identified the dysregulated hsa_circ_0001936 was associated with tumor size also. In addition, while among the hsa_circ_0001936 putative miRNA, miR-486-5p was downregulated and contributes to tumor progression by targeting protumorigenic ARHGAP5 in lung cancer.26

### TABLE 4 Four differently expressed circRNAs for qRT-PCR validation

| CircRNA name            | IA_vs_IP (raw intensity) | IIA_vs_IIP (raw intensity) | IIIA_vs_IIIP (raw intensity) | Regulation | Length | Fold change (microarray) | Type   |
|-------------------------|--------------------------|----------------------------|-------------------------------|------------|--------|---------------------------|--------|
| hsa_circRNA_104600      | 2970 428                 | 5691 458.5                 | 1787 268                      | Up         | 379    | 9.6217641                 | Exonic |
| hsa_circRNA_069397      | 2074.5 417               | 3744.5 353                | 1198 227                      | Up         | 1646   | 7.342784                  | Exonic |
| hsa_circRNA_105013      | 339 5288                 | 361 2671.5                | 604 1284.5                    | Down       | 676    | -10.4451322               | Exonic |
| hsa_circRNA_406010      | 322.5 3478               | 1452 2871                 | 629 849                       | Down       | 1242   | -7.0959405                | Intronic |

RT-qPCR showed that hsa_circ_0001936 was significantly downregulated in LCXW, which was not consistent with the microarray result. The possible reason was that this study employed a method of mix samples when hybridized microarray. However, this method may contribute to losing personality characteristics of the individual sample; it was a benefit for acquiring a general trend. However, RT-qPCR was a further verification for microarray, and the result from RT-qPCR was more credible. There is no report about hsa_circ_0001936 with disease so far. However, this study identified the dysregulated hsa_circ_0001936 was associated with tumor size also. In addition, while among the hsa_circ_0001936 putative miRNA, miR-486-5p was downregulated and contributes to tumor progression by targeting protumorigenic ARHGAP5 in lung cancer.26

### TABLE 5 Correlation between circRNA and clinicopathological features

| Parameter                        | Number | Percentage (%) | hsa_circRNA_104600 | hsa_circRNA_105013 | hsa_circRNA_406010 |
|----------------------------------|--------|----------------|--------------------|--------------------|--------------------|
| Total                            | 50     |                |                    |                    |                    |
| Sex                              |        |                |                    |                    |                    |
| Male                             | 26     | 52             | .4186              | .9442              | .5136              |
| Female                           | 24     | 48             |                    |                    |                    |
| Age (y)                          |        |                |                    |                    |                    |
| ≤50                              | 27     | 54             | .4831              | .3091              | .0567              |
| ≥51                              | 23     | 46             |                    |                    |                    |
| Smoking history                  |        |                |                    |                    |                    |
| Yes                              | 22     | 44             | .1035              | .1660              | .8326              |
| No                               | 28     | 56             |                    |                    |                    |
| Pathologic stage                 |        |                |                    |                    |                    |
| I                                | 12     | 24             | .4609              | .5018              | .2474              |
| II                               | 26     | 52             |                    |                    |                    |
| III                              | 12     | 24             |                    |                    |                    |
| Lymph node metastasis            |        |                |                    |                    |                    |
| Yes                              | 28     | 56             | .2952              | .8326              | .8326              |
| No                               | 22     | 44             |                    |                    |                    |
| Tumor size (cm)                  |        |                |                    |                    |                    |
| ≤3.0                             | 18     | 36             | .0145*             | .0373*             | .7067              |
| >3.0                             | 32     | 64             |                    |                    |                    |

*Means $P \leq .05$.
Therefore, hsa_circ_0001936 may also serve as a potential biomarker for LCXW.

Hsa_circRNA_406010 was statistically downregulated in LCXW, and there are no reports showed hsa_circRNA_406010 is related to diseases. According to literature, intron circRNAs can directly regulate the expression of the host gene, due to hsa_circRNA_406010 belongs to intron circRNA, and expression of its host gene is also studied. It was found that mRNA HECW2 was downregulated in 37 paired LCXW tissues, although the expression trend was the same with hsa_circRNA_406010, there was no correlation between HECW2 and hsa_circRNA_406010. Due to the limited number of samples, the direct role of hsa_circRNA_406010 and mRNA HECW2 still needs further experimental confirmation. Besides, this study also found that the prognosis of LCXW patient with low expression of hsa_circRNA_406010 was poor. Additionally, among the hsa_circRNA_406010 putative miRNA, miR-619-5p was upregulated in prostatic cancer.29 Thus, hsa_circRNA_406010 may be a potential biomarker for LCXW and even serve as a biomarker for evaluating the prognosis of LCXW.

We selected eight circRNAs as the candidates for RT-qPCR verification; however, the successful verification rate of circRNA was not ideal because of after trying various primers, and half of candidates cannot be amplified. There are two reasons may be responsible for: firstly, variable splicing diversity in circRNAs; secondly, although the total amount of circRNAs could reach 10 times than linear RNA, some circRNA at a very low expression, which leads to amplify difficulty. In addition to appealing for two reasons, it may be due to the quality and specificity of the primers, or the quality and specificity of the enzymes involved in the reaction, or it may be due to the PCR cycling conditions set in the experiment. Huang et al30 have mentioned in their study of circRNAs, the reason for failure to design circRNA primers may cause by the pretty low expression of circRNA.

In summary, this study reveals the circRNAs expression profile in LCXW. hsa_circ_0005927, hsa_circ_0001936, and hsa_circRNA_406010 were differentially expressed in LCXW, and their predicted miRNAs took part in regulating the progression of many cancers, for example, dysregulated of these miRNAs were associated with tumor size or related to poor prognosis of LCXW(The predicted miRNA of hsa_circ_0005927 include hsa-miR-609, hsa-miR-758-5p, hsa-miR-548c-3p, hsa-miR-592, hsa-miR-452-3p; The predicted miRNA of hsa_circ_0001936 include hsa-miR-141-5p, hsa-miR-486-5p, hsa-miR-578, hsa-miR-135a-5p, and hsa-miR-135b-5p; The predicted miRNA of hsa_circRNA_406010 includes hsa-miR-4778-5p, hsa-miR-5690, hsa-miR-1200, hsa-miR-619-5p, and hsa-miR-212-5p). Thus, we may believe hsa_circ_0005927, hsa_circ_0001936, and hsa_circRNA_406010 may serve as potential biomarkers and estimate the prognosis for LCXW. However, the specific mechanisms including target gene of downstream of these circRNAs need further investigate.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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