Oncologie
DOI: 10.32604/oncologie.2020.012494
Article

Comprehensive Network Analysis of Different Subtypes of Molecular Disorders in Lung Cancer

Haoliang Zhang[1,*], Xiaowei Xing[2], Yang Liu[1], Shuangli Li[1] and Weiyuan Li[3]

[1]Department of Oncology, Tangshan Workers' Hospital, Tangshan, China
[2]Department of Cardiovascular Internal Medicine, Tangshan, China
[3]Graduate School of North China Institute of Technology, Tangshan, China
[*]Corresponding Author: Haoliang Zhang. Email: haoliangzhang@aliyun.com

Abstract: Lung cancer is the leading cause of death in cancer patients. Based on a modular and comprehensive analysis method, it is intended to identify their common pathogenesis. We downloaded data and analyzed differences in lung adenocarcinoma samples, lung squamous cell carcinoma samples, and normal samples. Co-expression analysis, enrichment analysis, and hypergeometric testing were used to predict transcription factors, ncRNAs, and retrospective target genes. We get 4596 differentially expressed genes in common differences in high multiples and clustered into 14 modules dysfunction. The 14 genes (including DOK2, COL5A1, and TSPAN8) have the highest connectivity in the dysfunction module and are identified as the core genes of the module. Module genes are also substantially involved in biological processes, including extracellular matrix, carbohydrate-binding and renal system development, and involved signal transduction including PPAR signal transduction, cGMP-PKG signal transduction, PI3K-Akt signal transduction, and Apelin signal transduction. We identified ncRNA pivot (miR-335-5p, ANCR, TUG1) and Transcription Factors pivot (RELA, SP1) to regulate dysfunction module genes primarily. The analysis showed that comprehensive co-expression analysis helped us to understand the transcription factor ncRNA. Moreover, it helps us understand the molecular pathogenesis of co-expression of modular genes that regulate lung adenocarcinoma and squamous cell carcinoma. It provides a precious resource and theoretical basis for further experiments by biologists.

Keywords: Lung adenocarcinoma; squamous cell carcinoma; core gene; transcription factor; ncRNA

1 Introduction

Lung cancer is characterized by high morbidity and high mortality [1]. Lung cancer is broadly classified into non-small cell lung cancer and small cell lung cancer, of which the major histological subtypes of NSCLS are lung adenocarcinoma and lung squamous cell carcinoma [2]. Lung cancer patients usually have complications. The most common paraneoplastic diseases are Lambert-Eaton myasthenic syndrome and multifocal paraneoplastic encephalomyelitis, and the most common neurological complication is tumor brain metastasis [3]. Environmental and genetic factors cause lung cancer. Environmental factors include exposure to alfalfa, cooking fumes, asbestos, heavy metals, and environmental tobacco fumes, human papillomavirus infection [4,5]. In patients with non-small cell lung cancer, smokers are found to be more connected with squamous cell carcinoma than adenocarcinoma, and adenocarcinoma is more common in nonsmokers [6]. In genetics, there is a significant association between SNP rs920778 and rs1899663 in HOTAIR and susceptibility to primary lung cancer [7]. Mir-196a2 polymorphism affects the susceptibility of lung cancer [8]. The polymorphism of MIRLET7BHG (the MIRLET7B host gene at 22q13.31) may be a significant

predictor of asbestos exposure-connected lung cancer [9].

The researchers studied the pathogenesis of lung cancer subtypes from all aspects and achieved specific results. For example, Jin Y's study confirmed that miR-375 is essentially up-regulated in lung adenocarcinoma and small cell lung cancer. However, the interpretation was down-regulated in squamous cell carcinoma, and it was found that miR-375 targets ITPKB to promote cell growth of small cell lung cancer [10]. The down-regulation of lncRNAs LINC00222 permanently inhibits the proliferation, migration, and invasion of lung adenocarcinoma cells [11]. Knockout USP33 inhibits migration, invasion, and metastasis of lung adenocarcinoma cells via IL-6 and SLIT2 / ROBO1 signal transductions [12]. MiR-372-3p targeting FGF9 promotes cell proliferation and metastasis in lung squamous cell carcinoma [13]. MicroRNA-588 targets GRN to inhibit tumor cell migration and invasion in lung squamous cell carcinoma [14]. These findings deepen our understanding of the pathogenesis of lung squamous cell carcinoma and lung adenocarcinoma and provide theories for the study of their common pathogenesis. We conducted a systematic modular analysis and exploration. We identified standard dysfunction modules and core molecules between lung adenocarcinoma and lung squamous cell carcinoma to explore further pathways involved in the pathogenesis of lung adenocarcinoma and lung squamous cell carcinoma.

2 Materials and Methods

2.1 Data Resource

It aims to apply high-throughput genomic analysis techniques to help people to obtain a better understanding of cancer while improving their ability to prevent, diagnose and treat cancer. The interpretation profile data of lung adenocarcinoma included 61 lung adenocarcinoma paired samples and 56 normal samples. Expression profile data for lung squamous cell carcinoma included 48 lung squamous cell carcinoma paired samples and 48 normal samples. We screened ncRNA-mRNA interaction pairs with score ≥ 0.5 from the RAID v2.0 database [15], including 431937 interaction pairs involving 5431 ncRNAs. The RAID v2.0 database enrolls more than 5.27 million RNA-related interactions, referring to 130 000 RNAs in 60 species/protein symbols, which can help us comprehensively observe various RNA-related interactions. At the same time, all human transcription factor target data was downloaded and used in the general database TRRUST v2 database [16] for transcriptional studies, involving 2492 transcription factors and 9396 interaction pairs.

2.2 Difference Analysis

For RNA-Seq data on lung adenocarcinoma and lung squamous cell carcinoma on TCGA, we used R language DEseq2 for differential analysis [17]. The R language DEseq2 analysis process has three main steps, including normalization, dispersion estimation, and differential interpretation testing. Normalization is performed using weighted conditional likelihoods. By simulating the dispersion in all samples, we detect and correct for too low dispersion estimates. BBSeq simulates the dispersion on the mean. DSS uses the Bayesian method to estimate the dispersion of individual genes. These genes can explain the heterogeneity of the dispersion values of different genes. BaySeq and ShrinkBayes estimate the a priori of the Bayesian model for all genes. The findings provide posterior probabilities and false discovery rates for differential interpretation. For differential genes, we screened for highly differential multiples of $p < 0.01$ and $|\log FC| > 1$.

2.3 Co-Expression Analysis

To explore the synergistic interpretation of high differential fold genes in 4596 lung adenocarcinomas and lung squamous cell carcinomas, we used WGCNA [18]. We analyzed the interpretation profile matrix of high differential multiple genes and looked for gene modules for synergistic interpretation. We use the correlation coefficient weighting value and the N-th power of the gene correlation coefficient to get the correlation coefficient between any two genes. The connections between genes in the network are subject to scale-free network distribution, making the algorithm more biologically meaningful. Correlation

coefficients between genes construct a hierarchical clustering tree. Various branches of the clustering tree represent various gene modules, and various colors represent various modules. We got the association between ME and clinical features to determine the relevant modules. Gene significance (GS) is defined as the log 10 conversion of the *p* value (GS = 1 g P) in a linear regression between gene interpretation and clinical information. Module significance (MS) is defined as the average GS of all genes in the module, as well as the modules defined as clinical traits.

2.4 Enrichment Analysis

The exploration of gene function and the exploration of its signal transduction are often effective methods to study the molecular mechanism of disease. The function and pathway involved in the module gene can characterize the dysfunction mechanism of the module during the disease process. For the genes of 14 important modules of lung adenocarcinoma and lung squamous cell carcinoma, we used the R language Clusterprofiler package [19] for enrichment analysis on Go function (*p*-value cutoff = 0.05, qvalueCutoff = 0.05) and KEGG pathway (*p*-value cutoff = 0.05, qvalueCutoff = 0.05).

2.5 Transcription Factors and Ncrnas That Regulate Dysfunctional Modules

For each dysfunctional module, we specify that the pivot regulator refers to the number of targeted adjustments between each regulator and each module exceeding two. We obtained the interaction between the regulator and the module based on the hypergeometric test *p*-value <0.01. In the study, we used the ncRNA target data as the background set prediction. We wrote the R program to predict and obtained the pivotal regulator of the dysfunction module. Based on the human ncRNA-mRNA interaction pair in the RAID v2.0 database and all human transcription factor target data in the TRRUST v2 database, we retrospected the predicted ncRNA and TF back to the target gene. We obtained the target gene and the high difference in multiple gene intersections.

2.6 Verification of Key Genes by qPCR

Human blood samples were chosen according to international ethical guidelines for biomedical research involving humans and subjects. The study was approved by the Ethics Committee of Tangshan Workers' Hospital and conducted following the provisions of the Ethics Committee.

Specifically, total RNA in the blood was extracted, transcribed into cDNA using a reverse transcription kit, and qPCR reaction was carried out using the SYBR qPCR Detection Kit. The qPCR program begins the initial 3 minutes denaturation step at 95°C to activate the hot-start iTaqTM DNA polymerase. This was followed by 45 cycles of denaturation at 95°C for 10 seconds and annealing and extension at 60°C for 45 seconds. The internal reference gene is beta-actin.

3 Results

3.1 Determining the Typical Expression of Dysregulated Molecules in Lung Squamous Cell Carcinoma and Lung Adenocarcinoma

Differential interpretation analysis is an effective method for identifying the underlying genetic basis of disease. To screen for genes that may cause ADC and SCC, based on microarray interpretation profiling data, we performed differential gene screening for ADC, SCC, and normal samples in TCGA. The results showed that there were 5952 differentially differential genes in lung adenocarcinoma, and there were 8055 differentially differential genes in lung squamous cell carcinoma (Figs. 1A, 1B). The intersection of the two differential genomes showed that 4,596 differences were common (Fig. 1C). Among these genes, there may be related genes that have a significant effect on the development of ADC and SCC, which requires further analysis.

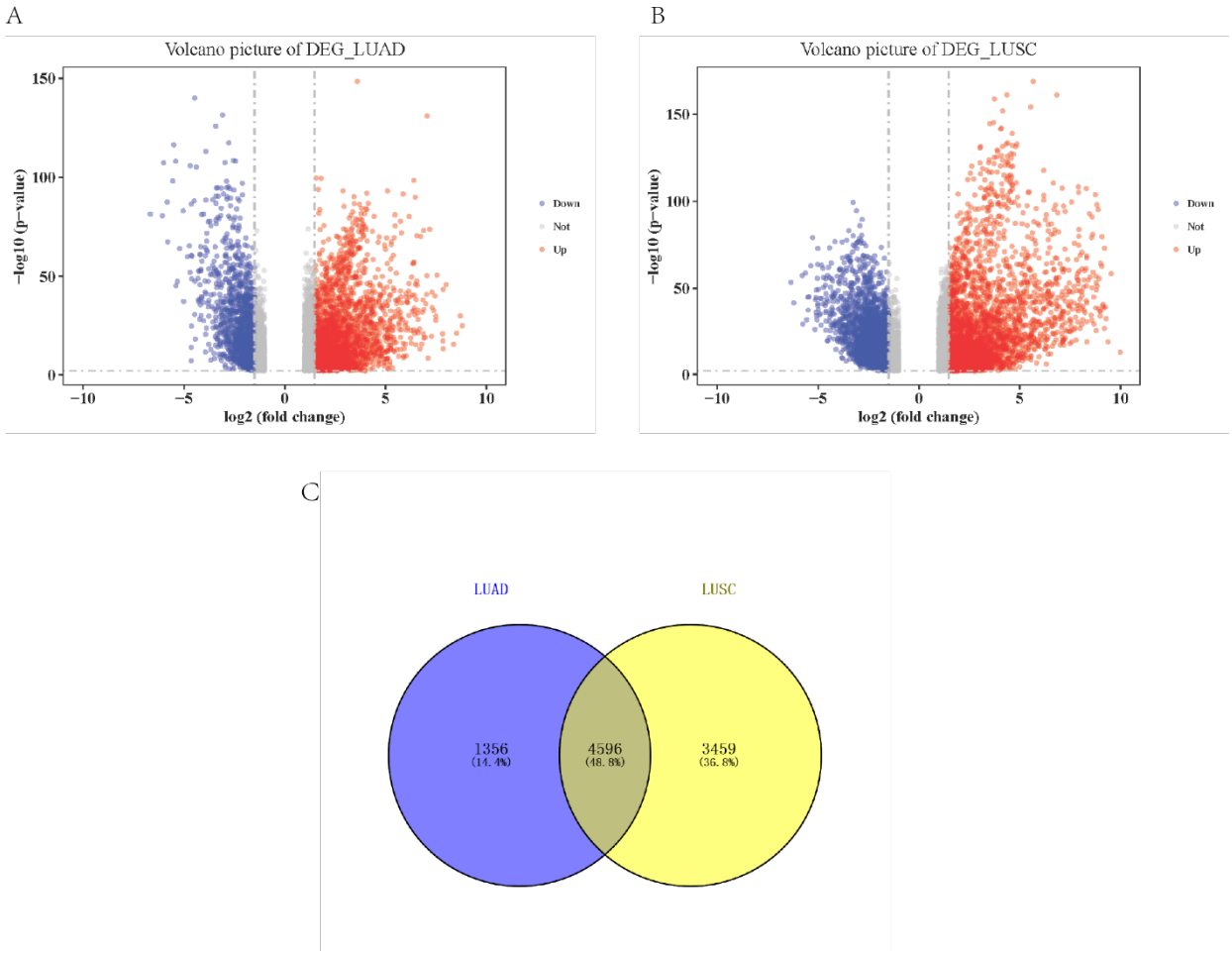

Figure 1: Differential interpretation of lung adenocarcinoma and lung squamous cell carcinoma. A & B is a volcano map of lung squamous cell carcinoma of lung adenocarcinoma, respectively. C. Differential gene Venn diagram of lung adenocarcinoma and lung squamous cell carcinoma

3.2 Construction of Weighted Co-Expression Network and Identification of Key Genes

An interpretation profile matrix was constructed in patient samples based on 4596 differential genes and their interaction genes. Based WGCNA, we learned that the genes exhibited significant group co-expression in disease samples. By identifying the co-expression panel as a module, we obtained 14 functional disorder modules, including 2965 differential genes (Figs. 2A, 2B). Based on the degree of gene connectivity of the gene co-expression network, the central gene with the highest connectivity in each module was identified, and 14 central genes including DOK2, COL5A1, and TSPAN8 were obtained. The co-expression network indicates that the central gene is an essential gene of the dysfunction module. The blue module and lung adenocarcinoma were negatively correlated with lung squamous cell carcinoma (Fig. 2C). This suggests that the turquoise module may have functions in tumorigenesis of SCC, and the blue module has contributions in the development of ADC. Furthermore, the expression level of key genes was verified by qPCR. We found that the expression trend of key genes was consistent with the previous results.

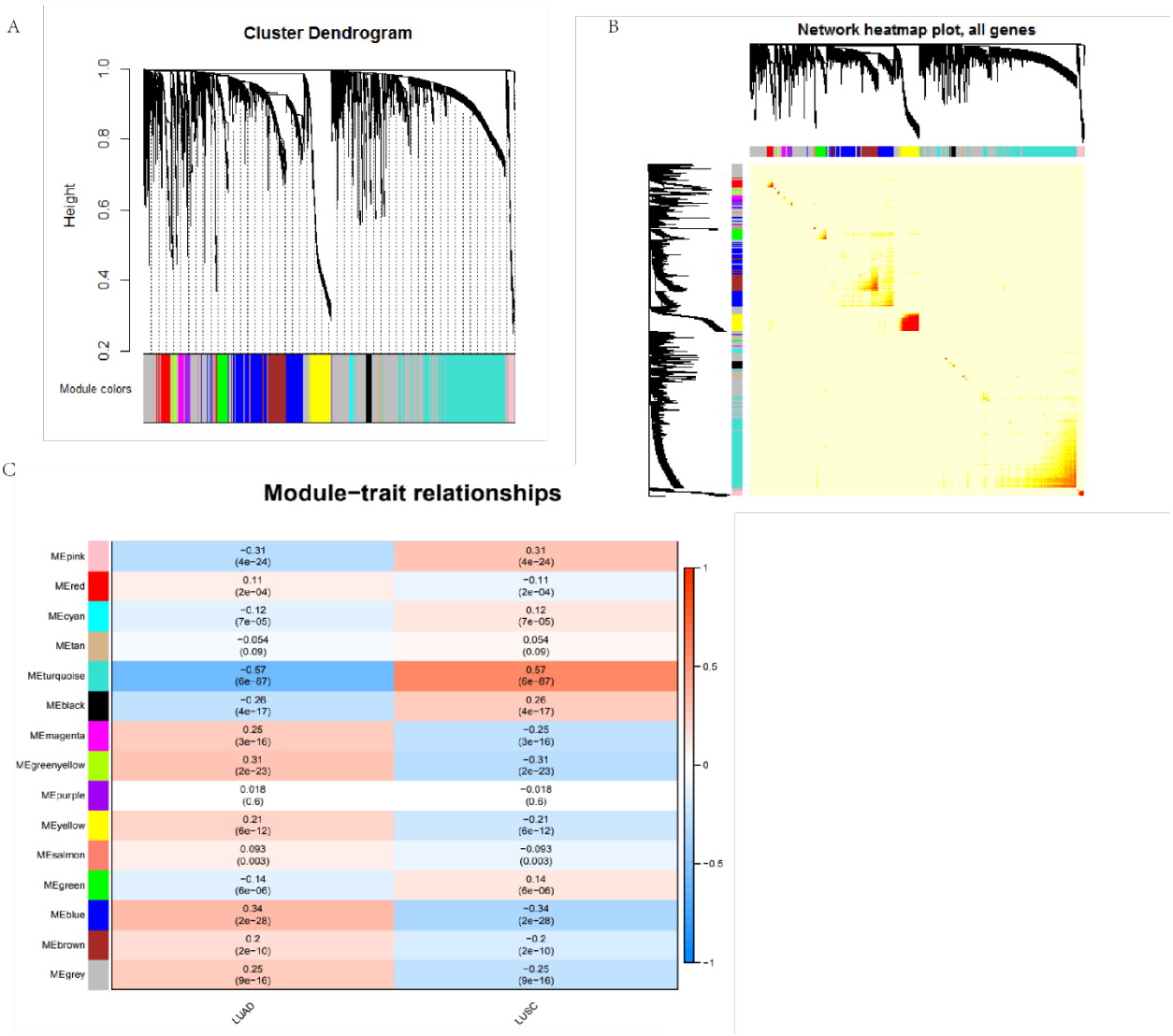

Figure 2: Synergistic interpretation of high differential fold genes in lung adenocarcinoma and lung squamous cell carcinoma in patient samples. A. The 14 co-expression panels obtained by clustering were identified as modules, and 14 colors represent 14 co-expression modules. B. Expression heat map of all genes in the sample and their interpretation behavior is clustered into seven co-expression modules. C. Each row represents a module, each column represents a phenotype, the color of each cell is mapped by the corresponding correlation coefficient, the value is from -1 to 1, the color transitions from blue to white, and then transitions to red

3.3 Module Genes Involved in Functions and Pathways

Function and pathway are essential mediators of the physiological response of the disease. The exploration of functional pathways involved in the exploration of dysfunctionality modules helps determine the relationship of genes in the same pathway within a module. They are also conducive to building molecular bridges between modules and diseases in systems biology and deepening the understanding of the underlying molecular mechanisms of disease. Enrichment analysis was performed on 14 modules and obtained 6431 biological processes, 728 cells, 1290 molecular functions, and 180 KEGG pathways. It was found that the genes of the six modules essentially participated in related biological processes such as extracellular matrix, carbohydrate binding and renal system development (Fig. 3A). Also, two modules of

genes are involved in PPAR signal transduction, cGMP-PKG signal transduction, PI3K-Akt signal transduction, and Apelin signal transduction (Fig. 3B).

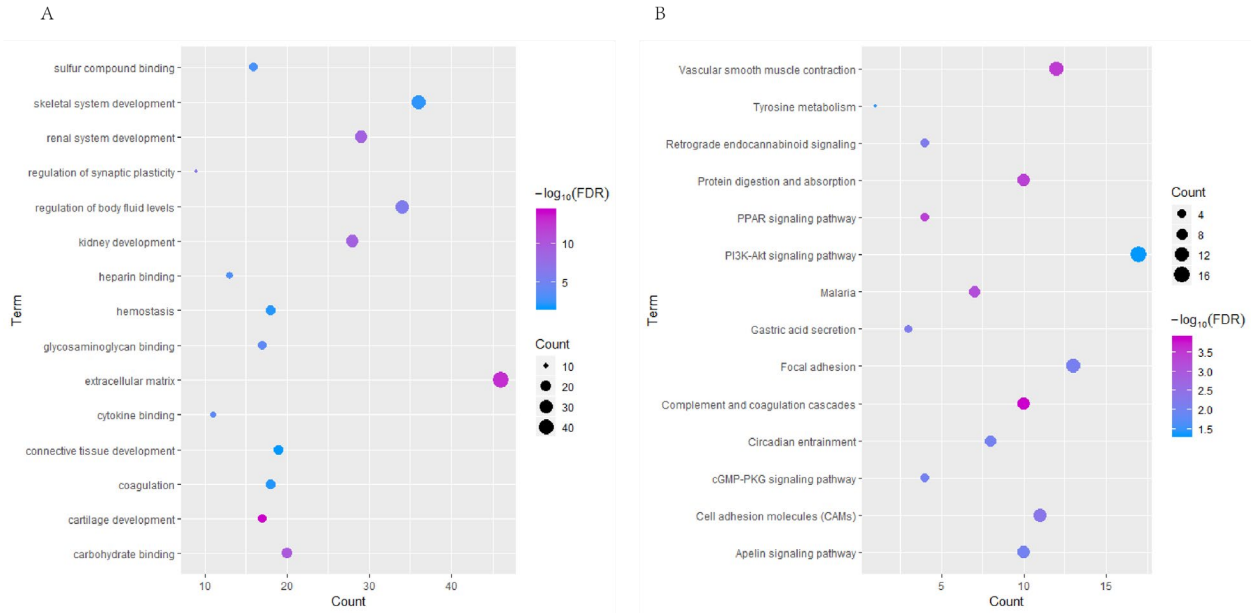

Figure 3: Functional and pathway enrichment analysis excerpts of the module gene. A. Module gene GO function enrichment analysis excerpt. The color increases from blue to purple, and the enrichment increases permanently. The larger the circle, the more significant the proportion of the gene in the module that accounts for the GO function. B. Module gene KEGG pathway enrichment analysis excerpt. The color increases from blue to purple, and the enrichment increases permanently. The larger the circle, the more significant the proportion of the gene in the KEGG pathway entry

3.4 TF and ncRNA That Drive Lung Cancer Subtype Progression

Transcription and post-transcriptional regulation of genes have always been considered as a critical regulator of disease occurrence and development. Transcription factors and ncRNA are regulators of common interpretation and function. We performed a pivotal analysis of the co-modules based on the targeted regulatory relationship of TF and ncRNA to the modular genes and explored vital transcriptional regulators that regulate the progression of lung adenocarcinoma and lung squamous cell carcinoma. The predicted results showed that a total of 341 ncRNAs involved 360 ncRNA-module regulatory pairs and 57 transcription factors involved 62 TF-module target pairs. Besides, the number of pivot control modules was statistically analyzed, and the most dysfunctional modules with ncRNA (miR-335-5p, ANCR, TUG1, miR-29c-3p) and TF (RELA, SP1) were obtained. By mediating dysfunctional modules, these transcription factors and ncRNAs regulate the progression of lung adenocarcinoma and lung squamous cell carcinoma. Potential regulators are identified as dysfunctional molecules of lung adenocarcinoma and lung squamous cell carcinoma.

3.5 Retrospective Target Gene

The snRNA, asRNA, snoRNA, miRNA, and piRNA in the cell are all synthesized by non-coding genes. The biological processes they participate in include gene activation, gene silencing, gene imprinting, does compensation, protein synthesis, and function regulation, and metabolic regulation. Finally, a combination of four core ncRNAs and five core TFs that drive the lung adenocarcinoma and lung squamous cell carcinoma co-expression modules, which regulate genes and genes involved in the pathway (Figs. 4A, 4B). We observed a relationship between the regulation of common differential gene regulation (transcriptional

and post-transcriptional) and gene-dependent pathways in lung adenocarcinoma and lung squamous cell carcinoma. By modulating the genes of modules 1, 2, 3, 6, 7, 13, we observed four core ncRNAs while understanding their involvement in the regulation of multiple signal transductions. Among the five core TF regulatory modules, genes 1, 2, 3, 7, and 10 participate in the regulation of multiple pathways. Among them, the most regulated genes of TUG1 and SP1 have the highest connectivity and has contributions in the pathogenesis of lung adenocarcinoma and lung squamous cell carcinoma, and are considered to be the most central regulatory factors.

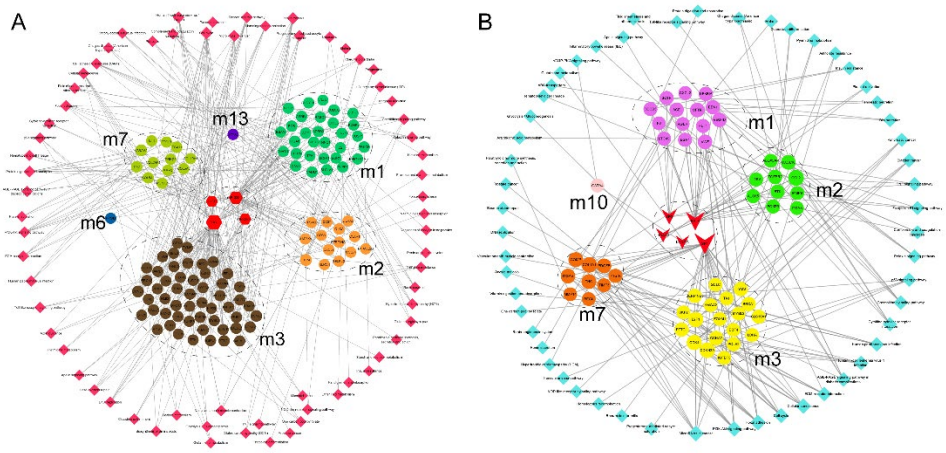

Figure 4: Regulation of the core regulatory factor regulatory module genes involved in signal transduction. A. Core ncRNA-Gene-pathway network diagram. The red hexagon represents the core ncRNA, the light red diamond represents the signal transduction, and the rest represents the genes within the module. B. Core TF-Gene-pathway network diagram. The red arrow represents the core TF, the light blue diamond represents the signal pathway, and the rest represents the genes within the module

4 Discussion

Lung cancer is a heterogeneous disease [20,21]. Pathologists performed subtype analysis to classify non-small cell lung cancer into lung adenocarcinoma, lung squamous cell carcinoma, and large cell neuroendocrine carcinoma [22]. In this study, we collected the RNA-Seq data of lung adenocarcinoma and lung squamous cell carcinoma on TCGA for differential analysis, and obtained differential gene with high difference fold. Therefore, these differential genes with high differential multiples are considered significant contributions in the dysfunctional mechanisms of lung adenocarcinoma and lung squamous cell carcinoma. Combining the weighted gene co-expression network, we identified 14 co-expression modules. The genes have synergistic interpretation behavior, and we believe that the synergistic interpretation of these genes promotes the occurrence of the disease. We observed the functions and pathways involved in the module, and the signal transductions involved in the genes of the two modules include PPAR signal transduction, cGMP-PKG signal transduction, PI3K-Akt signal transduction, and Apelin signal transduction. TGFβ induces PPAR γ signal transduction to promote EMT and has essential contributions in the invasion and migration of lung cancer cells [23]. For patients with advanced lung adenocarcinoma, signal transductions (MAPK, PI3K-Akt, Ras, and cGMP-PKG) are thought to be most likely connected with platinum resistance [24].

Also, CK2α may regulate the invasion and migration of lung adenocarcinoma cells through the PI3K-Akt signal transduction [25]. The silencing receptor tyrosine kinase ROR1 inhibits the proliferation of lung adenocarcinoma cells via the PI3K/AKT/mTOR signal transduction [26]. P53 regulates the survival of squamous cell carcinoma cells by inhibiting PI3K/AKT signaling [27]. At the molecular level, we identified 14 central genes, such as DOK2, COL5A1, and TSPAN8, through a co-expression network. These genes

are essentially differentially expressed and have critical regulatory contributions in the dysfunction module. DOK2 has been identified as a gene for tumor suppressor of EGFR mutant lung adenocarcinoma [28]. Liu W's study found that COL5A1 may promote the metastasis of lung adenocarcinoma cells [29]. The up-regulation of TSPAN8 promotes cell viability and proliferation, leading to non-small cell lung cancer [30]. We also identified 341 ncRNA-driven modules that function, including the long non-coding gene (ANCR, TUG1) and the small non-coding gene (miR-335-5p, miR-29c-3p). ANCR downregulates the TGF-β1 pathway to inhibit migration and invasion of NSCLC cells [31].

The upregulation of TUG1 is connected with increased tumor size, a degree of differentiation, lymph node metastasis, distant metastasis, and TNM staging. It is the most promising diagnostic marker for patients with lung adenocarcinoma [32]. TUG1-mediated HOXB7 interpretation affects cell proliferation in non-small cell lung cancer [33]. Increased interpretation of miR-335-5p inhibits cell proliferation in NSCLC cells [34]. We explored 57 transcription factors (HDAC2, NANOG, RELA, SP1, and SP3) that mediate differential gene co-expression networks in lung adenocarcinoma and lung squamous cell lung cancer, thereby regulating the pathogenesis of lung cancer subtypes. HDAC2 upregulates fibronectin by NF-κB to initiate migration and invasion of NSCLC cells [35]. NANOG is expressed in various cancers, and its interpretation is connected with poor survival in cancer patients [36]. With platinum-based chemotherapy, advanced non-small cell lung cancer, NANOG can be a poor predictor [37]. Co-expression of RELA and ACTN4 induces apoptosis in non-small cell lung cancer cells [38]. In the early stage of lung cancer, SP1 mediates the interpretation of miR-182, which inhibits the interpretation of FOXO3 and stimulates the proliferation of lung cancer cells. Down-regulation of advanced SP1 and miR-182 increases the interpretation of FOXO3 leading to metastasis of lung cancer cells [38-40]. SP1 regulates cell proliferation during the development of non-small cell lung cancer [41]. Key regulatory factors have regulatory contributions and have an essential impact on the formation of lung adenocarcinoma and lung squamous cell carcinoma.

Funding Statement: The author(s) received no specific funding for this study.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Hirsch, F. R., Scagliotti, G. V., Mulshine, J. L., Kwon, R., Curran, W. J. et al. (2017). Lung cancer: current therapies and new targeted treatments. *Lancet, 389,* 299–311.
2. Blandin Knight, S., Crosbie, P. A., Balata, H., Chudziak, J., Hussell, T. et al. (2017). Progress and prospects of early detection in lung cancer. *Open Biology,* 7.
3. Dropcho, E. J. (2014). Neurologic complications of lung cancer. *Handb Clin Neurol, 119,* 335–361.
4. Linares, I., Molina-Portillo, E., Exposito, J., Baeyens, J. A., Suarez, C. et al. (2016). Trends in lung cancer incidence by histologic subtype in the south of Spain, 1985-2012: a population-based study. *Clinical Translational Oncology, 18,* 489–496.
5. Subramanian, J., Govindan, R. (2007). Lung cancer in never smokers: a review. *Journal of Clinical Oncology, 25,* 561–570.
6. Saito, S., Espinoza-Mercado, F., Liu, H., Sata, N., Cui, X. et al. (2017). Current status of research and treatment for non-small cell lung cancer in never-smoking females. *Cancer Biology & Therapy, 18,* 359–368.
7. Wang, C., Li, Y., Li, Y. W., Zhang, H. B., Gong, H. et al. (2018). HOTAIR lncRNA SNPs rs920778 and rs1899663 are associated with smoking, male gender, and squamous cell carcinoma in a Chinese lung cancer population. *Acta Pharmacologica Sinica, 39,* 1797–1803.
8. Xu, L., Tang, W. (2015). The associations of nucleotide polymorphisms in mir-196a2, mir-146a, mir-149 with lung cancer risk. *Cancer Biomark, 15,* 57–63.
9. Liu, C. Y., Stucker, I., Chen, C., Goodman, G., McHugh, M. K. et al. (2015). Genome-wide gene-asbestos

exposure interaction association study identifies a common susceptibility variant on 22q13.31 associated with lung cancer risk. *Cancer Epidemiology Biomarkers Prevention, 24,* 1564–1573.

10. Jin, Y., Liu, Y., Zhang, J., Huang, W., Jiang, H. et al. (2015). The expression of miR-375 is associated with carcinogenesis in three subtypes of lung cancer. *PLoS One, 10,* e0144187.
11. Zhang, H., Wang, Y., Lu, J., Zhao, Y. (2018). Long non-coding RNA LINC00222 regulates GSK3beta activity and promotes cell apoptosis in lung adenocarcinoma. *Biomed Pharmacother, 106,* 755–762.
12. Wang, Y., Zhang, S., Mu, S., Zhang, B., Ma, S. (2018). USP33 suppresses lung adenocarcinoma lung cell invasion and metastasis by down-regulating SLIT2/ROBO1 signaling pathway. *Journal of Southern Medical University Research, 38,* 956–961.
13. Wang, Q., Liu, S., Zhao, X., Wang, Y., Tian, D. et al. (2017). MiR-372-3p promotes cell growth and metastasis by targeting FGF9 in lung squamous cell carcinoma. *Cancer* Medicine*, 6,* 1323–1330.
14. Qian, L., Lin, L., Du, Y., Hao, X., Zhao, Y. et al. (2016). MicroRNA-588 suppresses tumor cell migration and invasion by targeting GRN in lung squamous cell carcinoma. *Molecular Medicine Reports, 14,* 3021–3028.
15. Yi, Y., Zhao, Y., Li, C., Zhang, L., Huang, H. et al. (2017). RAID v2.0: an updated resource of RNA-associated interactions across organisms. *Nucleic Acids Research, 45,* D115–D118.
16. Han, H., Cho, J. W., Lee, S., Yun, A., Kim, H. et al. (2018). TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Research, 46,* D380–D386.
17. Love, M. I., Huber, W., Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology, 15,* 550.
18. Langfelder, P., Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics, 9,* 559.
19. Yu, G., Wang, L. G., Han, Y., He, Q. Y. (2012). ClusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS, 16,* 284–247.
20. Hensing, T., Chawla, A., Batra, R., Salgia, R. (2014). A personalized treatment for lung cancer: molecular pathways, targeted therapies, and genomic characterization. *Advances in Experimental Medicine and Biology, 799,* 85–117.
21. Zhang, Y., Wang, D. C., Shi, L., Zhu, B., Min, Z. et al. (2017). Genome analyses identify the genetic modification of lung cancer subtypes. *Semin Cancer Biology, 42,* 20–30.
22. Kayser, G. (2015). Non-small cell lung cancer. New biomarkers for diagnostics and therapy. *Pathologe, 36 (Suppl 2),* 189-193.
23. Lin, L. C., Hsu, S. L., Wu, C. L., Hsueh, C. M. (2014). TGFbeta can stimulate the p(38)/beta-catenin/PPARgamma signaling pathway to promote the EMT, invasion and migration of non-small cell lung cancer (H460 cells). *Clinical & Experimental Metastasis, 31,* 881–895.
24. Xu, X., Yu, S., Sun, W., Qin, X., Chen, Y. et al. (2018). MiRNA signature predicts the response of patients with advanced lung adenocarcinoma to platinum-based treatment. *Journal of Cancer Research and Clinical Oncology, 144,* 431–438.
25. Wu, A., Li, M., Mai, Z., Li, S., Yang, Z. (2017). CK2alpha regulates the metastases and migration of lung adenocarcinoma A549 cell line through PI3K/Akt/GSK-3beta signal pathway. *Chinese Journal of Lung Cancer, 20,* 233–238.
26. Liu, Y., Yang, H., Chen, T., Luo, Y., Xu, Z. et al. (2015). Silencing of receptor tyrosine kinase ROR1 inhibits tumor-cell proliferation via PI3K/AKT/mTOR signaling pathway in lung adenocarcinoma. *PLoS One, 10,* e0127092.
27. Singh, B., Reddy, P. G., Goberdhan, A., Walsh, C., Dao, S. et al. (2002). p53 regulates cell survival by inhibiting PIK3CA in squamous cell carcinomas. *Genes & Development, 16,* 984–993.
28. Berger, A. H., Chen, M., Morotti, A., Janas, J. A., Niki, M. et al. (2013). DOK2 inhibits EGFR-mutated lung adenocarcinoma. *PLoS One, 8,* e79526.
29. Liu, W., Wei, H., Gao, Z., Chen, G., Liu, Y. et al. (2018). COL5A1 may contribute the metastasis of lung adenocarcinoma. *Gene, 665,* 57–66.
30. Dong, Z., Zhao, L., Lu, S., Xiong, J., Geng, Z. (2016). Overexpression of TSPAN8 promotes tumor cell viability and proliferation in nonsmall cell lung cancer. *Cancer Biother Radiopharm, 31,* 353–359.

31. Wang, S., Lan, F., Xia, Y. (2018). lncRA ANCR inhibits non-small cell lung cancer cell migration and invasion by inactivating TGF-beta pathway. *Medical Science Monitor, 24,* 6002–6009.

32. Liu, H., Zhou, G., Fu, X., Cui, H., Pu, G. et al. (2017). Long noncoding RNA TUG1 is a diagnostic factor in lung adenocarcinoma and suppresses apoptosis via epigenetic silencing of BAX. *Oncotarget, 8,* 101899–101910.

33. Zhang, E. B., Yin, D. D., Sun, M., Kong, R., Liu, X. H. et al. (2014). P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression. *Cell Death & Disease, 5,* e1243.

34. Tang, H., Zhu, J., Du, W., Liu, S., Zeng, Y. et al. (2018). CPNE1 is a target of miR-335-5p and plays an important role in the pathogenesis of non-small cell lung cancer. *Journal of Experimental and Clinical Cancer Research, 37,* 131.

35. Li, L., Mei, D. T., Zeng, Y. (2016). HDAC2 promotes the migration and invasion of non-small cell lung cancer cells via upregulation of fibronectin. *Biomed Pharmacother, 84,* 284–290.

36. Gong, S., Li, Q., Jeter, C. R., Fan, Q., Tang, D. G. et al. (2015). Regulation of NANOG in cancer cells. *Molecular Carcinogenesis, 54,* 679–687.

37. Chang, B., Park, M. J., Choi, S. I., In, K. H., Kim, C. H. et al. (2017). NANOG as an adverse predictive marker in advanced non-small cell lung cancer treated with platinum-based chemotherapy. *Onco Targets and Therapy, 10,* 4625–4633.

38. Lomert, E., Turoverova, L., Kriger, D., Aksenov, N. D., Nikotina, A. D. et al. (2018). Co-expression of RelA/p65 and ACTN4 induces apoptosis in non-small lung carcinoma cells. *Cell Cycle, 17,* 616–626.

39. Yang, W. B., Chen, P. H., Hsu, T. S., Fu, T. F., Su, W. C. et al. (2014). Sp1-mediated microRNA-182 expression regulates lung cancer progression. *Oncotarget, 5,* 740–753.

40. Hsu, T. I., Wang, M. C., Chen, S. Y., Yeh, Y. M., Su, W. C. et al. (2012). Sp1 expression regulates lung tumor progression. *Oncogene, 31,* 3973–3988.

41. Zhu, W., Li, Z., Xiong, L., Yu, X., Chen, X. et al. (2017). FKBP3 promotes proliferation of non-small cell lung cancer cells through regulating Sp1/HDAC2/p27. *Theranostics, 7,* 3078–3089.