Potential Therapeutic Value of Mangiferin for Lung Adenocarcinoma (Luad): A Comprehensive Study Based on in Vitro Experiments and Bioinformatics

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Research article

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Abstract

Background: Lung adenocarcinoma (LUAD), which is the most common lung cancer type in never-smokers and primarily occurs in women, requires effective treatment methods. Mangiferin is a polyphenol widely found in mango trees and has been reported to have chemotherapeutic and preventive potential against various types of cancer. Thus, we investigated the potential therapeutic value of mangiferin for LUAD.

Methods: Cell lines A549, H2030 and H1299 were processed with mangiferin to detect and screen for differentially expressed lncRNAs and mRNAs. Close associations between extracted common lncRNAs and mRNAs were identified for lncRNA-mRNA network construction. Based on the network and an online database, target lncRNAs, target genes and meaningful lncRNA-mRNA pairs were identified, and signaling pathway analysis was performed.

Results: The top 200 lncRNA-mRNA pairs were used to construct the network. We identified 12 target lncRNAs and 18 target genes. Gene nodes in the network were mostly visualized on the PI3K-Akt signaling pathway (P < 0.01). Furthermore, lncRNA-mRNA pairs that contained the genes \textit{ARHGAP29}, \textit{BRIX1}, \textit{CD109}, \textit{CDK1}, \textit{CTNNAL1}, \textit{DAB2IP}, \textit{DDIT4L}, \textit{GPR162}, \textit{ICAM5}, \textit{KCNAB3} and \textit{MMP7} were considered to be meaningful lncRNA-mRNA pairs affected by mangiferin in LUAD.

Conclusions: Our study provided a comprehensive understanding of the potential therapeutic value of mangiferin for LUAD.

Introduction

Lung adenocarcinoma (LUAD), which belongs to the category of non-small-cell lung cancer and arises from the distal alveolar epithelium, is the most common lung cancer type. LUAD can be detected by radiography, fine needle aspiration biopsy and pathological analysis, while the expression variations of signaling molecules in LUAD also provide new candidates for diagnosis\cite{1,2}. Recently, treatment methods for LUAD, including surgery, chemotherapy or molecular-targeted therapy, and even immunotherapy, have been employed\cite{3–7}. Interestingly, traditional Chinese medicine (TCM) treatment can alleviate the symptoms caused by LUAD and ease multidrug resistance, has a synergistic and detoxifying effect on radiotherapy and chemotherapy, and has become an important component of comprehensive treatment\cite{8,9}. Active ingredients in TCMs for LUAD treatment have attracted considerable attention in recent years\cite{10–13}.

Mangiferin, an attractive TCM, is also known as 1,3,6,7-tetrahydroxyxanthone-C2-β-d glucoside and is one kind of polyphenol primarily derived from the Anacardiaceae and Gentianaceae families and is widely found in mango trees. Mangiferin has attracted considerable attention for its promising chemotherapeutic and preventive potential against various types of cancer, such as lung, brain, breast, cervix, ovarian, prostate cancers, glioma and leukemia, by single administration or in combination with known anticancer chemicals. In terms of antineoplastic mechanisms, mangiferin has been shown to act
on multiple molecular targets, which mediate the underlying biological signaling processes that inhibit the initiation, promotion, and metastasis of cancer[14–20]. For lung cancer, studies have found that decreased activities of some biochemical pathways in lung cancer-bearing animals were prevented after pre- and posttreatment with mangiferin and even approached normal control animal values[21–25]. It was also shown that the anticancer effect of mangiferin was more pronounced when used as a chemopreventive agent, rather than as a chemotherapeutic agent, against B(a)P-induced lung carcinogenesis[26]. In addition, mangiferin was able to decrease the tumor mass by helping the cisplatin with antiproliferative effects on A549 cells[25]. According to recent findings by Grauzdytė D et al., mangiferin ameliorates oxidative stress, accelerates the wound healing process and restores the proliferation rate in polycyclic aromatic hydrocarbon-exposed bronchial epithelium[27]. Positive effects of mangiferin stimulate further research on this promising phytochemical. Therefore, for LUAD, we are also optimistic about the potential therapeutic value of mangiferin.

On the other hand, molecular biological methods for exploring the origin, development and sequelae of cancers are attracting increasing interest; among these methods, the use of long noncoding RNA (lncRNA) carries great weight. LncRNAs, which are primarily located in the nucleus, are a class of RNA molecules that measure over 200nt and contain many types of transcripts. The expression of lncRNAs is tissue- and cell-specific. LncRNA can bind to DNA/RNA and thus is considered to perform important regulatory functions, and this RNA and is closely related to disease development. Several important lncRNAs have been identified in lung cancer. For example, Castillo J et al. summarized multiple approaches for discovering and providing consensus rankings of deregulated lncRNAs in carcinoma, especially focusing on lncRNAs that have improved the efficacy of LUAD early diagnosis, clinical assessment, and prognosis analysis[28].

Therefore, taking advantage of the characteristics of signaling molecules, we attempted to evaluate the expression levels of lncRNAs and messenger RNAs (mRNAs) in LUAD cell lines before and after treatment with mangiferin. Differentially expressed lncRNAs or mRNAs were identified for further exploration with online databases to verify whether mangiferin has potential inhibitory effects and even therapeutic value on LUAD.

**Materials And Methods**

1. Cell culturing and processing

We obtained the wild-type epidermal growth factor receptor (EGFR) and KRAS-mutated cell lines A549, H2030 and H1299 [29–31] from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and performed high-throughput screening before and after mangiferin treatment[32–34]. We stored the cell lines in the Dulbecco-modified Eagle's medium. All medias were added with 10% FBS, 100 mg/ml streptomycin, 100 U/ml penicillin and 0.03% L-glutamine. All cells were cultured under 37 ºC in a humid incubator with 5% CO₂. The responses of these cell lines to mangiferin were tested by detecting lncRNA and mRNA expression[25].
2. Statistical analysis and network construction.

The lncRNA and mRNA expression rates in A549, H2030 and H1299 cells before and after mangiferin treatment were compared using paired sample t-tests. To acquire the differentially expressed lncRNAs and mRNAs in mangiferin-processed LUAD cells, we intersected the lists of upregulated or downregulated lncRNAs and mRNAs from three cell lines. Next, we measured the relevance between the extracted common lncRNAs and mRNAs to obtain the closely associated lncRNA-mRNA pairs, which were used to construct the underlying lncRNA-mRNA regulatory network by Cytoscape v3.6.1[35].

3. Exploration of potential mechanisms in the lncRNA-mRNA network

Based on the network that contains the top 200 related lncRNA-mRNA pairs, we further subjected the gene nodes to protein-protein interaction (PPI) analysis via STRING v11.0 (https://string-db.org/), which was last updated on January 19, 2019 and provides online interaction evidence regarding proteins of various organisms. In addition, to explore the potential biological pathways that influenced mangiferin in LUAD, gene nodes from the network were also used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis by the online Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/, last updated in 2019).

4. Identification of significant lncRNA-mRNA pairs

Gene Expression Profiling Interactive Analysis 2 (GEPIA2, http://gepia2.cancer-pku.cn/) is an updated online tool to analyze the RNA sequencing expression data from totally over 18,000 tumor and normal specimens, of the TCGA and GTEx projects origin (http://xena.ucsc.edu) [36]. We fully utilized GEPIA2 to download differentially expressed genes (DEGs) of LUAD by $|\text{Log}_{2}\text{FC}|$ Cutoff $\geq 1$ and q-value Cutoff $\leq 0.01$. Furthermore, two gene groups from the lncRNA-mRNA network and GEPIA2 were intersected to obtain genes that were mangiferin-affected, as well as dysregulated in LUAD, of which the expression levels and survival analysis with hazard ratio (HR) in LUAD were also acquired from GEPIA2. Among the intersections, the dysregulated genes, which indicated poor or good prognosis, but were found to be inhibited or promoted by mangiferin toward the positive direction for LUAD treatment, were identified as significant target genes. In addition, according to the intersected group, those lncRNA-mRNA pairs that consisted of those target genes were considered to be significant pairs in mangiferin-processed LUAD cell lines.

Results

1. Differentially expressed lncRNAs and mRNAs

We collected differentially expressed lncRNAs and mRNAs in the A549, H2030 and H1299 cell lines. Next, those targets were intersected by Venn gram (Fig. 1), and we acquired 44 upregulated and 52 downregulated intersecting lncRNAs in three cell lines, while 229 upregulated mRNAs and 423 downregulated mRNAs were also obtained, among which the top 10 differentially expressed lncRNAs and
mRNAs are shown in Table 1. Furthermore, correlations between common differentially expressed targets, which combined the upregulated and downregulated groups by IncRNAs or mRNAs, were calculated by R language. Consequently, the top 200 closely related IncRNA-mRNA pairs were extracted to built the IncRNA-mRNA network (Fig. 2). With degrees not less than 10 inclusive, we also identified \textit{NONHSAT024774.2} and \textit{NONHSAT077537.2} from the network. Thus, in addition to the top 10 differentially expressed IncRNAs, we obtained 12 target IncRNAs (Table 1).
Table 1
Target IncRNAs and mRNAs of mangiferin in LUAD

| Type                        | Sources                        | Name                  | Average logFC | Average p-value |
|-----------------------------|--------------------------------|-----------------------|---------------|-----------------|
| Target IncRNAs              | top 10 differentially expressed | NONHSAT093921.2       | -6.944        | 1.91E-07        |
|                             |                                | NONHSAT094064.2       | 6.164         | 2.66E-08        |
|                             |                                | NONHSAT066294.2       | 6.136         | 1.02E-05        |
|                             |                                | NONHSAT112849.2       | -6.028        | 1.12E-03        |
|                             |                                | NONHSAT108944.2       | -5.874        | 1.93E-07        |
|                             |                                | NONHSAT099161.2       | -5.745        | 2.04E-05        |
|                             |                                | NONHSAT176210.1       | -5.674        | 4.47E-07        |
|                             |                                | NONHSAT039670.2       | 5.295         | 7.18E-08        |
|                             |                                | NONHSAT121497.2       | 5.267         | 1.99E-05        |
|                             |                                | NONHSAT093922.2       | -5.123        | 8.66E-05        |
|                             | IncRNA-mRNA network            | NONHSAT024774.2       | -1.908        | 1.94E-05        |
|                             |                                | NONHSAT077537.2       | -4.131        | 1.44E-03        |
| target mRNAs                | top 10 differentially expressed | NGFR                  | 7.140         | 2.09E-72        |
|                             |                                | RP11-203J24.9         | 6.452         | 2.03E-27        |
|                             |                                | ANXA3                 | -4.806        | 1.98E-05        |
|                             |                                | TSPAN8                | -4.430        | 1.76E-06        |
|                             |                                | RP11-473I1.9          | -4.292        | 4.04E-18        |
|                             |                                | SEMA3A                | -3.823        | 2.15E-07        |
|                             |                                | RP11-1072A3.3         | 3.606         | 1.60E-03        |
|                             |                                | FRK                   | -3.576        | 1.98E-08        |
|                             |                                | TOMM6                 | -3.564        | 6.83E-07        |
|                             |                                | FGF11                 | 3.521         | 1.36E-10        |
|                             | IncRNA-mRNA network            | DLX2                  | 2.155         | 2.91E-05        |
|                             |                                | EFNB3                 | 1.500         | 1.09E-03        |
|                             |                                | GEMIN2                | -1.134        | 1.10E-03        |
|                             | PPI network                    | CDK1                  | -2.098        | 7.03E-13        |
| Type | Sources | Name   | Average logFC | Average p-value |
|------|---------|--------|---------------|-----------------|
|      |         | CXCL8  | -1.279        | 3.84E-11        |
|      |         | CCL2   | -1.454        | 3.64E-12        |
|      |         | HMGB1P5| -1.829        | 1.36E-07        |
|      |         | DDX58  | -2.057        | 7.31E-06        |

2. PPI network constructed by gene nodes in the lncRNA-mRNA network

Intuitively displayed by the lncRNA-mRNA correlation network, all 115 mRNA nodes were further involved in PPI network construction. The PPI network showed that there was protein interactions among 58 items (Fig. 3), in which the genes CDK1, CXCL8, CCL2, HMGB1 and DDX58 were considered to be preliminary mangiferin-affected hub genes in this network with degrees not less than 5 inclusive. By adding the top 10 differentially expressed mRNAs and the genes DLX2, EFNB3 and GEMIN2 directly screened from the lncRNA-mRNA network, we finally obtained 18 target genes in total (Table 1).

3. GO and KEGG analysis of gene nodes in the lncRNA-mRNA network

The biological meanings behind 115 mRNA nodes in the lncRNA-mRNA network were explained by GO and KEGG analysis via DAVID v6.8. According to Table 2, we identified enriched GO terms, particularly extracellular matrix organization, cell adhesion and cellular response to interleukin-1 terms of biological process (BP), cell surface of cellular component (CC) (P < 0.01) and NAD binding and single-stranded RNA binding of molecular function (MF) (P < 0.05). In addition, genes were visualized on KEGG pathway maps, indicating that the most centralized PI3K-Akt signaling pathway (P < 0.01) in the network was influenced by mangiferin and that the involved lncRNAs may participate in LUAD cell lines.
Table 2  
GO and KEGG analysis of gene nodes in lncRNA-mRNA network

| Category  | Term                                                                 | Count | %      | P Value  | Genes                                                                 | FDR     |
|-----------|----------------------------------------------------------------------|-------|--------|----------|------------------------------------------------------------------------|---------|
| BP terms  | GO:0030198 ~ extracellular matrix organization                        | 7     | 0.044603 | 5.74E-04 | ERO1B, FGB, ICAM5, ADAMTS14, ITGB6, COL6A2, LOXL1                    | 0.862776|
|           | GO:0007155 ~ cell adhesion                                            | 9     | 0.057347 | 0.00279  | CTNNAL1, ACHE, CCL2, CYP1B1, ICAM5, ITGB6, COL6A2, KITLG, CD164       | 4.12949 |
|           | GO:0071347 ~ cellular response to interleukin-1                        | 4     | 0.025487 | 0.006149 | DAB2IP, CCL2, FGB, CXCL8                                              | 8.889712|
|           | GO:0006954 ~ inflammatory response                                     | 7     | 0.044603 | 0.014607 | HMGB1, DAB2IP, CCL2, AOX1, ITGB6, CXCL8, NGFR                        | 19.91837|
|           | GO:0043065 ~ positive regulation of apoptotic process                 | 6     | 0.038231 | 0.020706 | HMGB1, DAB2IP, CYP1B1, EEF1E1, ADAMTS14, NGFR                       | 27.08268|
|           | GO:0031623 ~ receptor internalization                                 | 3     | 0.019116 | 0.021334 | ACHE, NEDD4, CXCL8                                                   | 27.78522|
|           | GO:0030948 ~ negative regulation of vascular endothelial growth factor receptor signaling pathway | 2     | 0.012744 | 0.036119 | DAB2IP, NEDD4                                                        | 42.61011|
|           | GO:0045732 ~ positive regulation of protein catabolic process         | 3     | 0.019116 | 0.0395   | DAB2IP, APC2, NEDD4                                                  | 45.57497|
|           | GO:0016525 ~ negative regulation of angiogenesis                      | 3     | 0.019116 | 0.041919 | DAB2IP, CCL2, APOH                                                   | 47.60785|
| Category                          | Term                                                                 | Count | %     | P Value    | Genes                                                                 | FDR      |
|----------------------------------|-----------------------------------------------------------------------|-------|-------|------------|------------------------------------------------------------------------|----------|
| GO:0008283 ~ cell proliferation   | 6                                                                     | 0.038231 | 0.043219 | CDK1, ACHE, APC2, DUSP22, KITLG, EHF                                  | 48.6709  |
| GO:0046330 ~ positive regulation of JNK cascade | 3         | 0.019116 | 0.045649 | HMG1B1, DAB2IP, DUSP22                                              | 50.60397 |
| GO:0031639 ~ plasminogen activation | 2             | 0.012744 | 0.046199 | FGB, APOH                                                        | 51.03196 |
| GO:0070493 ~ thrombin receptor signaling pathway | 2         | 0.012744 | 0.046199 | IQGAP2, HPGD                                               | 51.03196 |
| CC terms                          | GO:0009986 ~ cell surface                                          | 9     | 0.057347 | 0.007821 | HMG1B1, MICB, ACHE, FGB, CD109, APOH, MMP7, IQGAP2, NGFR          | 8.988876 |
|                                  | GO:0005788 ~ endoplasmic reticulum lumen                           | 5     | 0.031859 | 0.018716 | KDELC2, ADAMTSL4, COL6A2, ESD, ERAP2                                | 20.27943 |
|                                  | GO:0005615 ~ extracellular space                                   | 14    | 0.089206 | 0.024181 | HMG1B1, ACHE, CCL2, CD109, MMP7, CXCL8, KITLG, FGB, APOH, COL6A2, C1QL4, LOXL1, MTUS1, MDH1 | 25.44538 |
|                                  | GO:0005576 ~ extracellular region                                  | 15    | 0.095578 | 0.042271 | HMG1B1, GALNT1, ACHE, CCL2, CAPZA2, FGF11, MMP7, CXCL8, KITLG, CD164, FGB, APOH, COL6A2, NGFR, LOXL1 | 40.43527 |
### Table 1: Gene Ontology and KEGG Pathway Analysis of LUAD

| Category   | Term                                                                 | Count | %     | P Value    | Genes                                                                 | FDR     |
|------------|----------------------------------------------------------------------|-------|-------|------------|-----------------------------------------------------------------------|---------|
| GO:0048471 | perinuclear region of cytoplasm                                       | 8     | 0.050975 | 0.045595   | CYB5R4, ACHE, GALNT1, CCL2, APC2, NEDD4, EIF4A2, GBP3                 | 42.86866|
| MF terms   | GO:0051287 ~ NAD binding                                             | 3     | 0.019116 | 0.017816   | AOX1, HPGD, MDH1                                                      | 20.95155|
|            | GO:0003727 ~ single-stranded RNA binding                             | 3     | 0.019116 | 0.022491   | DDX58, HMGB1, DLX2                                                   | 25.73369|
| KEGG pathway | hsa04151:PI3K-Akt signaling pathway                                  | 7     | 0.044603 | 0.019323   | CCNE2, ITGB6, FGF11, COL6A2, KITLG, NGFR, GNG12                     | 19.90123|
|            | hsa00380:Tryptophan metabolism                                       | 3     | 0.019116 | 0.02562    | KYNU, CYP1B1, AOX1                                                   | 25.55937|
|            | hsa04810:Regulation of actin cytoskeleton                             | 5     | 0.031859 | 0.041191   | APC2, ITGB6, FGF11, IQGAP2, GNG12                                   | 38.02111|
|            | hsa05200:Pathways in cancer                                          | 6     | 0.038231 | 0.096474   | CCNE2, APC2, FGF11, CXCL8, KITLG, GNG12                              | 68.45528|

### 4. Differentially expressed gene analysis of LUAD on GEPIA2

On the strength of GEPIA2, which combines the advantages of large datasets, a comprehensive exploration of recognized dysregulated genes in LUAD was performed to characterize the cancer. Based on GEPIA2, we generated 1111 higher expressed genes and 3134 lower expressed genes in LUAD compared to paired normal samples, with filter criteria of |Log2FC| Cutoff ≥ 1 and q-value Cutoff ≤ 0.01 being applied (Fig. 4).

### 5. Significant lncRNA-mRNA pairs affected by mangiferin in LUAD

We organized DEGs of LUAD from GEPIA2 and gene nodes from the lncRNA-mRNA network as gene groups A and B, respectively. The intersecting genes of group A and group B included 23 genes (Fig. 5) whose expression levels were downloaded (Table 3). We collected changes in the expression levels of
these proteins in our experimental cell lines treated with mangiferin. In addition, overall survival analysis of these targets was performed by employing Kaplan-Meier curves, which showed that aberrant expression of 12 genes, specifically $\text{ADAMTSL4, ARHGAP29, BRIX1, CD109, CDK1, COL6A2, CTNNAL1, DDIT4L, EFHD1, MMP7, ITPKA}$ and $\text{FGF11}$, in LUAD may indicate an optimistic survival rate (HR > 1) (Fig. 6). Therefore, according to Table 3, we hypothesized that the corresponding IncRNA-mRNA pairs, which contain the genes $\text{ARHGAP29, BRIX1, CD109, CDK1, CTNNAL1, DAB2IP, DDIT4L, GPR162, ICAM5, KCNAB3}$ and $\text{MMP7}$, are significant IncRNA-mRNA pairs affected by mangiferin in LUAD (Table 4).
| Gene Symbol | Log2FC from GEPIA | adjp    | HR   | Prognosis for LUAD | Mangiferin Effect | Supposed Treatment Effect |
|-------------|-------------------|---------|------|--------------------|-------------------|--------------------------|
| ABCA8       | -3.574            | 6.06E-203 | 0.73 | poor               | down-regulated    | No                       |
| ADAMTSL4    | -2.77             | 7.61E-146 | 1.1  | positive           | up-regulated      | No                       |
| AOX1        | -2.926            | 3.26E-120 | 0.94 | poor               | down-regulated    | No                       |
| ARHGAP29    | -1.625            | 3.83E-90  | 1.1  | positive           | down-regulated    | Yes                      |
| BRIX1       | 1.059             | 1.08E-93  | 1.2  | poor               | down-regulated    | Yes                      |
| CD109       | -1.041            | 9.42E-20  | 1.4  | positive           | down-regulated    | Yes                      |
| CDK1        | 2.016             | 1.35E-106 | 1.9  | poor               | down-regulated    | Yes                      |
| COL6A2      | -1.639            | 2.06E-46  | 1.1  | positive           | up-regulated      | No                       |
| CTNNAL1     | -1.045            | 9.15E-54  | 1.1  | positive           | down-regulated    | Yes                      |
| DAB2IP      | -1.273            | 1.16E-84  | 0.78 | poor               | up-regulated      | Yes                      |
| DDIT4L      | 1.167             | 2.63E-50  | 1.1  | poor               | down-regulated    | Yes                      |
| EFHD1       | -1.378            | 2.09E-13  | 1.1  | positive           | up-regulated      | No                       |
| EHF         | 1.332             | 4.76E-34  | 0.98 | positive           | down-regulated    | No                       |
| FAT3        | -1.007            | 5.54E-89  | 0.11 | poor               | down-regulated    | No                       |
| FGF11       | 1.198             | 7.68E-61  | 1.6  | poor               | up-regulated      | No                       |
| GPR162      | -2.662            | 1.34E-129 | 0.46 | poor               | up-regulated      | Yes                      |
| HOOK1       | 1.078             | 3.56E-71  | 0.84 | positive           | down-regulated    | No                       |
| Gene Symbol | Log2FC from GEPIA | adjp    | HR  | Prognosis for LUAD | Mangiferin Effect | Supposed Treatment Effect |
|-------------|-------------------|---------|-----|--------------------|-------------------|--------------------------|
| HPGD        | -1.086            | 1.63E-17| 0.83| poor               | down-regulated    | No                       |
| ICAM5       | -1.97             | 2.79E-68| 0.71| poor               | up-regulated      | Yes                      |
| ITPKA       | 1.495             | 4.00E-52| 1.2 | poor               | up-regulated      | No                       |
| KCNAB3      | -1.157            | 1.49E-34| 0.93| poor               | up-regulated      | Yes                      |
| MMP7        | 2.697             | 1.35E-48| 1.1 | poor               | down-regulated    | Yes                      |
| MYCBP       | 1.053             | 4.47E-76| 0.98| positive           | down-regulated    | No                       |
Table 4
Significant lncRNA-mRNA pairs

| lncRNA ID       | mRNA ID   | Correlation | p-value   |
|-----------------|-----------|-------------|-----------|
| NONHSAT203419.1 | ARHGAP29  | 2.42E-02    | 9.64E-01  |
| NONHSAT101516.2 | ARHGAP29  | 1.25E-02    | 9.81E-01  |
| NONHSAT157365.1 | ARHGAP29  | 7.26E-05    | 1.00E+00  |
| MSTRG.49165.1   | ARHGAP29  | -2.45E-03   | 9.96E-01  |
| NONHSAT024774.2 | MMP7      | -3.20E-02   | 9.52E-01  |
| NONHSAT180188.1 | KCNAB3    | 6.62E-03    | 9.90E-01  |
| NONHSAT159653.1 | KCNAB3    | -2.53E-02   | 9.62E-01  |
| NONHSAT068057.2 | ICAM5     | 3.10E-02    | 9.53E-01  |
| NONHSAT159653.1 | GPR162    | 1.55E-02    | 9.77E-01  |
| NONHSAT066294.2 | GPR162    | -4.28E-03   | 9.94E-01  |
| NONHSAT105444.2 | DDIT4L    | -1.12E-02   | 9.83E-01  |
| NONHSAT180188.1 | DDIT4L    | -2.35E-02   | 9.65E-01  |
| NONHSAT135605.2 | DDIT4L    | -3.08E-02   | 9.54E-01  |
| NONHSAT055695.2 | DAB2IP    | 2.67E-02    | 9.60E-01  |
| NONHSAT157365.1 | CTNNAL1   | -8.21E-03   | 9.88E-01  |
| NONHSAT055695.2 | CDK1      | 1.25E-02    | 9.81E-01  |
| NONHSAT024774.2 | CDK1      | -2.38E-02   | 9.64E-01  |
| NONHSAT009703.2 | CD109     | 2.96E-02    | 9.56E-01  |
| NONHSAT024774.2 | BRIX1     | 1.89E-02    | 9.72E-01  |

Discussion

In this research, we analyzed the potential therapeutic value of mangiferin for LUAD. Cell lines A549, H2030 and H1299 were processed with mangiferin, and bioinformatic analysis was employed, which helped us to construct the top 200 lncRNA-mRNA pairs network. We also obtained 12 lncRNAs and 18 mRNAs from differential expression calculations, degrees of connection in the lncRNA-mRNA network and PPI network, which were considered to be underlying targets of mangiferin. Additionally, mangiferin was mostly thought to affect the PI3K-Akt signaling pathway in LUAD. Furthermore, lncRNA-mRNA pairs that contained the genes ARHGAP29, BRIX1, CD109, CDK1, CTNNAL1, DAB2IP, DDIT4L, GPR162, ICAM5,
KCNAB3 and MMP7 were determined to be meaningful lncRNA-mRNA pairs affected by mangiferin in LUAD. Generally, our study helped to elucidate the potential therapeutic value of mangiferin for LUAD.

In recent years, traditional Chinese medicine (TCM) or products have exhibited the effects of tumor prevention and treatment. Studies employing molecular biology and biochemical pharmacology to investigate the underlying mechanism governing these effects have opened new approaches for clinical antineoplastic drug development. In our study, mangiferin is a widely sourced treatment option that has shown therapeutic value in such cancer types as liver cancer, ovarian cancer, gastric cancer, and lung cancer[15, 17, 37, 38], while potential targets, such as NFκB, PPARγ, MMP-7, MMP-9 and EMT[14], were predicted. Similarly, we adopted the experimental method of a self-control study treated with mangiferin to explore the medicinal effect of mangiferin in LUAD cell lines.

Regarding how to evaluate the potential value of mangiferin for treating cancer, the trend of antitumor chemotherapy prefers targeted medicine development, which provides a useful reference for further research on this subject. A large amount of research has been devoted to the molecular mechanism of tumorigenesis and development, which encourage and contribute to the development of molecularly targeted treatment. Given several examples, Wang et al. have considered W934, a novel PI3K/Akt pathway inhibitor, to be a potential therapeutic drug candidate to treat the non-small-cell lung cancer (NSCLC)[39]. Research from Yang et al. showed that antipsychotic chlorpromazine has the potential to be a repurposed drug for breast cancer treatment[40]. Among the potentially affected molecular targets, the lncRNA-mRNA interaction network has been heavily researched, as systematic analysis indicated that an lncRNA-mRNA co-expression relevant to platinum resistance in advanced serous ovarian cancer[41]. Research of miRNA-lncRNA-mRNA interconnections also support significant academic, practical and clinical basis to gastric cancer[42]. Moreover, Xiao et al. found that LINC0092 and chromosome 2 open reading frame 71 were correlated with better prognosis of breast cancer by analyzing the resultant ER subtype-related miRNA-lncRNA-mRNA network in breast and gastric cancer[43]. Drawing on previous research, our research attempted to elucidate the mechanism of mangiferin in LUAD by analyzing the differentially expressed lncRNAs and mRNAs and constructing the network.

Thus, we considered three committed steps to achieve the goal. First, the lncRNAs and mRNAs that are differentially expressed after mangiferin treatment should be screened out. Second, a close association between identified common lncRNAs and mRNAs should be determined for lncRNA-mRNA network construction. Third, identification of target lncRNAs, target genes and meaningful lncRNA-mRNA pairs should be performed. Additionally, we investigated the potential mechanism by which the nodes in lncRNA-mRNA networks may be involved in the effects of mangiferin on LUAD.

Target lncRNAs and genes should be characterized as the probable targets affected by mangiferin in the network. Therefore, differentially expressed lncRNAs that have abundant connections with the mRNA group were considered. Similarly, to identify target genes, we added the protein interaction condition, as regulatory relationships among proteins have also been verified by large databases[44]. However, when targeting these nodes, it is not clear whether mangiferin has an anticancer effect. Furthermore, for
significant lncRNA-mRNA pair identification, which was predicted to be beneficial for LUAD prognosis after mangiferin treatment, DEGs of LUAD downloaded in GEPIA2 and displayed in Venn diagrams help establish links between laboratory results and databases. To elaborate, mangiferin should promote the dysregulated genes that suggest positive prognosis and inhibit those suggesting poor prognosis by upregulating or downregulating the expression levels. Consequently, the eligible genes and corresponding lncRNAs became meaningful targets for mangiferin to produce anticancer effects in LUAD.

According to the results, 12 lncRNAs were presented as targets. Based on NONCODE v5 (http://www.noncode.org)[45], exosome expression profile analysis indicated that NONHSAT094064.2 is highly expressed in hepatocellular carcinoma cell lines, human umbilical vein endothelial cell lines and normal human blood. NONHSAT112849.2, NONHSAT099161.2 and NONHSAT176210.1 showed significant overexpression in the human umbilical vein endothelial cell line and the foreskin fibroblast cell line, while NONHSAT077537.2 was significantly present in the squamous cell carcinoma cell line and normal human blood. However, no studies have discussed these lncRNAs in detail, and to date, we have not found any basis in the published literature for demonstrating associations of these 12 lncRNAs with LUAD or mangiferin.

In addition, we extracted 18 target genes of mangiferin in LUAD, among which NGFR, RP11-203J24.9, RP11-1072A3.3, FGF11, DLX2 and EFNB3 were upregulated, while ANXA3, TSPAN8, RP11-473I1.9, SEMA3A, FRK, TOMM6, GEMIN2, CDK1, CXCL8, CCL2, HMGB1 and DDX58 were downregulated, by mangiferin treatment. NGFR was suggested to be involved in the switching of KRAS + LUAD to squamous cell carcinoma when highly expressed[46]. Studies have shown that suppression of EFNB3 decreases NSCLC progression, but no evidence has been found in LUAD[47, 48]. ANXA3 knockdown was found to inhibit the growth, migration, invasion, and metastasis of LUAD via in vitro and in vivo experiments[49, 50]. Moreover, research has indicated TSPAN8 to be a diagnostic biomarker in lung cancer [51]. Zhou et al. found that A549 cells secrete SEMA3A to inhibit the maturation and functions of dendritic cells, which might be associated with the unidentified mechanism of immune evasion by tumor cells[52]. Interestingly, FRK was considered to be a underlying treatment target for drug discovery, as it has a carcinogenic effect in lung cancer cells via inducing metabolic reprogramming and finally promoting epithelial-mesenchymal transition and metastasis[53]. TOMM6 is an upregulated mRNA-related lncRNA UCA1 that might affect cisplatin resistance in LUAD[54]. CDK1 was found to be an adverse prognostic and diagnostic biomarker for LUAD[55, 56]. Liu et al. found that CXCL8 played an adverse role which accelerating cancer progression and bad outcome of LUAD, while human Dachshund homologue 1 antagonized CXCL8 to enhance the survival of LUAD patients[57]. Moreover, high levels of CCL2 predict unfavorable survival in lung adenocarcinoma[58]; and HMGB1-regulated autophagy was proven to be a significant contributor to docetaxel resistance in LUAD cells[59]. In summary, most of these genes play roles in LUAD, at least in lung cancer. However, few or no publications have reported the associations of these genes with mangiferin. Only HMGB1, whose protein expression rate was decreased by mangiferin, effectively prevented alcohol hepatitis [60]. Therefore, further validation is needed.
In the significant lncRNA-mRNA pairs that contained ARHGAP29, BRIX1, CD109, CDK1, CTNNAL1, DAB2IP, DDIT4L, GPR162, ICAM5, KCNAB3 and MMP7, Shukla et al. presented the first RNA-seq prognostic signature for LUAD, including CD109 [61]. The genovariation of DAB2IP/AIP1 is related to increasing the risk of lung cancer in Chinese males[62]. Similarly, another study revealed that an elevated circulating tumor cell count and overexpression of MMP7 correlate with metastasis and clinical progression in LUAD patients[63]. However, most of the significant genes predicted in our study, which are related to neither LUAD nor mangiferin, still lack literature validation, and the regulatory mechanism governing the lncRNA-mRNA pairs warrants further exploration.

Potential pathway analysis under the lncRNA-mRNA network was also performed. KEGG analysis demonstrated that mangiferin was mostly centralized in the PI3K-Akt signaling pathway (P < 0.01) when LUAD cells were treated. The PI3K-Akt signaling pathway, involving the key proteins phosphatidylinositol 3-kinase (PI3K) and Akt/Protein Kinase B, is an intracellular signal transduction road that promotes metabolism, hyperplasia, cell survival, growth and angiogenesis through responding to extracellular signals. Dysregulation of the PI3K/Akt pathway is implicated in a number of human cancers[64–67]. Studies of the PI3K-Akt signaling pathway may also help to develop targeted medicine for LUAD. For example, Zhang et al. suggested 20(S)-protopanaxadiol (PPD) as a promising chemopreventive agent that downregulated the PI3K/Akt signaling pathway in A549 cells[68]. It was also indicated that allicin may inhibit invasion of LUAD by reducing the activity of the PI3K/AKT signaling pathway[69], and baicalein may increase cisplatin sensitivity of A549 cells via the PI3K/Akt/NF-κB pathway[70]. On the other hand, it was reported that mangiferin relieves lipopolysaccharide-induced injury by activating the PI3K/AKT pathway[71]. mangiferin also inhibits the MMP-9 gene in phorbol myristate acetate-stimulated human astrogliaoma, in which the PI3K/AKT pathway is involved[72]. Therefore, we demonstrated that mangiferin may influence the prognosis of LUAD patients by regulating the PI3K/AKT signaling pathway.

Conclusions

this study employed expression profile analysis in cell lines and composited data from a large database and demonstrated that mangiferin could be a promising drug for LUAD therapy by affecting lncRNA-mRNA pairs and the PI3K/AKT signaling pathway. However, several aspects warrant further research: whether mangiferin could play a therapeutic role requires further analysis of the parameters from clinical specimens, as aberrant expression of partial genes cannot represent a whole situation. In addition, the scarcity of interactions among genes from hub genes in the network and significant lncRNA-mRNA pairs increased the difficulty of mangiferin-targeted node verification, for which more samples are needed for validation. Finally, the association between lncRNAs and mRNAs warrants further experimental confirmation. Despite these limitations, the lncRNA-mRNA interaction network, as well as the predicted hub nodes and significant targeted lncRNA-mRNA pairs, also provided a comprehensive understanding of the potential therapeutic value of mangiferin for LUAD. In future research, we plan to further verify our results by employing larger sample sizes and animal model experiments and by employing more accurate bioinformatic methods, such as molecular docking, to evaluate the overall clinical value of mangiferin for LUAD.
Abbreviations

LUAD
lung adenocarcinoma
TCM
traditional Chinese medicine
IncRNA
long noncoding RNA
mRNA
messenger RNA
EGFR
epidermal growth factor receptor
PPI
protein-protein interaction
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
DAVID
Database for Annotation, Visualization and Integrated Discovery
GEPIA2
Gene Expression Profiling Interactive Analysis
BP
biological process
CC
cell surface of cellular component
MF
molecular function
DEGs
differentially expressed genes
NSCLC
non-small-cell lung cancer

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

**Availability of data and materials**

The datasets analysed during the current study are available in the STRING,

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

The study was conceived and designed by LD, CXL, MJJ and WRH. All authors participated in the experiments and data mining. LD and CXL analyzed, interpreted data and drafted the manuscript. MJJ, WRH participated in drafting the manuscript. All authors read and approved the final manuscript.

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**References**

1. Meng QC, Gao PR, Ren PF, Song YP, Li HL. [Early diagnosis of subtype in early clinical stage lung adenocarcinoma by using an autoantibody panel and computed tomography]. Zhonghua Yi Xue Za Zhi. 2019;99(3):204–8.

2. Zhuhong H, Zhenyu B, Xiangyuan C, Tingzhen X, Libin S. Genome-wide isoform-level analysis reveals tumor-specific isoforms for lung adenocarcinoma diagnosis and prognosis. Cancer Genet. 2019;230:58–65.

3. Chalela R, Curull V, Enriquez C, Pijuan L, Bellosillo B, Gea J. Lung adenocarcinoma: from molecular basis to genome-guided therapy and immunotherapy. J Thorac Dis. 2017;9(7):2142–58.

4. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL, Paz-Ares L. Lung cancer: current therapies and new targeted treatments. Lancet. 2017;389(10066):299–311.
5. Li C, Wang J, Shao JB, Zhu LM, Sun ZG, Zhang N. Microwave ablation combined with chemotherapy improved progression free survival of IV stage lung adenocarcinoma patients compared with chemotherapy alone. Thorac Cancer. 2019;10(7):1628–35.

6. Saito M, Suzuki H, Kono K, Takenoshita S, Kohno T. Treatment of lung adenocarcinoma by molecular-targeted therapy and immunotherapy. Surg Today. 2018;48(1):1–8.

7. Zhang G, Zeng R, Wang K, A Y, Li L, Gong K. Clinical efficacy and safety evaluation of pemetrexed combined with radiotherapy in treatment of patients with lung adenocarcinoma brain metastasis. Oncol Lett. 2019;17(3):2874–80.

8. Lou JS, Yao P, Tsim KWK. Cancer Treatment by Using Traditional Chinese Medicine: Probing Active Compounds in Anti-multidrug Resistance During Drug Therapy. Curr Med Chem. 2018;25(38):5128–41.

9. Wang Y, Feng L, Piao B, Zhang P. Review on Research about Traditional Chinese Medicine in Cancer Stem Cell. Evid Based Complement Alternat Med. 2017;2017:4505194.

10. Cao P, Liu B, Du F, Li D, Wang Y, Yan X, Li X, Li Y. Scutellarin suppresses proliferation and promotes apoptosis in A549 lung adenocarcinoma cells via AKT/mTOR/4EBP1 and STAT3 pathways. Thorac Cancer. 2019;10(3):492–500.

11. Li CL, Hsia TC, Li CH, Chen KJ, Yang YH, Yang ST. Adjunctive Traditional Chinese Medicine Improves Survival in Patients With Advanced Lung Adenocarcinoma Treated With First-Line Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors (TKIs): A Nationwide, Population-Based Cohort Study. Integr Cancer Ther. 2019;18:1534735419827079.

12. Xia Y, Shi L, Ai ZZ, Zhang DZ, Liu YW, You PT. Chinese medicine formula "Shenqi San" extract inhibits proliferation of human lung adenocarcinoma A549 cells via inducing apoptosis. J Huazhong Univ Sci Technolog Med Sci. 2017;37(5):766–71.

13. Zhang J, Gao Y, Ma C, Wang Y. Murrayanine Induces Cell Cycle Arrest, Oxidative Stress, and Inhibition of Phosphorylated p38 Expression in A549 Lung Adenocarcinoma Cells. Med Sci Monit. 2019;25:2002–8.

14. Gold-Smith F, Fernandez A, Bishop K. Mangiferin and Cancer: Mechanisms of Action. Nutrients 2016, 8(7).

15. Nunez Selles AJ, Daglia M, Rastrelli L. The potential role of mangiferin in cancer treatment through its immunomodulatory, anti-angiogenic, apoptopic, and gene regulatory effects. Biofactors. 2016;42(5):475–91.

16. Rajendran P, Rengarajan T, Nandakumar N, Divya H, Nishigaki I. Mangiferin in cancer chemoprevention and treatment: pharmacokinetics and molecular targets. J Recept Signal Transduct Res. 2015;35(1):76–84.

17. He W, You Y, Du S, Lei T, Wang H, Li X, He X, Tong R, Wang Y. Anti-neoplastic effect of mangiferin on human ovarian adenocarcinoma OVCAR8 cells via the regulation of YAP. Oncol Lett. 2019;17(1):1008–18.
18. Xiao J, Liu L, Zhong Z, Xiao C, Zhang J. Mangiferin regulates proliferation and apoptosis in glioma cells by induction of microRNA-15b and inhibition of MMP-9 expression. Oncol Rep. 2015;33(6):2815–20.
19. Deng Q, Tian YX, Liang J. Mangiferin inhibits cell migration and invasion through Rac1/WAVE2 signalling in breast cancer. Cytotechnology. 2018;70(2):593–601.
20. Li M, Ma H, Yang L, Li P. Mangiferin inhibition of proliferation and induction of apoptosis in human prostate cancer cells is correlated with downregulation of B-cell lymphoma-2 and upregulation of microRNA-182. Oncol Lett. 2016;11(1):817–22.
21. Rajendran P, Ekambaram G, Sakthisekaran D. Cytoprotective effect of mangiferin on benzo(a)pyrene-induced lung carcinogenesis in swiss albino mice. Basic Clin Pharmacol Toxicol. 2008;103(2):137–42.
22. Rajendran P, Ekambaram G, Sakthisekaran D. Effect of mangiferin on benzo(a)pyrene induced lung carcinogenesis in experimental Swiss albino mice. Nat Prod Res. 2008;22(8):672–80.
23. Rajendran P, Rengarajan T, Nishigaki I, Ekambaram G, Sakthisekaran D. Potent chemopreventive effect of mangiferin on lung carcinogenesis in experimental Swiss albino mice. J Cancer Res Ther. 2014;10(4):1033–9.
24. Rajendran P, Jayakumar T, Nishigaki I, Ekambaram G, Nishigaki Y, Vetriselvi J, Sakthisekaran D. Immnomodulatory Effect of Mangiferin in Experimental Animals with Benzo(a)Pyrene-induced Lung Carcinogenesis. Int J Biomed Sci. 2013;9(2):68–74.
25. Shi W, Deng J, Tong R, Yang Y, He X, Lv J, Wang H, Deng S, Qi P, Zhang D, et al. Molecular mechanisms underlying mangiferin-induced apoptosis and cell cycle arrest in A549 human lung carcinoma cells. Mol Med Rep. 2016;13(4):3423–32.
26. Rajendran P, Ekambaram G, Magesh V, Sakthisekaran D. Chemopreventive efficacy of mangiferin against benzo(a)pyrene induced lung carcinogenesis in experimental animals. Environ Toxicol Pharmacol. 2008;26(3):278–82.
27. Grauzdyte D, Raudoniute J, Kulvinskiene I, Bagdonas E, Stasiulaitiene I, Martuzevicius D, Bironaite D, Aldonyte R, Venskutonis PR. Cytoprotective Effects of Mangiferin and Z-Ligustilide in PAH-Exposed Human Airway Epithelium in Vitro. Nutrients 2019, 11(2).
28. Castillo J, Stueve TR, Marconett CN. Intersecting transcriptomic profiling technologies and long non-coding RNA function in lung adenocarcinoma: discovery, mechanisms, and therapeutic applications. Oncotarget. 2017;8(46):81538–57.
29. Cai X, Luo J, Yang X, Deng H, Zhang J, Li S, Wei H, Yang C, Xu L, Jin R, et al. In vivo selection for spine-derived highly metastatic lung cancer cells is associated with increased migration, inflammation and decreased adhesion. Oncotarget. 2015;6(26):22905–17.
30. Miao J, Hsu PC, Yang YL, Xu Z, Dai Y, Wang Y, Chan G, Huang Z, Hu B, Li H, et al. YAP regulates PD-L1 expression in human NSCLC cells. Oncotarget. 2017;8(70):114576–87.
31. Wilson MR, Hou Z, Yang S, Polin L, Kushner J, White K, Huang J, Ratnam M, Gangjee A, Matherly LH. Targeting Nonsquamous Nonsmall Cell Lung Cancer via the Proton-Coupled Folate Transporter with
6-Substituted Pyrrolo[2,3-d]Pyrimidine Thienoyl Antifolates. Mol Pharmacol. 2016;89(4):425–34.

32. Dearden S, Stevens J, Wu YL, Blowers D. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). Ann Oncol. 2013;24(9):2371–6.

33. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst. 2005;97(5):339–46.

34. Tsao AS, Scagliotti GV, Bunn PA Jr, Carbone DP, Warren GW, Bai C, de Koning HJ, Yousaf-Khan AU, McWilliams A, Tsao MS, et al. Scientific Advances in Lung Cancer 2015. J Thorac Oncol. 2016;11(5):613–38.

35. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.

36. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47(W1):W556–60.

37. Du M, Wen G, Jin J, Chen Y, Cao J, Xu A. Mangiferin prevents the growth of gastric carcinoma by blocking the PI3K-Akt signalling pathway. Anticancer Drugs 2017.

38. Yang G, Shang X, Cui G, Zhao L, Zhao H, Wang N. Mangiferin Attenuated Diethylnitrosamine-Induced Hepatocellular Carcinoma in Sprague-Dawley Rats via Alteration of Oxidative Stress and Apoptotic Pathway. J Environ Pathol Toxicol Oncol. 2019;38(1):1–12.

39. Wang J, Wang HY, Shen Y, Liang D, Wang HY, Zhang SQ, Cao YX, Cao L. A novel small-molecule PI3K/Akt signaling inhibitor, W934, exhibits potent antitumor efficacy in A549 non-small-cell lung cancer. Anticancer Drugs 2019.

40. Yang CE, Lee WY, Cheng HW, Chung CH, Mi FL, Lin CW. The antipsychotic chlorpromazine suppresses YAP signaling, stemness properties, and drug resistance in breast cancer cells. Chem Biol Interact. 2019;302:28–35.

41. Fang L, Wang H, Li P. Systematic analysis reveals a IncRNA-mRNA co-expression network associated with platinum resistance in high-grade serous ovarian cancer. Invest New Drugs. 2018;36(2):187–94.

42. Mao Y, Liu R, Zhou H, Yin S, Zhao Q, Ding X, Wang H. Transcriptome analysis of miRNA-IncRNA-mRNA interactions in the malignant transformation process of gastric cancer initiation. Cancer Gene Ther. 2017;24(6):267–75.

43. Xiao B, Zhang W, Chen L, Hang J, Wang L, Zhang R, Liao Y, Chen J, Ma Q, Sun Z, et al. Analysis of the miRNA-mRNA-IncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. Gene. 2018;658:28–35.

44. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45(D1):D362–8.

45. Fang S, Zhang L, Guo J, Niu Y, Wu Y, Li H, Zhao L, Li X, Teng X, Sun X, et al. NONCODEv5: a comprehensive annotation database for long non-coding RNAs. Nucleic Acids Res.
46. Zhang H, Fillmore Brainson C, Koyama S, Redig AJ, Chen T, Li S, Gupta M, Garcia-de-Alba C, Paschini M, Herter-Sprie GS, et al. Lkb1 inactivation drives lung cancer lineage switching governed by Polycomb Repressive Complex 2. Nat Commun. 2017;8:14922.

47. Efazat G, Novak M, Kaminskyy VO, De Petris L, Kanter L, Juntti T, Bergman P, Zhivotovsky B, Lewensohn R, Haag P, et al. Ephrin B3 interacts with multiple EphA receptors and drives migration and invasion in non-small cell lung cancer. Oncotarget. 2016;7(37):60332–47.

48. Stahl S, Branca RM, Efazat G, Ruzzene M, Zhivotovsky B, Lewensohn R, Viktorsson K, Lehtio J. Phosphoproteomic profiling of NSCLC cells reveals that ephrin B3 regulates pro-survival signaling through Akt1-mediated phosphorylation of the EphA2 receptor. J Proteome Res. 2011;10(5):2566–78.

49. Liu YF, Liu QQ, Zhang YH, Qiu JH: Annexin A3 Knockdown Suppresses Lung Adenocarcinoma. Anal Cell Pathol (Amst) 2016, 2016:4131403.

50. Liu YF, Xiao ZQ, Li MX, Li MY, Zhang PF, Li C, Li F, Chen YH, Yi H, Yao HX, et al. Quantitative proteome analysis reveals annexin A3 as a novel biomarker in lung adenocarcinoma. J Pathol. 2009;217(1):54–64.

51. Sandfeld-Paulsen B, Jakobsen KR, Baek R, Folkeresen BH, Rasmussen TR, Meldgaard P, Varming K, Jorgensen MM, Sorensen BS. Exosomal Proteins as Diagnostic Biomarkers in Lung Cancer. J Thorac Oncol. 2016;11(10):1701–10.

52. Zhou XL, Huang Y, Wang F, Cai LF, Zhang LH, Shi LY. [Effects of Sema3A derived from tumor cells on functions of dendritic cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2010;39(4):364–9.

53. Zhang L, Yang Y, Chai L, Bu H, Yang Y, Huang H, Ran J, Zhu Y, Li L, Chen F, et al. FRK plays an oncogenic role in non-small cell lung cancer by enhancing the stemness phenotype via induction of metabolic reprogramming. Int J Cancer. 2020;146(1):208–22.

54. Zhou H, Shen Q, Fu J, Jiang F, Wang L, Wang Y. Analysis of IncRNA UCA1-related downstream pathways and molecules of cisplatin resistance in lung adenocarcinoma. J Clin Lab Anal 2020:e23312.

55. Liu WT, Wang Y, Zhang J, Ye F, Huang XH, Li B, He QY. A novel strategy of integrated microarray analysis identifies CENPA, CDK1 and CDC20 as a cluster of diagnostic biomarkers in lung adenocarcinoma. Cancer Lett. 2018;425:43–53.

56. Shi YX, Zhu T, Zou T, Zhuo W, Chen YX, Huang MS, Zheng W, Wang CJ, Li X, Mao XY, et al. Prognostic and predictive values of CDK1 and MAD2L1 in lung adenocarcinoma. Oncotarget. 2016;7(51):85235–43.

57. Liu Q, Li A, Yu S, Qin S, Han N, Pestell RG, Han X, Wu K. DACH1 antagonizes CXCL8 to repress tumorigenesis of lung adenocarcinoma and improve prognosis. J Hematol Oncol. 2018;11(1):53.

58. Li L, Liu YD, Zhan YT, Zhu YH, Li Y, Xie D, Guan XY. High levels of CCL2 or CCL4 in the tumor microenvironment predict unfavorable survival in lung adenocarcinoma. Thorac Cancer. 2018;9(7):775–84.
59. Pan B, Chen D, Huang J, Wang R, Feng B, Song H, Chen L. HMGB1-mediated autophagy promotes docetaxel resistance in human lung adenocarcinoma. Mol Cancer. 2014;13:165.

60. Li M, Wu C, Guo H, Chu C, Hu M, Zhou C. Mangiferin improves hepatic damage-associated molecular patterns, lipid metabolic disorder and mitochondrial dysfunction in alcohol hepatitis rats. Food Funct. 2019;10(6):3514–34.

61. Shukla S, Evans JR, Malik R, Feng FY, Dhanasekaran SM, Cao X, Chen G, Beer DG, Jiang H, Chinnaiyan AM. Development of a RNA-Seq Based Prognostic Signature in Lung Adenocarcinoma. J Natl Cancer Inst 2017, 109(1).

62. Yang L, Li Y, Ling X, Liu L, Liu B, Xu K, Bin X, Ji W, Lu J. A common genetic variant (97906C > A) of DAB2IP/AIP1 is associated with an increased risk and early onset of lung cancer in Chinese males. PLoS One. 2011;6(10):e26944.

63. Sun Y, Chen Y, Li S, Lei Y, Xu D, Jiang N, Zhang Y, Cao J, Ke Z. NanoVelcro-captured CTC number concomitant with enhanced serum levels of MMP7 and MMP9 enables accurate prediction of metastasis and poor prognosis in patients with lung adenocarcinoma. Int J Nanomedicine. 2017;12:6399–412.

64. Liu J, Liu X, Ma W, Kou W, Li C, Zhao J. Anticancer activity of cucurbitacin-A in ovarian cancer cell line SKOV3 involves cell cycle arrest, apoptosis and inhibition of mTOR/PI3K/Akt signaling pathway. J BUON. 2018;23(1):124–8.

65. Liu JS, Huo CY, Cao HH, Fan CL, Hu JY, Deng LJ, Lu ZB, Yang HY, Yu LZ, Mo ZX, et al. Aloperine induces apoptosis and G2/M cell cycle arrest in hepatocellular carcinoma cells through the PI3K/Akt signaling pathway. Phytomedicine. 2019;61:152843.

66. Luo Y, Zha L, Luo L, Chen X, Zhang Q, Gao C, Zhuang X, Yuan S, Qiao T. [6]-Gingerol enhances the cisplatin sensitivity of gastric cancer cells through inhibition of proliferation and invasion via PI3K/AKT signaling pathway. Phytother Res. 2019;33(5):1353–62.

67. Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. Ann Med. 2014;46(6):372–83.

68. Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004;9(6):667–76.

69. Zhang YL, Zhang R, Xu HL, Yu XF, Qu SC, Sui DY. 20(S)-protopanaxadiol triggers mitochondrial-mediated apoptosis in human lung adenocarcinoma A549 cells via inhibiting the PI3K/Akt signaling pathway. Am J Chin Med. 2013;41(5):1137–52.

70. Huang L, Song Y, Lian J, Wang Z. Allicin inhibits the invasion of lung adenocarcinoma cells by altering tissue inhibitor of metalloproteinase/matrix metalloproteinase balance via reducing the activity of phosphoinositide 3-kinase/AKT signaling. Oncol Lett. 2017;14(1):468–74.

71. Ma Y, Liu Y, Ma Y, Jiang N, Wang L, Wang B, Niu W, Hu Y, Lin Q, Yu B. Mangiferin Relieves Lipopolysaccharide-Induced Injury by Up-Regulating miR-181a via Targeting PTEN in ATDC5 Cells. Front Pharmacol. 2020;11:137.
72. Jung JS, Jung K, Kim DH, Kim HS. Selective inhibition of MMP-9 gene expression by mangiferin in PMA-stimulated human astrogliaoma cells: involvement of PI3K/Akt and MAPK signaling pathways. Pharmacol Res. 2012;66(1):95–103.

**Figures**

**Figure 1**

Venn gram for extracting common lncRNAs and mRNAs among three cell lines.
Figure 2

LncRNA-mRNA network by top200 related LncRNA-mRNA pairs.
Figure 3

PPI network constructed by gene nodes in lncRNA-mRNA network.
Figure 4

Volcanic map for DEGs in LUAD.
Figure 5

Venn gram of DEGs from GEPIA2 and gene nodes from lncRNA-mRNA network.
Figure 6

Kaplan-Meier curves of intersected genes.