Verification of expression of LINC00648 in the serum of lung cancer patients by TCGA database

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Abstract: TCGA data were used to verify the expression of LINC00648 in lung cancer patients to provide a reference for clinical practice. Lung cancer transcriptome data were downloaded by the TCGA database and LINC00648 data were extracted for analysis. Fifty-two patients with lung cancer diagnosed in our hospital from May 2014 to March 2016 were collected as the patient group and 30 normal people as the control group. RT-qPCR was used to detect the expression of LINC00648 in serum, follow up of patients was carried out, and bioinformatics was used to analyze the potential mechanism of LINC00648. LINC00648 was highly expressed in lung cancer. Lymphatic metastasis and probability of low differentiation were significantly increased, and the overall survival rate of highly expressed patients with lung cancer was reduced and the prognosis was poor. LINC00648 had 17 potential miR-targeted and 78 miR-targeted mRNAs. LINC00648 was found to have participated in SMAD binding, transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding, PDZ domain binding, cytokine binding, activin binding, RNA polymerase II activating transcription factor binding, transforming growth factor-beta receptor binding, etc. LINC00648 participated in the signal pathways of the Hippo signaling pathway, Transcriptional misregulation in cancer, MAPK signaling pathway, Proteoglycans in cancer. There were 55 co-expression pairs in PPI protein co-expression analysis, of which KIF11 was the most common.  High expression of LINC00648 in lung cancer patients indicates poor prognosis of patients and is expected to become a potential diagnostic marker for lung cancer.

Key words: TCGA; LINC00648; Lung cancer; Bioinformatics analysis.

Introduction

As one of the most common causes of cancer-related deaths in the world, lung cancer has become the most serious health and public safety problem in the world (1). A recent epidemiological statistic showed that in 2018 (2), there were more than 2 million new lung cancer patients and 1.7 million death in the world. The incidence rate of lung cancer is increasing year by year and shows a younger trend. Lung cancer can be divided into small cell lung cancer and non-small cell lung cancer according to pathological types, with the proportions of 15-20 and 80-85% respectively (3, 4). Although targeted therapy of molecular biological tumors has improved the survival rate of lung cancer in recent years, the survival rate of lung cancer patients is still not ideal. Studies have found that most patients have progressed to the middle and late stages after being admitted to the hospital for pathological diagnosis, which makes surgical treatment difficult and leads to poor prognosis (5–7). Data showed that the 5-year survival rate of lung cancer patients is only 16%, and how to improve this problem has become one of the main problems that clinicians need to solve (8).

At present, besides the pathological biopsy, the most accurate diagnostic scheme for lung cancer is imaging detection (9). Previous studies reported (10) that in large randomized trials using low-dose CT, it was found that the mortality rate of low-dose CT was 20% lower than that of chest X-ray. This is also an important reason why low-dose CT is recommended for early diagnosis of lung cancer. However, due to the radiation and high price of low-dose CT, it is difficult for patients to carry out multiple tests (11). Therefore, clinicians urgently need to find an economical and effective noninvasive biomarker for early lung cancer to solve this problem.

Non-coding RNA has been a hot topic discussed by various disciplines in recent ten years (12). Previously, due to technical and instrument defects, research on non-coding RNA was limited. With the continuous improvement of technology, more and more non-coding RNA has been found to be associated with the occurrence and development of various diseases (13). Among them, non-coding short-chain RNA (miR), cyclic RNA (circRNA), Long non-coding RNA (LncRNA) are the most prominent (14-16). LncRNA is a long-chain non-coding RNA with a length of more than 200nt. Previous studies found that (17) LncRNAs are differentially expressed in various tumors, such as lung cancer, gastric cancer, liver cancer, colon cancer and other tumors (18-21), and have certain diagnostic value and is expected to become a potential diagnostic marker for tumors. LINC00648 is one of the members of LncRNA. Previous studies on LINC00648 are very few and there is little research in
lung cancer. In this study, we found that LINC00648 is highly expressed in lung cancer patients through TCGA database analysis, and is expected to become a potential diagnostic marker for lung cancer patients.

Therefore, in this study, we verified the diagnostic value and potential mechanism of LINC00648 in lung cancer patients through the TCGA database combined with clinical experiments, providing potential directions for clinical diagnosis and treatment.

Materials and Methods

TCGA database analysis

We logged on to the https://portal.gdc.cancer.gov to download mRNA data of Lung adenocarcinoma and Lung squamous cell carcinoma transcripts. The data were integrated by Perl script, and then LINC00648 was retrieved from lung cancer patients. The extracted data were transformed into the log (x+1,2), and then the difference analysis was carried out. Altogether 1145 samples were downloaded, of which 1037 were cancer samples and 108 were adjacent samples.

Clinical data collection

Fifty-two patients with lung cancer diagnosed and treated in our hospital from May 2014 to March 2016 were collected as the study group, including 40 male patients and 12 female patients, with an average age of 62.5± 6.2 years. Another 30 normal people examined during the same period were collected as the control group, including 22 males and 8 females, with an average age of 61.5± 5.3 years. This study was approved by the Medical Ethics Committee of our hospital. Inclusion criteria were as follows: Altogether 52 patients were confirmed as primary non-small cell lung cancer through histological examination, and targeted radiotherapy and chemotherapy were not carried out before the study; patients were classified into different stages according to the 8th Union for International Cancer Control (UICC); patients and their families in the study were informed and signed informed consent forms. Exclusion criteria were as follows: patients with other tumors and with a survival period of fewer than 3 months; patients did not cooperate with follow-up; patients with congenital diseases and immune deficiency diseases; patients had a serious infection before this study.

Collection and analysis of serum samples

A 5 ml of peripheral blood from two groups of subjects were collected, placed for 30 min, centrifuged at 3000 rpm for 10 min, and then the supernatant was collected for RT-qPCR amplification.

RT-qPCR detection

Total RNA was extracted by TRIzol kit (Invitrogen Company, USA), and the purity, concentration and integrity of total RNA were detected by UV spectrophotometer and agarose gel electrophoresis. Subsequently, reverse transcription was performed by the TaqMan™ Reverse reverse transcription kit (Invitrogen Company, USA), and the transcription steps were strictly operated according to the kit instructions. The cDNA was subjected to subsequent research. PCR amplification was carried out using the PrimeScript RT Master Mix kit (Takara Bio Company, Japan). Amplification system: 10 μL of SYBR qPCR Mix, 0.8 μL of upstream and downstream primers, 2 μL of cDNA product, 0.4 μL of 50× Rox reference dye, and finally RNase-free water was used to make up to 20 μL. PCR reaction conditions: pre-denaturation at 95 ℃ for the 60s, denaturation at 95 ℃ for 30s, annealing and extension at 60 ℃ for 40s, with a total of 40 cycles. In the experiment, three parallel repeating wells were designed, and all specimens were repeatedly tested for 3 times. GENE and GADPH were used as internal references, the data were analyzed with 2^−ΔΔct (22). The PCR instrument was ABI 7500PCR, the upstream primer of LINC00648: 5'-TCCCCAGTACCCCT-3', and the downstream primer: 5'-GCC TAACCGGTCTGCTG-3'; GADPH upstream primer: 5'-GAGAGAGAGAGAGAGACCTCACCCTAC-3', downstream primer: 5'-ACTGTGAGAGAGAGAGAGAGAGATTC-3'.

Follow-up

The patients were followed up until March 2019. The follow-up was counted through telephone and outpatient electronic medical records at the 1st, 3rd, 6th, 9th and 12th months of each year.

Bioinformatics analysis

Http://starbase.sysu.edu.cn/ was adopted to predict LINC00648 targeted miR. miRDB, miRTarBase and TargetScan online websites were adopted to predict targeted mRNA for the predicted targeted miR, and Cytoscape to visualize ceRNA (competing for endogenous RNAs, Endogenous competitive RNA) network. The R language ClusterProfiler package was used to enrich GO and KEGG, and String was used to visualizing the protein co-expression network for targeting RNA.

Statistical analysis

In this study, the SPSS20.0 software package was applied to perform statistical analysis of the data. GraphPad 7 software package was used to visualize the required pictures. K-S test was used to analyze the distribution of measurement data. Normal distribution data were expressed by mean± standard deviation (Meas±SD), an inter-group comparison was conducted by an independent sample t-test, a multi-group comparison was conducted by one-way analysis of variance, expressed by F, and afterward, pairwise comparisons were conducted by LSD-t-test. ROC was used to visualize the diagnostic value of LINC00648 in lung cancer, Pearson test was used to analyze the correlation of various genes, K-M survival curve was used to plot the total survival condition of patients, Log-rank test was applied for analysis, and multivariate Cox regression was applied to analyze the prognosis of patients. When p<0.05, there was a statistical difference.

Results

Baseline data

First, comparing the clinical data of the two groups of patients, it was discovered that there were statistical differences in gender, age, BMI, smoking history and drinking history between the two groups (p> 0.05), indicating that the two groups were comparable. Fifty-two...
High expression of LINC00648 can be used as a prognostic marker of lung cancer.

In order to confirm the diagnostic value of LINC00648 in lung cancer, we further visualized the ROC curve. The result showed that the area under the curve of LINC00648 in the diagnosis of lung cancer was 0.886, which had high diagnostic value. In order to observe the expression of LINC00648 in patients with early lung cancer, we further compared the expression of LINC00648 in patients with different TNM stages. The results showed that LINC00648 was differentially expressed in different stages, and the ROC curve was visualized to find that LINC00648 has certain clinical value in diagnosing early lung cancer (Figure 1 and Table 2).

Table 2. Relationship between LINC00648 and pathological data of patients.

| Factor                | High expression (n=26) | Low expression (n=26) | \( \chi^2 \) value | p value |
|-----------------------|-----------------------|-----------------------|---------------------|---------|
| Gender                |                       |                       |                     |         |
| Male                  | 22 (84.62)            | 18 (69.23)            | 1.733               | 0.188   |
| Female                | 4 (15.38)             | 8 (30.77)             |                     |         |
| Age                   |                       |                       |                     |         |
| ≥60 years old (n=37)  | 17 (65.38)            | 20 (76.92)            | 0.843               | 0.359   |
| <60 years old (n=15)  | 9 (34.62)             | 6 (23.08)             |                     |         |
| Tumor size            |                       |                       |                     |         |
| ≥3cm (n=25)           | 14 (53.85)            | 11 (42.31)            | 0.693               | 0.405   |
| <3cm (n=27)           | 12 (46.15)            | 15 (57.69)            |                     |         |
| Lymphatic metastasis  |                       |                       |                     |         |
| Yes (n=20)            | 14 (53.85)            | 6 (23.08)             | 5.200               | 0.023   |
| No (n=32)             | 12 (46.15)            | 20 (76.92)            |                     |         |
| Differentiation       |                       |                       |                     |         |
| Low differentiation   | 16 (61.54)            | 6 (23.08)             | 7.879               | 0.005   |
| Medium+High           | 10 (38.46)            | 20 (76.92)            |                     |         |
| TNM staging           |                       |                       |                     |         |
| I+II (n=32)           | 11 (42.31)            | 21 (80.77)            | 8.125               | 0.004   |
| III+IV (n=20)         | 15 (57.69)            | 5 (19.23)             |                     |         |

Figure 1. Expression of LINC00648 in lung cancer patients. A: LINC00648 expression increased in lung cancer samples in the TCGA database. B: LINC00648 expression increased in the patient's serum. *** indicates that \( p > 0.001 \).
High expression of LINC00648 can be used as a prognostic marker of lung cancer.

Table 3).

Relationship between LINC00648 and survival of lung cancer patients

Altogether 52 patients were followed up. The overall survival rate of the patients was 21.15% (11 cases). According to the death of the patients, the patients were divided into a survival group and death group. Comparing the expression of LINC00648 in the two groups of patients, it was found that the expression of LINC00648 in the death group was significantly higher than that in the survival group. ROC curve analysis showed that the area of LINC00648 under the predicted death curve was 0.747, which has certain clinical value. According to the median value of LINC00648, the patients were further divided into high and low expression groups. Observing the survival of patients, it was found that the overall survival rate of patients in the high expression group was significantly reduced (p = 0.001, Figure 3).

Prognosis analysis of lung cancer patients

The pathological data of lung cancer patients were collected for the univariate Cox regression analysis. The results showed that lymph metastasis, differentiation, TNM staging and LINC00648 were independent factors affecting the prognosis of patients. Further multivariate Cox regression analysis found that lymph metastasis and LINC00648 were independent prognostic factors of patients (Table 4).

Bioinformatics analysis

Through bioinformatics analysis, the relevant mechanism of LINC00648 was further explored. First, a total of 17 targeted miRs were found by predicting

Table 3. ROC curve parameters.

| Indicators                                  | AUC   | 95CI%  | Specificity | Sensitivity | Youden index | Cut-off |
|---------------------------------------------|-------|--------|-------------|-------------|--------------|---------|
| LINC00648 diagnosis of lung cancer          | 0.886 | 0.817-0.955 | 96.67%   | 76.92%   | 73.59%       | > 1.169 |
| LINC00648 distinguishing early lung cancer from healthy people | 0.759 | 0.619-0.900 | 96.67%   | 50.00%   | 46.67%       | > 1.169 |
| LINC00648 diagnosis of early and advanced lung cancer | 0.907 | 0.823-0.990 | 100.00%  | 79.41%   | 79.41%       | > 1.297 |

Figure 3. Relationship between LINC00648 and patient survival; A: LINC00648 expression increased in dead patients. B: Predicting curve area of LINC00648 on patient death. The best specificity and sensitivity were 100.00% and 48.78% when the cut-off was 1.353. C: The overall survival rate of patients with high expression of LINC00648 decreased.

Table 4. Prognostic factors of lung cancer patients.

| Factor                                      | Univariate Cox regression | Multivariate Cox regression |
|---------------------------------------------|---------------------------|-----------------------------|
|                                        | p-value | HR     | HR(95CI%) | p-value | HR     | HR(95CI%) |
| Gender (male VS female)                    | 0.178   | 1.704  | 0.785-3.699 |  |  | |
| Age (≥60VS < 60)                           | 0.353   | 0.730  | 0.376-1.418 |  |  | |
| Tumor size (≥3cmVS < 3cm)                  | 0.357   | 0.747  | 0.402-1.389 |  |  | |
| Lymphatic metastasis (yes VS no)           | 0.006   | 2.471  | 1.301-4.692 | 0.019 | 2.200 | 1.141-4.24 |
| Differentiation (low differentiation VS medium+high differentiation) | 0.018   | 2.147  | 1.141-4.041 | 1.393 | 0.704 | 0.704-2.756 |
| TNM phase (I+II VS III+IV)                 | 0.007   | 0.421  | 0.223-0.793 | 0.203 | 0.627 | 0.306-1.286 |
| LINC00648 (high VS low)                    | 0.001   | 2.882  | 1.52-5.467 | 0.004 | 2.647 | 1.375-5.095 |
High expression of LINC00648 can be used as a prognostic marker of lung cancer.

Discussion

In this study, we have proved through experiments that LINC00648 is highly expressed in lung cancer patients. Patients with high expression showed at stage III+IV, lymphatic metastasis and the probability of low differentiation significantly increased. Patients showed poor prognosis. LINC00648 was expected to become a potential biomarker for the diagnosis and pro-

Table 5. Potential miR targeting LINC00648.

| LncRNA       | miR                  |
|--------------|----------------------|
| LINC00648    | miR-223-3p, miR-199a-5p, miR-199b-5p, miR-186-5p, miR-4761-3p, miR-3179, miR-224-5p, miR-5687, miR-766-5p, miR-9-5p, miR-580-3p, miR-380-3p, miR-369-3p, miR-374c-5p, miR-655-3p, miR-605-3p, and miR-625-3p |

Table 6. Potential mRNA targeting 17 miR.

| miR              | mRNA                                                                 |
|------------------|----------------------------------------------------------------------|
| miR-223-3p, miR-199a-5p, miR-199b-5p, miR-186-5p, miR-4761-3p, miR-3179, miR-224-5p, miR-5687, miR-766-5p, miR-9-5p, miR-580-3p, miR-380-3p, miR-369-3p, miR-374c-5p, miR-655-3p, miR-605-3p, and miR-625-3p | MAP3K3, CDK5R1, KLF13, WNT2, PTX3, LINGO1, ZNF107, PRELP, TWF1, HOXD10, EFNA3, CAV1, TRIM2, IGF2BP3, JUNB, MAGI2, ADM, PODXL, IL6ST, DRAM1, PABPC1L2B, KIF14, ZBED3, FZD5, DEPDC1, RHOB, ATAD2, TMEM164, CFTR, TRIM67, PLAG2, ARHGAP6, KIAA1462, CREB5, BCL2L1, CSTF2, PABPC1L2A, RBL1, MAP1B, TRIB1, LMO2, CYB5A, EPB41L3, TGFBR3, DCL1, EYA4, LIN28B, SESN3, PPM1F, FBK2, PHACTR2, FZD4, SLCL1A, FAM60A, DSN1, SMAD7, BTG2, MTIF2, CSF1, EN2, TNFRSF21, ZNF106, TGFBR2, ZEB1, ZFHX4, ZNF365, ZWINT, UNG, NETO2, ONECUT2, C15orf52, FRY, ECT2, FOXP1, KIF11, TXNIP, MEF2C, and ACSL4 |

Table 7. GO terminology. See the text for more information.

| ID           | Description                                                                 | p-value | geneID | Count |
|--------------|------------------------------------------------------------------------------|---------|--------|-------|
| GO:0046332   | SMAD binding                                                                | 2.971E-04 | SMAD7/TGFBR2/TGFBR3/MAGI2 | 4     |
| GO:0001228   | transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding | 4.370E-04 | HOXD10/JUNB/MEO2/MEO2C/ONECUT2/KLF13/BCL11BCREB3 | 8     |
| GO:0030165   | PDZ domain binding                                                           | 5.59E-04 | CFTR/TGFBR3/FZD4/KIF14 | 4     |
| GO:0019955   | cytokine binding                                                             | 1.039E-03 | IL6ST/TGFBR3/TGFBR3/FZD4 | 4     |
| GO:0048185   | activin binding                                                              | 1.188E-03 | SMAD7/TGFBR3 | 2     |
| GO:0001102   | RNA polymerase II activating transcription factor binding                     | 1.246E-03 | LMO2/MEO2C/RBL1 | 3     |
| GO:0005160   | transforming growth factor beta receptor binding                              | 1.246E-03 | SMAD7/TGFBR2/TGFBR3 | 3     |

Table 8. KEGG terminology. See the text for more information.

| ID          | Description                        | p-value | geneID | Count |
|-------------|------------------------------------|---------|--------|-------|
| hsa04390    | Hippo signaling pathway            | 5.297E-04 | SMAD7/TGFBR2/WNT2/FZD5/FZD4 | 5     |
| hsa05202    | Transcriptional misregulation in cancer | 1.214E-03 | LMO2/MEO2C/ZEB1/TGFBR2/BCL11B | 5     |
| hsa04010    | MAPK signaling pathway             | 1.66E-03 | CSF1/EFNA3/MEO2C/MAP3K3/TGFBR2/PLA2G4D | 6     |
| hsa05205    | Proteoglycans in cancer            | 1.874E-03 | CAV1/HOXD10/WNT2/FZD5/FZD4 | 5     |
gnosis of lung cancer patients. Further bioinformatics analysis showed that LINC00648 participates in the progress of several important biological pathways and tumor signaling pathways, which is also an important direction for our future research.

LncRNA is a long-chain non-coding RNA. In recent years, many studies have found that LncRNA is involved in the occurrence and development of lung cancer (22, 23). For example, Nie et al. (24) found that LncRNA-UCA1 plays a carcinogenic role in non-small cell lung cancer by targeting miR-193a-3p, and Cui et al. (25) found that up-regulation of LncRNA SNHG1 can inhibit miR-101-3p and activate Wnt/β-catenin signaling pathway to promote the progress of non-small cell lung cancer, while Jiang et al. (26) found that circulating LncRNA XLOC_009167 can be used as a biomarker for lung cancer diagnosis. LINC00648 is also a member of the LncRNA family and located on human chromosome 14q21.3. Previous studies on LINC00648 were rare, but we found that LINC00648 was highly expressed in lung cancer samples through the TCGA database, which indicates that LINC00648 is expected to become a potential marker for lung cancer diagnosis and prognosis. Therefore, we further confirmed the role of LINC00648 in lung cancer through clinical research.

In this study, we detected the expression of LINC00648 in serum samples of clinical patients and found that it increased significantly in patients with lung cancer, and further compared the expression in patients with different stages. We found that the expression of LINC00648 also showed an upward trend with the continuous increase of TNM stages. LINC00648 had a high clinical diagnostic value in the diagnosis of lung cancer and early lung cancer and was expected to become a potential diagnostic marker of lung cancer by the ROC curve. Lung cancer is one of the top three malignant tumors in clinical mortality and morbidity. Prognosis and survival of lung cancer patients have always been a difficult problem for clinicians to solve (27). Previous studies found that (28) early diagnosis can effectively improve the survival rate of lung cancer patients, but there were relatively few markers for predicting the survival prognosis of lung cancer patients. In this study, we have followed up the patients and compared the expression of LINC00648 in patients’ serum according to the survival status of the patients. It was found that the expression of LINC00648 in patients’ serum in the death group was obviously increased. Moreover, the area of LINC00648 under the prediction death curve of lung cancer patients was more than 0.7 through ROC curve analysis, which has certain clinical value. After further observation of the survival of patients in the high and low expression groups, it was found that the overall survival rate of patients in the high expression group was significantly reduced, which suggested that LINC00648 could be used as a potential observation indicator for the survival of lung cancer patients, and also found that LINC00648 could be used as an independent prognostic factor for lung cancer patients through Cox regression analysis.

Through the above research, we could preliminarily explain that LINC00648 has high clinical value in the diagnosis and prognosis of lung cancer, but the relevant mechanism is still unclear. Therefore, in this study, we determined the possible relevant mechanisms through bioinformatics, laying the foundation for our subsequent research. In this study, we first predicted miR that may have binding sites with LINC00648, they further predicted the potential binding mRNA of miR and visualized the ceRNA network map. In recent years, studies found that crosstalk occurs between LncRNAs and mRNAs through competition and sharing of miR response elements (MRE), called ceRNAs (29). And there were many studies indicated that (30, 31) ceRNAs play different roles in the occurrence and development of various diseases. In this study, we found a total of 17 miRs that may be combined with LINC00648.

Through literature search, we found that miR-223-3p, miR-199a-5p, miR-199b-5p are relatively more studied in lung cancer (32-34). Further, online prediction software was used to analyze the predicted miR downstream mRNA, and 78 potential mRNA were found. Through GO enrichment analysis, it was found that LINC00648 may participate in the biological functions of SMAD binding, Transcription Activator Activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding, PDZ domain binding, cytokine binding, activin binding, RNA polymerase II activating transcription factor binding, transforming growth factor-beta receptor binding. However, KEGG enrichment analysis found that LINC00648 participated in the occurrence of the Hippo signaling pathway, Transcriptional misregulation in cancer, MAPK signaling pathway, Proteoglycans in cancer. Many previous kinds of literature reported that (35-38) Hippo signaling pathway, Transcriptional misregulation in cancer, MAPK signaling pathway, Proteoglycans in cancer signaling pathways play an important role in the occurrence and development of lung cancer, and these studies are the direction and cornerstone of our future research. Subsequently, we also predicted PPI protein co-expression of the predicted mRNA and found 55 co-expression pairs, of which KIF11 was the protein with the most over-expression pairs. KIF11 is called member 11 of the kinesin family and is located on a human 10q23.33 chromosome. Previous studies found that the differential expression of (39) KIF11 is closely related to the poor prognosis of lung cancer, but the specific mechanism has not been clearly studied, which may be our future research direction (40-55).

In this study, we verified the clinical value of LINC00648 in lung cancer through the TCGA database and clinical experiments and predicted the potential mechanism of LINC00648 through bioinformatics analysis. However, this study still has certain limitations. First, we did not collect serum samples of patients with benign lung lesions. Whether LINC00648 has diagnostic value in differentiating patients with benign lung lesions is unclear. Secondly, although we analyzed the potential mechanism of LINC00648 through bioinformatics, we did not carry out further experiments to verify it. Finally, more samples are needed to further verify whether LINC00648 can be popularized clinically for the small sample size. Therefore, we hope to carry out basic experiments in future research and collect different samples and cases to further demonstrate the results of this study. To sum up, high expression of LINC00648 in lung cancer patients indicates poor pro-
gnosis of patients and is expected to become a potential diagnostic marker for lung cancer.

**Author contribution**

YL and CW wrote the manuscript, analyzed and interpreted the patient general data. JL performed PCR. YT was responsible for observation indicators analysis. All authors read and approved the final manuscript.

**References**

1. Torre LA, Siegel RL, Jemal A. Lung cancer statistics[M]/Lung cancer and personalized medicine. Springer Cham 2016: 1-19.
2. 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: 394-424.
3. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JH, Beasley MB, Chirieac LR, Dacic S, Duhig E, Flieder DB, Geisinger K. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 2015; 10: 1243–1260.
4. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The eighth edition lung cancer stage classification. Chest 2017; 151: 193–203.
5. International Early Lung Cancer Action Program Investigators. Survival of patients with stage I lung cancer detected on CT screening. N Engl J Med 2006; 355: 1763-1771.
6. Dillman RO, Herndon J, Seagren SL, Eaton Jr WL, Green MR. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of cancer and leukemia group B (CALGB) 8433 trial. J Natl Cancer Inst 1996; 88: 1210-1215.
7. Nesbitt JC, Putnam JR JB, Walsh GL, Roth JA, Mountain CF. Survival in early-stage non-small cell lung cancer. Ann Thorac Surg 1995; 60: 466-472.
8. Cronin KA, Ries LA, Edwards BK. The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute. Cancer 2014; 120: 3755.
9. Henschke CI, Yip R, Smith JP, Wolf AS, Flores RM, Liang M, Salvatore MM, Liu Y, Xu DM, Yankelevitz DF. CT screening for lung cancer: part-solid nodules in baseline and annual repeat rounds. AJR Am J Roentgenol 2016; 207: 1176-1184.
10. National Lung Screening Trial Research Team. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011; 365: 395-409.
11. Xie Y, Zhang Y, Du L, Jiang X, Yan S, Duan W, Li J, Zhan Y, Wang L, Zhang S, Li S. Circulating long noncoding RNA act as potential novel biomarkers for diagnosis and prognosis of non-small cell lung cancer. Mol Oncol 2018; 12: 648-658.
12. Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks cross-talks across cardiovascular diseases. Sci Rep 2017; 7: 10185.
13. Liu C, Yang Z, Deng Z, Zhou Y, Gong Q, Zhao R, Chen T. Upregulated IncRNA ADAMTS9-AS2 suppresses progression of lung cancer by targeting miR-193a-3p. Cancer Lett 2016; 371: 99-106.
14. Cui Y, Zhang F, Zhu C, Geng L, Tian T, Liu H. Upregulated IncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/β-catenin signaling pathway. Oncotarget 2017; 8: 17785.
15. Jiang N, Meng X, Mi H, Chi Y, Li S, Jin Z, Tian H, He J, Shen W, Tian H, Pan J. Circulating IncRNA XLOC_009167 serves as a diagnostic biomarker to predict lung cancer. Clin Chim Acta 2018; 486: 26-33.
16. Chen W, Hang Y, Xu W, Wu J, Chen L, Chen J, Mao Y, Song J, Song J, Wang H. BLACAT1 predicts poor prognosis and serves as oncogenic IncRNA in small-cell lung cancer. J Cell Biochem 2019; 120: 2540-2546.
17. Bracht JW, Mayo-de-las-Casas C, Berenguer J, Karachaliou N, Rosell R. The present and future of liquid biopsies in non-small cell lung cancer: combining four biosources for diagnosis, prognosis, prediction, and disease monitoring. Curr Oncol Rep 2018; 20: 70.
18. He B, Bai Y, Kang W, Zhang X, Jiang X. LncRNA SNHG5 regulates imatinib resistance in chronic myeloid leukemia via acting as a CeRNA against MiR-205-5p. Am J Cancer Res 2017; 7: 1704.
19. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, Ma W. ceRNA in cancer: possible functions and clinical implications. J Med Genet 2015; 52: 710-718.
20. Song C, Zhang J, Qi H, Feng C, Chen Y, Cao Y, Ba L, Ai B, Wang Q, Huang W, Li C. The global view of mRNA-related ceRNA cross-talks across cardiovascular diseases. Sci Rep 2017; 7: 10185.
21. Liu C, Yang Z, Deng Z, Zhou Y, Gong Q, Zhao R, Chen T. Upregulated IncRNA ADAMTS9-AS2 suppresses progression of lung cancer through inhibition of miR-223-3p and promotion of TGFB3. IUBMB Life 2018; 70: 536-546.
22. Ahmad A, Khansarinejad B, Hosseinikhani S, Ghanei M, Mowlaw SJ. miR-199a-5p and miR-495 target GRP78 within UPR pathway of lung cancer. Gene. 2017; 620: 2540-2546.
23. Chen W, Zhang E, Zhong Z, Jiang M, Yang X, Zhou D. Dysregulated long noncoding RNAs (lncRNAs) in hepatocellular carcinoma: implications for tumorigenesis, disease progression, and liver cancer stem cells. Mol Cancer 2017; 16: 165.
24. Yue B, Qiu S, Zhao S, Liu C, Zhang D, Yu F, Peng Z, Yan D. LncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. J Gastroenterol Hepatol 2016; 31: 595-603.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods; 2001; 25: 402-408.
26. Chiu HS, Songmvan S, Patel E, Chen TW, Singh VP, Zorman B, Patil SL, Pan Y, Chatterjee SS, Caesar-Johnson SJ, Demchok JA. Pan-cancer analysis of IncRNA regulation supports their targeting of cancer genes in each tumor context. Cell Rep 2018; 23: 297-312.
27. Nie W, Ge HJ, Yang XQ, Sun X, Huang H, Tao X, Chen WS, Li B. LncRNA-UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p. Cancer Lett 2016; 371: 99-106.
High expression of LINC00648 can be used as a prognostic marker of lung cancer.

37. Kang KA, Piao MJ, Hewage SR, Ryu YS, Oh MC, Kwon TK, Chae S, Hyun JW. Fisetin induces apoptosis and endoplasmic reticulum stress in human non-small cell lung cancer through inhibition of the MAPK signaling pathway. Tumour Biol 2016; 37: 9615-9624.
38. Theocharis AD, Karamanou NK. Proteoglycans remodeling in cancer: underlying molecular mechanisms. Matrix Biol 2019; 75: 220-59.
39. Schneider MA, Christopoulos P, Muley T, et al. AURKA, DLGAP5, PX2, KIF11 and CKAP5: Five specific mitosis-associated genes correlate with poor prognosis for non-small cell lung cancer patients. Int J Oncol. 2017, 50: 365-372.
40. Lou Y, Shi J, Guo D, Qureshi AK, Song L. Function of PD-L1 in antitumor immunity of glioma cells. Saudi J Biol Sci 2017; 24(4): 803-807.
41. Lou Y, Yang J, Wang L, Chen X, Xin X, Liu Y. The clinical efficacy study of treatment to Chiari malformation type I with syringomyelia under the minimally invasive surgery of resection of Submeningeal cerebellar Tonsillar Herniation and reconstruction of Cisterna magna. Saudi J Biol Sci 2019; 26(8): 1927-1931.
42. Nie Y, Luo F, Lin Q. Dietary nutrition and gut microflora: A promising target for treating diseases. Trends Food Sci Technol 2018; 75: 72-80.
43. Nie Y, Luo F, Wang L, Yang T, Shi L, Li X, Shen J, Xu W, Guo T, Lin Q. Anti-hyperlipidemic effect of rice bran polysaccharide and its potential mechanism in high-fat diet mice. Food Funct 2017; 8(11): 4028-4041.
44. Ren Y, Jiao X, Zhang L. Expression level of fibroblast growth factor 5 (FGF5) in the peripheral blood of primary hypertension and its clinical significance. Saudi J Biol Sci 2018; 25(3): 469-473.
45. Wang L, Lin Q, Yang T, Liang Y, Nie Y, Luo Y, Shen J, Fu X, Tang Y, Luo F. Oryzanol Modifies High Fat Diet-Induced Obesity, Liver Gene Expression Profile, and Inflammation Response in Mice. J Agri Food Chem 2017; 65(38): 8374-8385.
46. Zhang T, Wu X, Shaheen SM, Zhao Q, Liu X, Rinklebe J, Ren H. Ammonium nitrogen recovery from digestate by hydrothermal pretreatment followed by activated hydrochar sorption. Chem Eng J 2020; 379: 1-54.
47. Zhu B, Pang R, Chevallier J, Wei YM, Vo DT. Including intangible costs into the cost-of-illness approach: a method refinement illustrated based on the PM2.5 economic burden in China. Europ J Health Econ 2019; 20(4): 501-511.
48. Chen H, Chen Y, Yang L. Intelligent early structural health prognosis with nonlinear system identification for RFID signal analysis. Comput Commun 2020; 157: 150-161.
49. Chen HX, Huang L, Yang L, Chen YT, Huang JM. Model-based method with nonlinear ultrasonic system identification for mechanical structural health assessment. Trans Emerg Telecommun Technol 2020; 1-15.
50. Li W, Jia MX, Wang JH, Lu JL, Deng J, Tang JX, Liu, C. Association of MMP9-1562C/T and MMP13-77A/G Polymorphisms with Non-Small Cell Lung Cancer in Southern Chinese Population. Biomolecules 2019; 9(3): 107-119.
51. Liang Y, Lin Q, Huang P, Wang Y, Li J, Zhang L, Cao J. Rice Bioactive Peptide Binding with TLR4 To Overcome H2O2-Induced Injury in Human Umbilical Vein Endothelial Cells through NF-kappa B Signaling. J Agri Food Chem 2018; 66(2): 440-448.
52. Lou Y, Guo D, Zhang H, Song L. Effectiveness of mesenchymal stems cells cultured by hanging drop vs. conventional culturing on the repair of hypoxic-ischemic-damaged mouse brains, measured by stemness gene expression. Open Life Sci 2016; 11(1): 519-523.
53. Chen X, Xu Y, Meng L, Chen X, Yuan L, Cai Q, Shi W, Huang G. Non-parametric partial least squares-discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data. Sens Actuators B Chem 2020; 311:127924-127931.
54. Guo T, Lin Q, Li X, Nie Y, Wang L, Shi L, Xu W, Hu T, Guo T, Luo F. Octacosanol Attenuates Inflammation in Both RAW264.7 Macrophages and a Mouse Model of Colitis. J Agri Food Chem 2017; 65(18): 3647-3658.
55. Jiang X, Zhu B, Chevallier J, Xie R. Allocating provincial CO2 quotas for the Chinese national carbon program. Australian J Agri Res Econ 2018; 62(3): 457-479.