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RESEARCH ARTICLE

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Screening and identification of biomarkers associated with the diagnosis and prognosis of lung adenocarcinoma

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Abstract

Background: In this study, we aimed to identify the pathogenesis and prognostic biomarkers of lung adenocarcinoma (LUAD).

Methods: Differentially expressed mRNAs (DEmRNAs) and single nucleotide polymorphism (SNP) mutant genes were screened. In addition, enrichment and protein-protein interaction (PPI) network analyses of the SNP-mutated genes were performed. Thereafter, the correlation between gene mutation and expression was analyzed. Finally, the mutated genes associated with LUAD prognosis were validated on the basis of The Cancer Genome Atlas (TCGA) database.

Results: A total of 2502 DEmRNAs were initially screened in this study. We identified 756 SNP-mutated genes from more than 30 cases. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the mutated genes involved in LUAD were mainly associated with the ECM-receptor interaction, focal adhesion, and calcium signaling pathways. Tumor protein p53 (*TP53*) and neurexin 1 (*NRXN1*) with the higher degree were chosen as the hub genes in the PPI network. In addition, the correlation analysis revealed six genes, including assembly factor for spindle microtubules (*ASPM*), centromere protein F (*CENPF*), contactin 3 (*CNTN3*), catenin delta 2 (*CTNND2*), PKHD1 like 1 (*PKHD1L1*), and semaphorin 6D (*SEMA6D*), and three SNP mutations at ASPM rs368020495, CENPF rs762653487, and *PKHD1L1* rs768349010 sites that were found to be associated with LUAD prognosis. Further validation showed that among the aforementioned six mutated genes, *CENPF* was upregulated and *SEMA6D* was downregulated.

Conclusion: *CENPF*, *SEMA6D*, *TP53*, and *NRXN1* were found to be closely associated with the development of LUAD.

KEYWORDS

bioinformatics analysis, biomarker, lung adenocarcinoma, prognosis, single nucleotide polymorphism

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1 | INTRODUCTION

Lung cancer is a type of malignant tumor that has resulted in high morbidity and mortality across the world. According to global data released by the International Agency for Research on Cancer (IARC) in 2018, lung cancer accounts for the vast majority of deaths worldwide.[1] Lung adenocarcinoma (LUAD) is one of the most common pathological types of lung cancer.[2,3] The number of LUAD patients has been shown to increase over time, accounting for almost half of the total number of current lung cancer patients.[4] Although the cause of LUAD is unclear, the associated risk factors include genetic factors, air pollution, ionizing radiation, and other factors, such as diet.[5] The clinical symptoms of LUAD are mainly dry cough and chest pain; however, no special symptoms appear in the early stage. Moreover, pain, hoarseness, and pleural effusion are the symptoms that appear only in the advanced stages of LUAD.[6] Surgery, chemotherapy, and radiotherapy are the main forms of treatment for LUAD. However, approximately 70% of the patients that have been diagnosed with LUAD are in stages III-IV at the time of diagnosis, implying that less than 20% of them are expected to survive for another 5 years.[7,8] Therefore, identification of effective biomarkers may be useful for investigating the pathogenesis and early prognosis of LUAD.

The Cancer Genome Atlas (TCGA) database allows access to information regarding copy number variations (CNVs), mRNA expression, single nucleotide polymorphisms (SNPs), and signaling pathways, which are available for numerous genes in various tumor conditions and their corresponding paracancerous tissues.[9,10] Moreover, the TCGA database can be used to extract data to perform high-throughput genomic analyses and better understand the genetic basis of a particular disease through the genome sequencing and bioinformatics analysis of gene mutations that may be responsible for the occurrence of cancer.[11] Based on the TCGA database, Gao et al investigated SNP-mutated genes for the diagnosis of breast cancer.[12] Previously, several studies have investigated the possible molecular mechanisms of the initiation and development of LUAD in order to identify novel therapeutic targets. For instance, Chou et al demonstrated the therapeutic

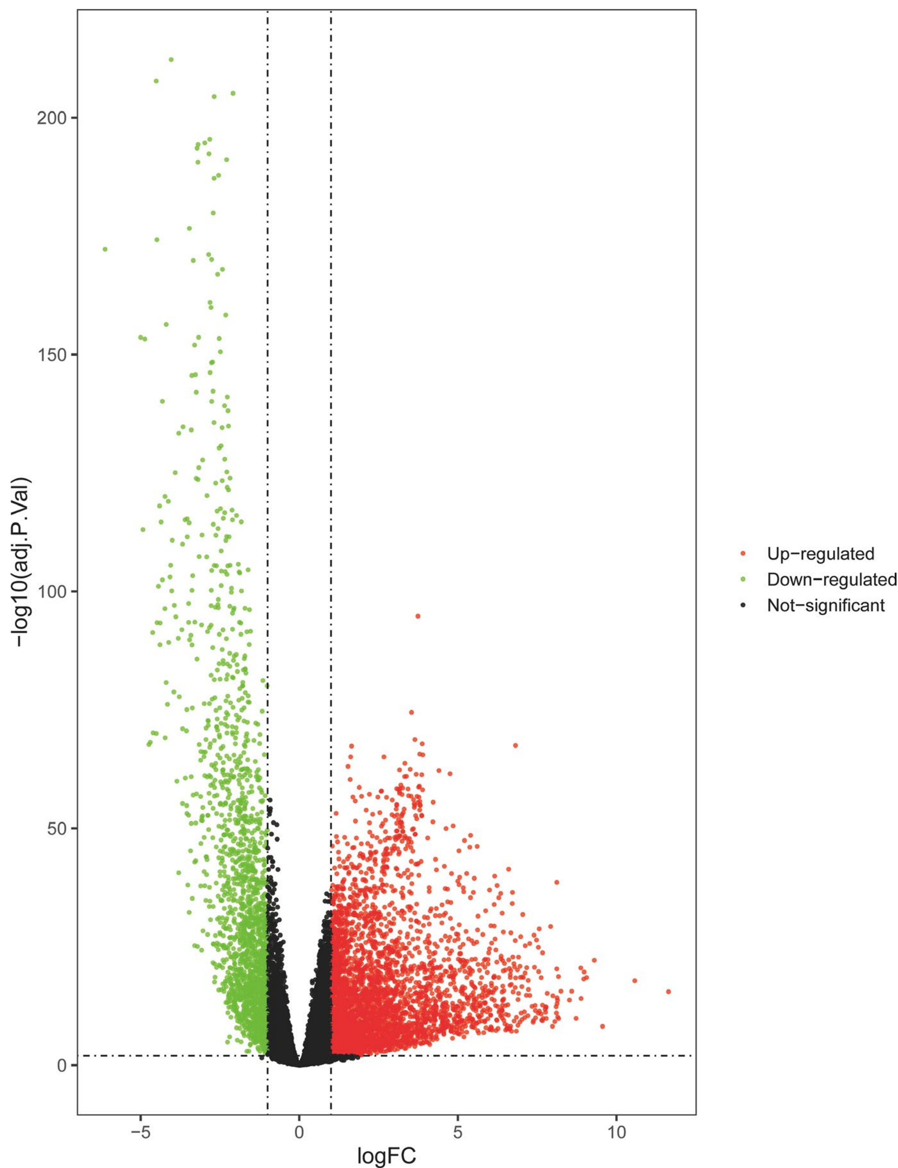

FIGURE 1 The volcano plot of the differentially expressed mRNAs. The red dots represent the upregulated mRNAs, and the green dots represent the downregulated mRNAs

effects of statins against LUAD via p53 mutant–mediated apoptosis.[13] Feng et al revealed that miRNA-147b promotes the aggressiveness of LUAD cells by negatively regulating MFAP4 and thus may act as a diagnostic marker of LUAD.[14] Dong et al showed that the lncRNA DGCR5 may act a diagnostic marker of LUAD.[15]

SNPs are an important genetic biomarker to investigate the characteristics of different types of cancers. However, studies based on SNP mutation-related genes in association with LUAD have not been performed yet. In this study, a total of 594 mRNA expression profiles and 503 SNP mutations data associated with LUAD were downloaded from the TCGA database. Thereafter, the differentially expressed mRNAs (DEmRNAs) and SNP-mutated genes were screened. In addition, enrichment and protein-protein interaction (PPI) network analyses of the mutated genes were performed. The correlation between gene mutation and expression was also analyzed. Finally, survival analysis of the SNP-mutated genes was performed, which affected the expression of their corresponding genes. In this study, we aimed to identify the pathogenesis and prognostic biomarkers of LUAD, which could potentially be used as new therapeutic targets for LUAD.

2 | MATERIALS AND METHODS

2.1 | Data processing and differential expression analysis

In total, 594 mRNA expression profile datasets associated with LUAD cases were downloaded from the TCGA Genomic Data Commons (GDC) Portal (https://gdc.cancer.gov/), which included data corresponding to 535 cases of LUAD samples and 59 cases of control samples. The differentially expressed mRNAs (DEmRNAs) between the tumor and control groups were analyzed using the edgeR tool in the R statistical software package (The R Foundation, Vienna, Austria). The DEmRNAs were screened using the screening criteria that was set as |log2 (fold-change [FC])|> 2, and $P < .01$. Based on the data of the 503 SNP mutations that was available on TCGA, "TCGA.LUAD.varscan." data were downloaded and analyzed using VarScan software (http://varscan.sourceforge.net/) to obtain further information regarding the mutated genes.

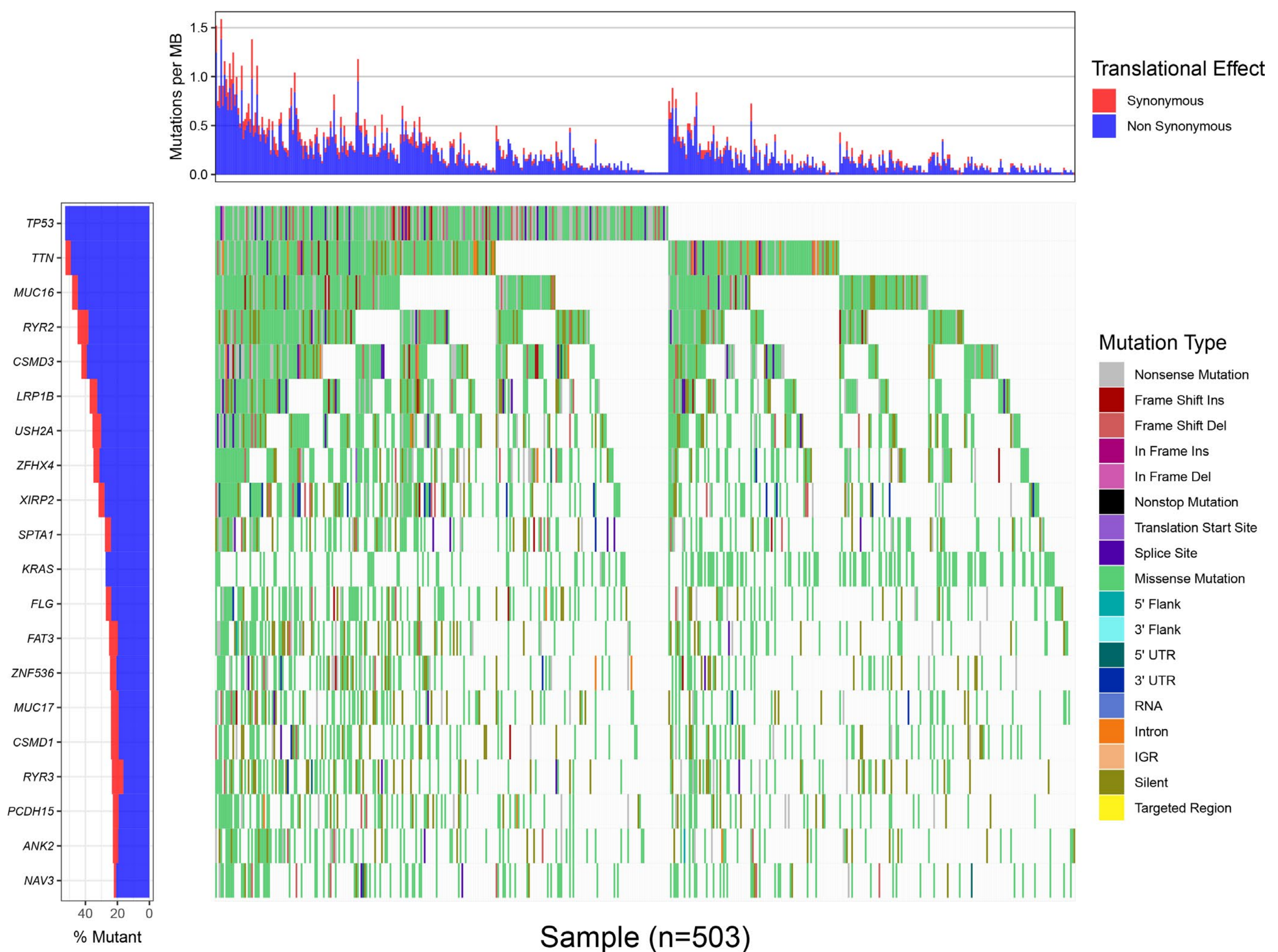

FIGURE 2 The waterfall map of the top 20 genes that were mutated in more than 30 samples. A, mutated genes, B, translational effect, and C, mutation types

2.2 | Enrichment analyses

Regarding analyses of the mutated genes, the web-based tool WebGestalt (WEB-based GEne SeT AnaLysis Toolkit) [16] (http://www.webgestalt.org/) was used to perform gene ontology (GO) analysis, and subsequently, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed with the cutoff value of $P < .01$. GO functional analysis was also performed, which included biological processes (BP), cellular components (CC), and molecular functions (MF).

2.3 | PPI network analysis of the mutated genes

The construction of biological networks can be represented in the form of an actual scale system and can provide a visual representation of molecular interactions. The Search Tool for the Retrieval of Interacting Genes (STRING) [17] (Version 10.0, http://www.string-db.org/) database was used to analyze the interactions between the proteins and proteins encoded by the mutated genes. The PPI score was set as 0.4 (which was referred to as "median confidence"). Thereafter, the PPI network was generated using Cytoscape software.[18]

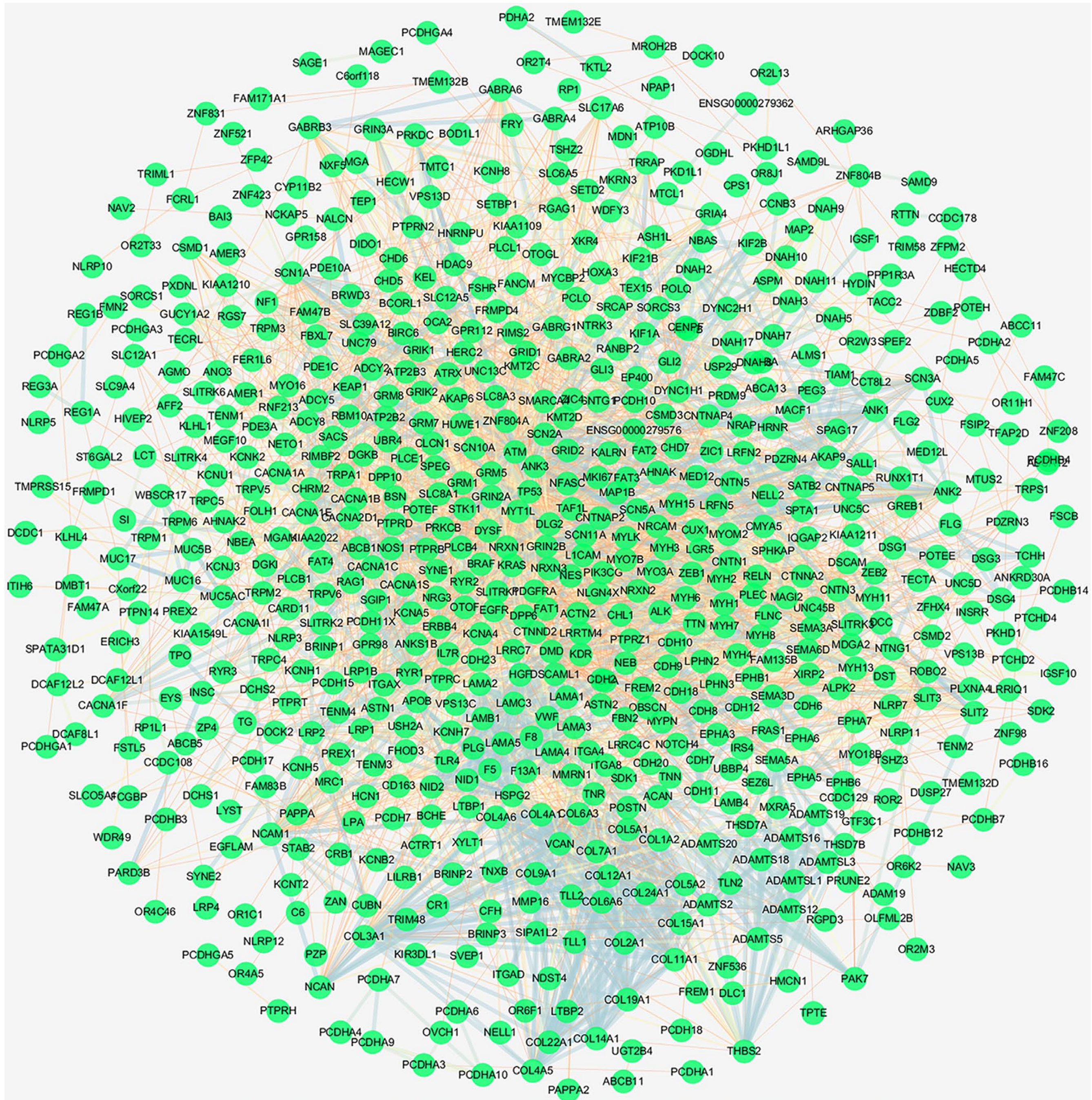

FIGURE 3 The protein-protein interaction (PPI) network of the 756 mutant genes associated with lung adenocarcinoma (LUAD)

2.4 | Correlation analysis

The mRNA expression profiles of the SNP-mutated genes were integrated with the SNP mutation data, and the correlation analysis between gene mutation and expression was analyzed using the Wilcox test with the cutoff value of $P < .01$. Then, the relationship between the SNP mutation sites and gene expression data was further analyzed.

2.5 | Survival analysis

The generation of Kaplan-Meier plots can help to evaluate the survival of cancer patients using gene expression data. Based on the TCGA database, the clinical data of patients with LUAD were downloaded to extract the corresponding survival data. Then, the survival analysis was performed for the prognosis of LUAD patients by generating Kaplan-Meier plots and performing a log-rank test. Here, the statistical significance level was defined as $P < .01$. The survival analysis was performed on the SNP-mutated genes that were found to affect the expression of related genes. Finally, the prognosis-related genes were screened as potential biomarkers of LUAD.

2.6 | Validation analysis

We further validated the mutated genes associated with LUAD prognosis by obtaining the expression values of these mutated genes from the TCGA database. Based on these values, the 542 samples were divided into two groups, including a tumor group (483 tumor samples) and a control group (59 normal samples). Then, the differentially expressed genes between the two groups were analyzed using the edgeR tool in the R statistical software package (The R Foundation) with the cutoff value of |log2 (fold-change [FC])|> 2 and $P < .01$.

3 | RESULTS

3.1 | Differential expression analysis

The DEmRNAs between the tumor and control groups were analyzed using the edgeR tool in the R statistical software package (The R Foundation). As a result, a total of 2502 DEmRNAs were screened with the cutoff value of |log2 (fold-change [FC])|> 2 and $P < .01$, including 1975 upregulated and 527 downregulated mRNAs (Figure 1). In addition, 756 SNP-mutated genes were identified in more than 30 samples. The top 20 out of 756 genes were analyzed for their mutation frequencies (Figure 2).

3.2 | GO functional and pathway enrichment analyses of the mutated genes

To further elucidate the functional role of the SNP-mutated genes in LUAD, we used the WebGestalt tool to perform functional enrichment and KEGG pathway analyses of the 756 SNP-mutated

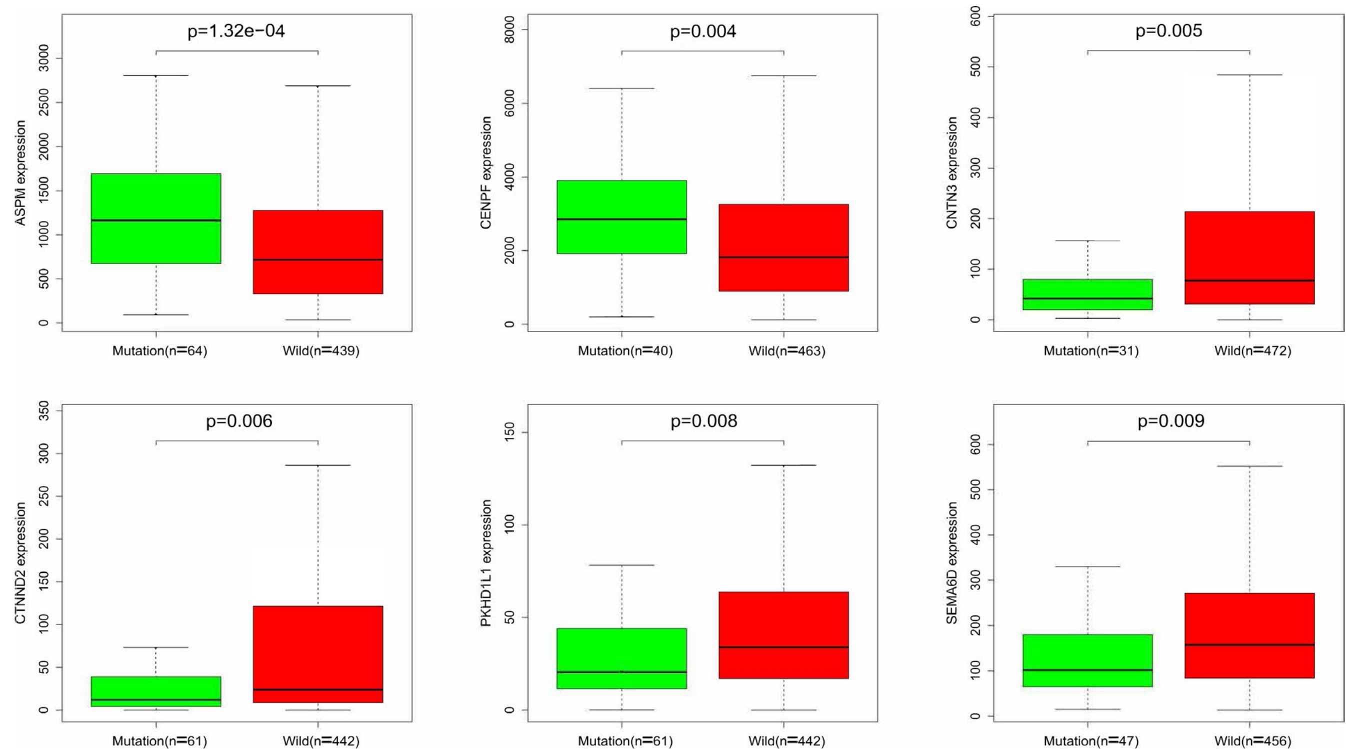

FIGURE 4 The relationship between the respective mutation and expression of six genes

genes. The functional analysis revealed that in the BP group, SNP-mutated genes were mainly enriched in the process corresponding to calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules, homophilic cell adhesion via plasma membrane adhesion molecules, and cell-cell adhesion via plasma membrane adhesion molecules. In the CC group, the genes were found to be enriched mainly in the components related to the basement membrane, contractile fibers, and sarcomeres. In the MF group, these genes were particularly enriched in ATP-dependent microtubule motor activity, which was minus-end-directed (Table S1A). In addition, the pathway analysis revealed the enrichment of SNP-mutated genes in many signaling pathways associated with cancer, including the ECM-receptor interaction, focal adhesion, and calcium signaling pathways (Table S1B).

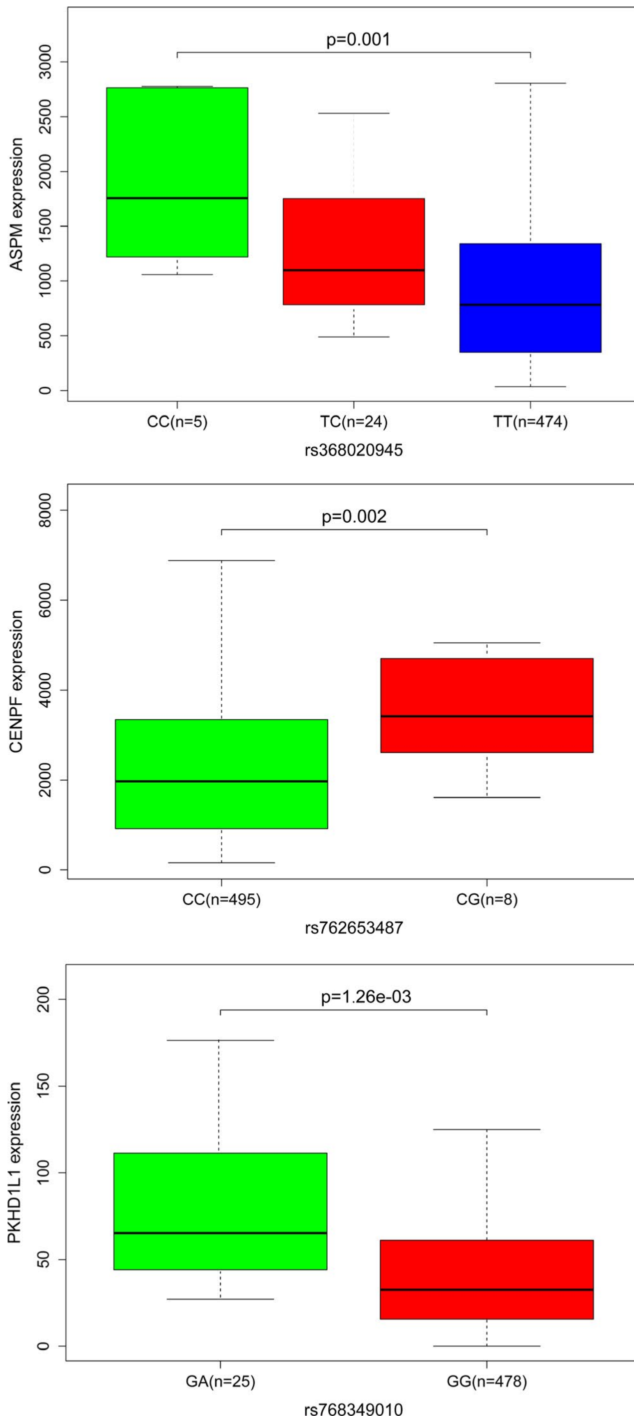

FIGURE 5 The relationship between the mutation sites and corresponding expression of the *ASMP*, *CENPF*, and *PKHD1L1* genes

3.3 | PPI network analysis of the mutated genes

To further investigate the potential link between the SNP-mutated genes, the STRING database was used to perform data mining and elucidate the interactions between the genes associated with LUAD. In total, 675 nodes and 3934 edges were formed in the PPI network that was generated using Cytoscape software (Figure 3). Tumor protein p53 (*TP53*) and neurexin 1 (*NRXN1*) with the higher degree were chosen as the hub genes (Table S2).

3.4 | Correlation analysis between gene mutation and expression

Based on the analysis of the SNP mutation-related data of the 756 mutated genes and their corresponding mRNA expression profiles, 42 mutated genes that correlated with their respective gene expression were identified. To further analyze the association between these 42 genes and the overall survival (OS) of patients with LUAD, we identified 6 genes, including assembly factor for spindle microtubules (*ASPM*), centromere protein F (*CENPF*), contactin 3 (*CNTN3*), catenin delta 2 (*CTNND2*), PKHD1 like 1 (*PKHD1L1*), and semaphorin 6D (*SEMA6D*) that were associated with the prognosis of LUAD (Figure 4). In addition, further analysis of these six genes for their mutation sites revealed three SNP mutation sites that were significantly related to their gene expression, which included ASPM rs368020495, CENPF rs762653487, and PKHD1L1 rs768349010 (Figure 5).

3.5 | Survival analysis of the mutated genes associated with the prognosis of LUAD

To screen the mutated genes that could be potentially used as prognostic biomarkers of LUAD, patients were divided into high and low expression groups according to the median expression value based on the Kaplan-Meier plots. We found that the OS levels of two genes (*ASPM* and *CENPF*) in the high expression group were decreased compared with those of the low expression group while the low expression of four genes (*CNTN3*, *CTNND2*, *PKHD1L1*, and *SEMA6D*) resulted in shorter OS compared with the high expression of the same genes (Figure 6). Therefore, it can be speculated that *ASPM* and *CENPF* are the oncogenes, and *CNTN3*, *CTNND2*, *PKHD1L1*, and *SEMA6D* are the tumor suppressor genes.

3.6 | Validation analysis

Based on the TCGA database, we further validated the mutated genes associated with LUAD prognosis by obtaining their respective expression values. Then, the differentially expressed genes between the tumor and control groups were analyzed with the cutoff value of |log2 (fold-change [FC])|> 2 and $P < .01$. We found that among the six mutated genes associated with the prognosis of LUAD, *CENPF* was upregulated, and *SEMA6D* was downregulated (Figure 7). These findings were in accordance with the results obtained from the survival analysis.

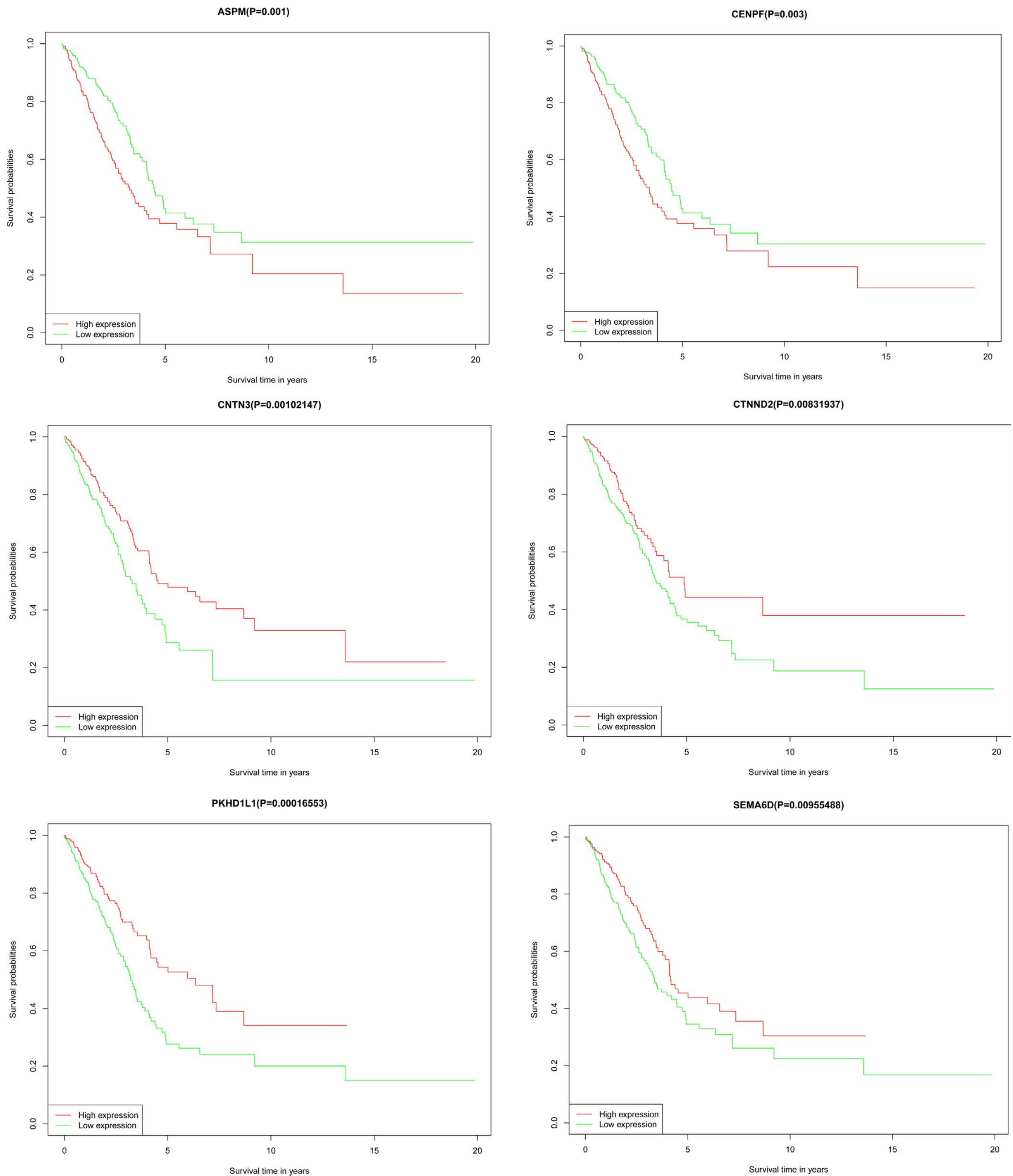

FIGURE 6 Kaplan-Meier survival curves of the six mutated genes

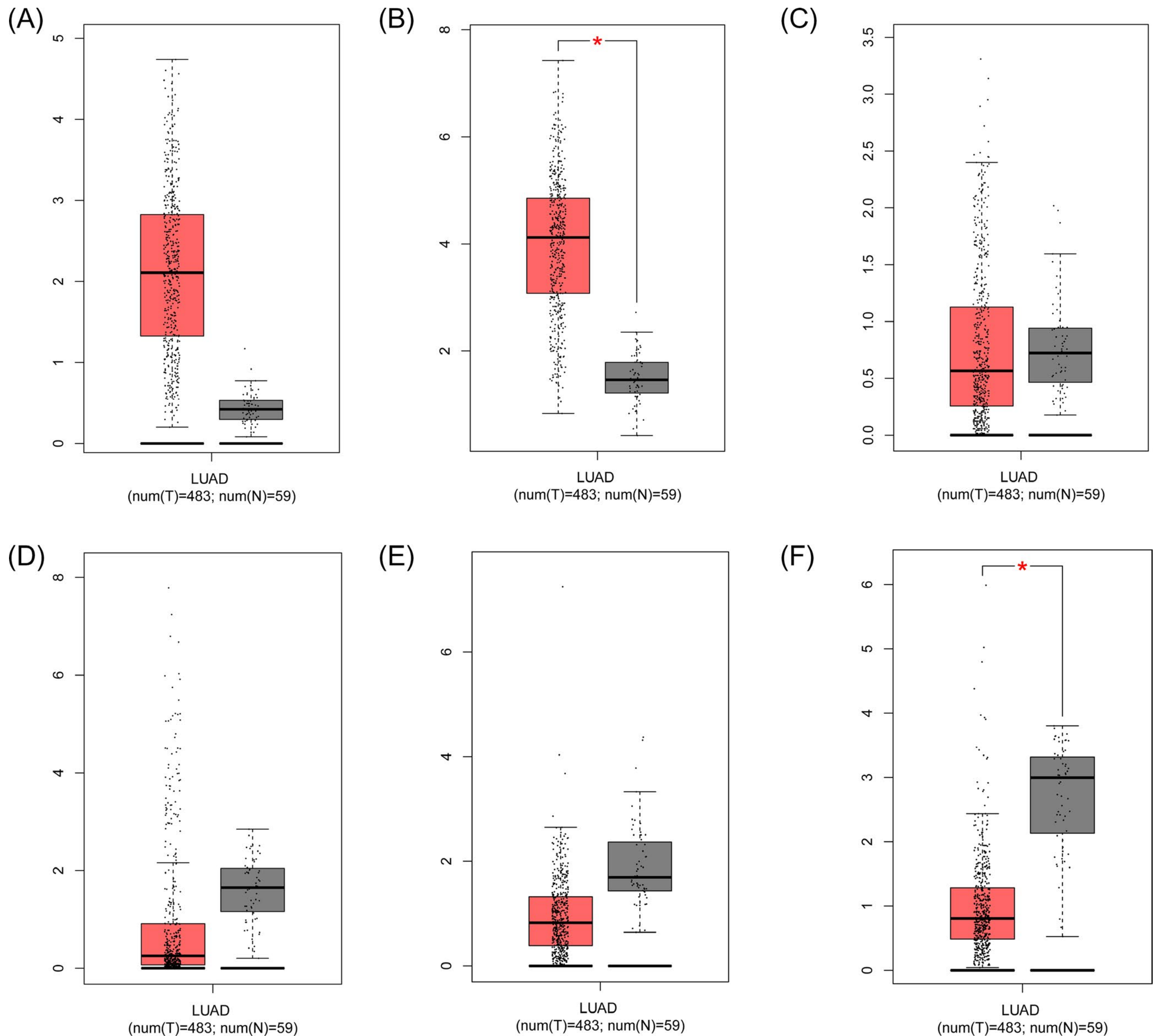

FIGURE 7 Validation of the mutated genes associated with the prognosis of LUAD based on The Cancer Genome Atlas (TCGA) database. A, *ASPM*, B, *CENPF*, (C) *CNTN3*, (D) *CTNND2*, (E) *PKHD1L1*, and (F) *SEMA6D*. (*$P < .01$)

4 | DISCUSSION

LUAD is the one of the most harmful malignant tumors that affects human health and life worldwide. In this study, we aimed to screen and identify the prognostic biomarkers associated with SNP-mediated expression through a series of bioinformatics analyses using LUAD-related data downloaded from the TCGA database. To further study the molecular mechanisms directly involved in these SNP-mutated genes, functional enrichment and KEGG pathway analyses were performed. The genes were found to be enriched in calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules, the basement membrane, and ATP-dependent microtubule motor activity. The pathway analysis indicated that LUAD-associated SNP-mutated genes were mainly involved in the ECM-receptor interaction, focal adhesion, and calcium signaling pathways related to cancer development. The functional enrichment and pathway analyses also revealed the molecular mechanisms associated with these SNP mutations in disease progression and the functional level interaction of these genes.

A total of 756 SNP-mutated genes in more than 30 samples were identified in this study, and the pathway analysis indicated that the mutated genes were mainly involved in the ECM-receptor interaction, focal adhesion, and calcium signaling pathways. Zhang et al identified the gene signature associated with the prognosis of smoking-related LUAD using bioinformatics analyses, and the functional enrichment analysis stressed that DEmRNAs were closely related to the carcinogenesis of smoking-related LUAD, such as the cell cycle, ECM-receptor interaction, and cell division.[19] Furthermore, Nakanishi et al uncovered that the focal

adhesion pathway and Src family kinases are targets for vinorelbine-resistant lung cancer.[20] In addition, Xu et al found that curcumin induces the apoptosis of non–small-cell lung cancer (NSCLC) cells through a calcium signaling pathway,[21] which was further validated in this study and indicates that the mutated genes have important biological functions in the development of LUAD.

In addition, the genes *TP53* and *NRXN1* with the higher degree were chosen as the hub genes in the PPI network. Assoun et al uncovered that TP53-mutated status was correlated with an immunotherapy OS benefit in advanced NSCLC.[22] Craig et al showed that a biomarker comprising *TP53*, *PIK3CA*, and *BRAF* somatic mutations may better stratify individuals for optimal lung cancer screening and prevention outcomes.[23] Furthermore, Yang et al found that *NRXN1* could efficiently function as a novel and independent prognosis biomarker and therapeutic target for colorectal cancer patients.[24] However, few studies on *NRXN1* associated with LUAD have been reported. Thus, we supposed that *TP53* and *NRXN1* may also be related to the development of LUAD.

CENPF was described for the first time as a binding partner of the retinoblastoma (Rb) protein in cancer cell lines.[25] Previous studies in the field of oncology have shown that *CENPF* is associated with OS in breast [26] and bladder cancers.[27] However, the role of *CENPF* in LUAD development has not been explored yet. In addition, bio-signal analysis has revealed that the *SEMA6D* gene may play an important role in promoting the OS of patients with breast cancer.[28] Studies have shown that the *SEMA6D* gene is directly related to the presence of *VEGFR2* in the angiogenesis of gastric cancer, which plays an important pathogenic role.[29] Studies on esophageal cancer have identified that *SEMA6D* is a target gene that is upregulated by *hsa-miR-1*.[30] Here, we identified two genes (*CENPF* and *SEMA6D*) that were found to be associated with LUAD prognosis. However, most of these identified genes have not been previously linked to LUAD. Our data indicate that these genes could serve as potential prognostic biomarkers of LUAD.

In the present study, SNP-mutated genes associated with the prognosis of LUAD were identified and the survival analysis of these mutated genes was performed, providing a theoretical basis for designing personalized precision medical treatment of LUAD. However, there were several limitations in this study. For instance, relevant experiments, including cell biology assays, as well as animal and clinical studies need to be performed in order to verify the candidate targets and signaling pathways that were identified from our bioinformatics analyses.

In conclusion, *CENPF*, *SEMA6D*, *TP53*, and *NRXN1* are likely related to the progression of LUAD. Thus, these molecular markers may be used as therapeutic targets in the treatment of LUAD.

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None.

CONFLICT OF INTEREST DISCLOSURE

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

SL and MH conceived and designed the study. LZ involved in data acquisition. YW and ML analyzed and interpreted the data. YC involved in statistical analysis. YW drafted the manuscript. SL revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

ETHICAL APPROVAL

This article does not contain any experiments that were performed using human participants or animals.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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