

A Novel E3-ubiquitin Ligase-Related LncRNA Signature for Predicting Prognosis in Patients with Lung Adenocarcinoma

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Research Article

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Abstract

Background: E3 ubiquitin ligase mRNA plays an important role in mediating tumor microenvironment, and is involved in tumor initiation and progression. However, few studies have realized the value of E3 ubiquitin ligase-related lncRNAs in lung adenocarcinoma (LUAD).

Methods: Herein, we comprehensively evaluated the E3-ubiquitination patterns including multiple tumor-related molecular phenotypes in LUAD samples using lncRNA profiling from GEO and TCGA database, identified a survival-related risk signature consisting of E3-ubiquitin ligase-related lncRNAs via LASSO and multivariate stepwise Cox regression analysis. Based on the risk score calculated for each sample, LUAD patients were divided into high- and low-risk groups. The predictive value of the signature in overall survival was explored, and a nomogram integrating the risk signature and clinical characteristics was identified and tested.

Results: A risk signature consisting of 7 specific E3-ubiquitin ligase-related lncRNAs was screened, and can be viewed as a reliable independent predictor of prognosis. We performed consensus clustering analysis and successfully identified 4 molecular subtypes significantly linked to the OS of LUAD, which validates the prognostic and predictive value of this signature to some extent. The ssGESA analysis revealed that the high-risk group was bound up closely with epithelial-mesenchymal transition, hypoxia, and PI3K/AKT/mTOR pathways, and had a worse outcome. Moreover, we created a nomogram consisting of pathological staging and risk score. Validation analysis demonstrated high conformity of nomogram predictive probability and actual overall survival in LUAD of TCGA and GEO datasets.

Conclusion: The model consisting of specific E3-ubiquitin ligase-related lncRNAs contributes to predicting the prognosis of LUAD patients.

Introduction

Lung adenocarcinoma (LUAD), a most common type of non-small cell lung cancer (NSCLC), accounts for about 40% of all lung cancers (Denisenko et al., 2018). The new therapeutic strategies such as molecular targeted agents and immune checkpoint inhibitors have demonstrated astounding durable efficacy in a small fraction of LUAD patients. However, the majority of patients still experience dismal prognosis, far from a satisfactory clinical need. The average five-year survival rate of LUAD patients is less than 18%, mainly due to late diagnosis, recurrence, and metastasis (Zhao et al., 2018). In this scenario, identifying promising diagnostic biomarkers and therapeutic targets for LUAD is of great importance.

Ubiquitination is a dynamic and reversible process in which ubiquitin covalently conjugates onto lysine (K) residues of target proteins. The process is catalyzed by sequential actions of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes. E3 ubiquitin ligases modulate the transfer of ubiquitin to substrate proteins determining their fate (Zhou and Sun, 2021; Kwon and Ciechanover, 2017). E3-mediated ubiquitin-proteasome system regulates the activation and repression of the innate and adaptive immune responses, mediates tumor microenvironment, and induces tumor initiation and

progression (Budroni and Versteeg, 2021; Liu et al., 2021). For example, Kohli et al. reported that UBE3A, also known as E6-associated protein (E6-AP), was the founding member of the HECT family of E3 ubiquitin ligases. UBE3A-mediated SIRT6 degradation promoted cell proliferation and invasiveness in liver cancer cells (Kohli et al., 2018). E3 ubiquitin ligase ITCH interacts with ASPP2 to suppress Smad7 degradation, and thus negatively regulates TGF- β 1-induced epithelial-mesenchymal transition (EMT) and ultimately inhibits gastric cancer invasion (Gen et al., 2017). Ubiquitin-mediated NOTCH1 protein (N1ICD) is abnormally activated via RNF8 degradation leading to increase the activation of Notch signaling, promoting the initiation and progression of breast cancer (Li et al., 2018a). The overexpression of E3 ubiquitin ligase NEDD4 leads to the increase of cell migration in lung cancer through facilitating EGF-induced cathepsin B secretion by lysosomal exocytosis (Shao et al., 2018).

Long non-coding RNA (lncRNA), characterized by more than 200 nucleotides in length, is believed to play an essential role in tumorigenesis and progression (Wu et al., 2019). Recently, several studies are starting to realize the relationship between lncRNA and E3 ubiquitin ligase and their interaction in lung cancer. For instance, lncRNA Meg3 can inhibit the expression of Skp2 (a component of the E3 ubiquitin ligase SCF) while promoting p27 expression (a CDK inhibitor), thereby hindering tumor development (Su et al., 2016). Zhu et al. reported that lncRNA DUXAP9-206 has a direct effect on E3 ubiquitin ligase Cbl-b, enticing enhanced EGFR signaling in NSCLC (Zhu et al., 2019). Likewise, lncRNA MIAT can regulate Pellino E3 Ubiquitin Protein Ligase Family Member 3 (PELI3) through competing endogenous RNA (ceRNA) mechanism to enhance the proliferation, migration, and invasion of NSCLC cells (Li et al., 2020). Although a growing number of lncRNAs have been annotated, the specific role of lncRNAs related to E3 ubiquitin ligase in the occurrence and development of cancers remains unclear. In the study, we are the first to comprehensively describe the biological function of E3-ubiquitin ligase-related lncRNAs in lung cancer, and boost the understanding of the roles and mechanism of action of E3-ubiquitin ligase-related lncRNAs in lung cancer using lncRNA profiling analysis with the intention of selecting important biomarkers. Moreover, we also identified for the first time the E3-ubiquitin ligase-related lncRNA signature for predicting the prognosis of LUAD patients.

Materials And Methods

Data Download and Preprocessing

RNA-Seq and corresponding clinical parameters were downloaded from The Cancer Genome Atlas (TCGA) database consisting of 535 tumor and 59 normal samples. Patients with complete follow-up were randomized into training and internal testing groups at a ratio of 6:4. LUAD samples from GSE412717 were also employed as an independent external validation set. A total of 196 genes encoding E3-ubiquitin ligase were obtained from the UbiBrowser, a bioinformatics platform providing a comprehensive and up-to-date dataset of proteome-wide human E3-substrate interaction network (Li et al., 2017). The study did not need the ethics committees' approval due to the open-access database including the TCGA or GEO. The workflow diagram of this study was presented in Figure 1.

Identification and Evaluation of Risk Signature

We extracted lncRNAs data (FPKM values) from TCGA and GEO databases respectively and performed the intersection of lncRNAs. Subsequently, we screened E3-ubiquitin ligase-related lncRNAs using Pearson correlation ($Cor > 0.5$ or $Cor < -0.5$, $P < 0.001$) after excluding genes with FPKM value less than 0.5. After then, to construct a specific signature composed of E3-ubiquitin ligase-related lncRNAs, the least absolute shrinkage and selection operator (LASSO) Cox regression with an estimation of penalty parameter through performing 10-fold cross-validations and multivariate stepwise Cox regression analysis were employed to recognize the key lncRNAs based on the above-mapped lncRNAs. On the basis of gene expression together with regression coefficients (β genes*expression level of genes), the risk score was computed. According to the median cutoff of the risk score in the training cohort, LUAD patients were dichotomized into high- and low-risk groups. The Kaplan-Meier survival curves and receiver operating characteristic (ROC) curves were performed to evaluate the predictive efficacy and reliability of the signature. Furthermore, together with other clinical variables, the created predictive signature was incorporated into the stepwise multivariate analyses. Relative expression of lncRNAs identified between normal and tumor samples was further validated.

Consensus Clustering for Risk Signature

To further validate the predictive performance of the signature above, we performed the cluster analysis to categorize LUAD patients into several subtypes using the R “ConsensusClusterPlus” package. Furthermore, corresponding Kaplan-Meier survival curves were delineated to determine the survival difference of different subtypes.

Analysis of Immune Cell Infiltration, Functional Enrichment, and Tumor-related Phenotypes Among Risk Signature

To explore the association between tumor immune microenvironment (TIM) and the risk signature, single sample gene set enrichment analysis (ssGSEA) was employed to evaluate the degree of immune infiltration in LUAD. The R package “GSVA” was utilized to calculate the proportion of 28 types of immune cells based on a specific gene set composed of 782 genes (Bindea et al., 2013; Hänzelmann et al., 2013). Furthermore, we also calculate the Immune Score and Stromal Score of LUAD samples in the TCGA cohort via ESTIMATE algorithm (Yoshihara et al., 2013). Enrichment analysis was utilized to evaluate the enrichment pathways of differentially expressed genes (DEGs) between low- and high-risk groups via R package “ClusterProfiler” (Yu et al., 2012). A P value of < 0.05 was considered as a threshold to judge significant categories in GO and KEGG analysis. Differences in top enriched signaling pathways and other tumor-related pathways between distinct risk signature groups from the TCGA cohort were assessed and compared using the GSEA algorithm.

Construction of nomogram

Nomogram integrating with the risk score and prognosis-related clinical parameters like pathological staging was established to illustrate the 1-, 2- and 3-year survival probabilities of I-III stage LUAD patients

according to the results of multivariate Cox regression analysis. Calibration plots delineated by bootstrapping method investigated the agreement between the survival probabilities between reality and prediction based on the constructed nomogram. Receiver operating characteristic curves (ROCs) with the area under the curve (AUC) values also were developed to evaluate the predictive ability of the nomogram compared with the TNM staging system.

Statistical Analyses

All statistical analyses were performed using R software (version 3.6.1) unless otherwise indicated. Univariate and multivariate Cox regression analyses were utilized to evaluate the independent prognostic value of E3-ubiquitin ligase-related lncRNAs in terms of OS. Kaplan–Meier curves were employed to compare the OS between high- and low-risk groups. Mann-Whitney-Wilcoxon test was utilized to compare the distribution of immune cell infiltration between high- and low-risk groups. The prognostic capacity of risk signature and nomogram was evaluated by receiver operating characteristic (ROC) curves (R package “timeROC”) and AUC values. In terms of the prognostic predictive accuracy and precision of the nomogram, the calibration plots also were performed. $P < 0.05$ was considered statistically significant unless otherwise stated.

Results

Construction of E3-ubiquitin Ligase-Related Signatures lncRNA Risk Signature

A total of 337 E3-ubiquitin ligase-related lncRNAs were extracted from 14,086 shared lncRNAs in both TCGA and GEO datasets, followed by LASSO regression analysis (Figure 2A and 2B). Then based on multivariate Cox regression analysis (Figure 2C), a 7-lncRNA risk signature was constructed (GABPB1-AS1, HCFC1-AS1, AC018529.1, LINC02178, AC106053.1, FRMD6-AS1, and AC107021.2). Based on regression coefficients and expression levels, the formula of risk score was as followed: $(-1.1461 \times \text{expression level of GABPB1-AS1}) + (-1.4099 \times \text{expression level of HCFC1-AS1}) + (-3.3698 \times \text{expression level of AC018529.1}) + (1.7381 \times \text{expression level of LINC02178}) + (5.1659 \times \text{expression level of AC106053.1}) + (3.4516 \times \text{expression level of FRMD6-AS1}) + (0.9922 \times \text{expression level of AC107021.2})$. The expression level of 7 lncRNAs was shown in Figure 2D. Patients were divided into high- and low-risk cohorts according to the median risk score of the training group. Meanwhile, the distribution of expression heat map, risk score, and survival status of LUAD patients in the three cohorts were plotted in Supplemental Figure 1 in detail. These genes are most likely to have important effects on LUAD.

Validation of Prognostic Value of Risk Signature

The Kaplan–Meier analysis for each dataset proved that high-risk group had worse survival status compared with the low-risk group ($P < 0.05$) among patients with LUAD (Figure 3A-3C). ROC curves suggested that the E3-ubiquitin ligase-related lncRNA signature had good prognostic prediction ability in LUAD patients. The AUC of 2-, 3-, and 5-year was 0.75, 0.75, and 0.76 in the TCGA training cohort, 0.59,

0.63, 0.71 in the TCGA testing cohort, and 0.6, 0.63, 0.63 in the GEO cohort, respectively (Figure 3D-3F). Forest plot of the multivariate analysis ($P < 0.01$, Figure 3G-3I) indicated that the risk signature was an independent prognostic factor compared to other predictors (age, gender, tumor stage, and smoking status).

Identifying Molecular Subtypes Based on E3-ubiquitin Ligase-related LncRNA Signature

To further investigate the prognostic and predictive value of the above signature, we then performed unsupervised clustering and found 4 stable subtypes in LUAD based on TCGA and GEO data (Figure 4A-4C and Supplemental Figure S3). Kaplan–Meier analysis demonstrated significant survival discrepancies among the four subgroups and cluster D had the worst outcome compared to the other three subgroups ($P < 0.001$, Figure 4D). This analysis further indicated that the 7 lncRNAs related to E3-ubiquitin ligase may be feature genes.

Correlation Assessment Between Immune Risk Signature and Immune Cell Infiltration and Functions Pathways

We further explored the potential relationship between the risk signature and the immune response in LUAD. A clear distinction of immune infiltration level between high- and low-risk groups was presented via ssGSEA (Figure 5A). The infiltration level of anti-tumor immunocyte including activated CD4⁺/CD8⁺ T cell and effect memory CD8⁺ T cell was higher in the low-risk group ($P < 0.01$) than that in the high-risk group. The levels of intermediate immunocyte subtypes including eosinophil, immature B cell, activated B cell, mast cell and T follicular helper cell were also elevated in the low-risk signature group ($P < 0.001$) (Figure 5A). There is no significant difference between high- and low-risk groups in terms of stromal and immune scores. Top KEGG enriched signaling pathways were depicted in Figure 5B, the enriched signaling pathways involving in the low-risk group were NOD-like receptor signaling pathway, neuroactive ligand-receptor, cytokine-cytokine receptor interaction, cAMP signaling pathway, cell adhesion molecules, and so on. In the high-risk group, there were two mainly enriched signaling pathways — viral carcinogenesis and necroptosis.

Characteristics of Transcriptome Traits in Tumor-related Regulation

To reveal the role of E3-ubiquitin ligase-related risk groups in the tumor-related regulation, we further studied the expression difference of genes associated with the transcripts of some chemokine and cytokine in different regulatory pathways including angiogenesis, apoptosis, epithelial-mesenchymal transition (EMT), hypoxia, and P53 signaling pathway. The ssGSEA analyses for the activity of multiple tumor-related regulations revealed that the high-risk group with worse survival were significantly related with EMT pathways, hypoxia pathways, Notch signaling, and PI3K/AKT/mTOR signaling pathways (Figure 6). Moreover, we compared the expression difference of multiple genes related to the regulation of

EMT and hypoxia. The result indicated that these genes relevant to EMT and hypoxia pathway were significantly upregulated in the high-risk group.

Construction and Validation of the Nomogram

A nomogram was established to predict the probability of the 1-, 2-, and 3-year overall survival of LUAD with I-III stage in the TCGA cohort (Figure 7A). In assessing the predicting efficacy at 1-year OS, the AUC for nomogram, tumor stage, and risk score was 0.780, 0.700, 0.691, respectively (Figure 7B). The 2 years AUC of the nomogram, tumor stage, and risk score was 0.810, 0.749, 0.702, respectively (Figure 7C). In terms of 3 years AUC, the value of nomogram, tumor stage, and risk score was 0.772, 0.717, 0.679, respectively (Figure 7D). The calibration curves for the 1-, 2- and 3-year survival revealed excellent agreement in both prediction and observation in training cohorts (Figure 7E-G). Validation analysis in the GSE412717 cohort showed identical results (Supplemental Figure S3).

Discussion

Here, we systematically for the first time analyzed the role of E3-ubiquitin ligase-related lncRNAs in LUAD prognostic prediction by bioinformatics and built a reliable prognostic signature based on seven specific lncRNAs. Further analyses validated the predictive efficacy in the TCGA and GEO datasets. This signature stratified the patients into high- and low-risk subgroups, characterized by distinct tumor immune infiltration level and tumor-related regulatory phenotypes such as EMT and hypoxia via ssGSEA analyses. Through multivariate Cox regression analysis, this signature was included in a fusion nomogram combined with the pathological staging to predict the outcome of LUAD with the I-III stage. Taken together, the newly identified risk model may serve as a promising tool for prognosis estimation of LUAD patients.

Mounting researchers have discovered that E3-ubiquitin ligase-related lncRNAs could mediate immunity and be involved in multiple tumor-related pathways. Our study evaluated 28 immune cell infiltrations and found that risk score was negatively associated with activated CD4⁺ T cells and effector memory CD8⁺ T cell that exerted an antineoplastic effect by releasing TNF- α and IFN- γ (Gabrilovich et al., 2012; Apetoh et al., 2011). Thus, low-risk samples tend to have a favorable prognosis. Meanwhile, the high-risk group presented a tight correlation with the pathways/phenotypes including epithelial-mesenchymal transition (EMT), hypoxia, Notch signaling, and PI3K/AKT/mTOR signaling pathways. E3-ubiquitin ligase-related lncRNAs had been described to regulate tumor progression via EMT. Pan et al. demonstrated that lnc-CTSLP4 could reverse EMT of GC cells by recruiting E3 ubiquitin ligase ZFP91 to decrease NRNPAB-mediated Snail transcription, thereby inhibiting GC progression (Pan et al., 2021). LINC00858 could sponge miR-134-5p to promote E3 ubiquitin ligase RAD18 elevation, resulting in enhancing EMT of cancer cells (Xue et al., 2020). Besides, the overexpression of lnc-DARS-AS1 was able to interfere with the interaction between RBM39 and E3 ubiquitin ligase RNF147 via hypoxia regulation, leading to myeloma genesis (Tong et al., 2020). In short, E3-ubiquitin ligase-related lncRNAs are inversely associated with the migration and invasion of LUAD.

We identified 7 E3-ubiquitin ligase-related lncRNAs included in the risk signature and further developed a nomogram. GABPB1-AS1 was highly expressed in normal tissue and acted as a favorable gene in renal carcinoma (Gao et al., 2020a), and its overexpression could inhibit proliferation, migration, and invasion. A previous study reported that LINC02178 may be a promising prognostic biomarker of cancers like LUAD (Li et al., 2018b). Other lncRNAs were reported for the first time and are worthy of further investigation. Nomogram, as a graphical representation tool, has been widely used to integrate a variety of variables especially clinical information and genomics in the evaluation of prognosis (Balachandran et al., 2015). According to the hazard ratio of the various factors, each variable can get a corresponding score, and clinicians will easily calculate the total score and offer the corresponding survival probability to individuals. Currently, researchers have performed nomograms to provide individualized prognosis predictions in multiple cancers like breast cancer, gastric cancer, lymphoma, and lung cancer (Wang et al., 2021; Geng et al., 2021; Gao et al., 2020b; Wu et al., 2021). Herein, we constructed a prognostic nomogram by combining pathological staging with E3-ubiquitin ligase-related lncRNA signature. The result revealed that the accuracy and clinical usefulness of our nomogram for predicting prognosis were excellent compared to the TNM staging system.

There were several limitations in this study. Our study included the TCGA and GEO datasets and all results were derived and validated using them. Given the limited number of patients with an advanced stage in the two datasets, we only established and validated a nomogram for predicting survival probability in stage I–III LUAD patients. Additionally, additional LUAD samples should be employed to estimate the predictive value of lncRNAs. Likewise, the roles of the lncRNAs and their interactions with E3-ubiquitin ligase genes should be determined in future researches in vitro and in vivo.

Conclusions

In conclusion, our study identified seven lncRNAs related to E3-ubiquitin ligase with stable survival predictive value through performing an integrated analysis of large-scale lncRNA expression profiles from the GEO and TCGA databases. Meanwhile, we developed the corresponding prognostic signature, which would contribute to the understanding of the molecular mechanism in LUAD.

Abbreviations

LUAD: Lung adenocarcinoma; AUC: Area under curve; NSCLC: Non-small cell lung cancer; lncRNA: Long non-coding RNA; ROC: Receiver operating characteristic; TIM \uparrow : Tumor immune microenvironment; ssGSEA: Single sample gene set enrichment analysis; DEGs \uparrow : Differentially expressed genes; LASSO: the least absolute shrinkage and selection operator; EMT \uparrow : Epithelial-mesenchymal transition.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

Not applicable.

Competing interests:

No conflict of interests.

Authors' contributions:

Conception and design, as well as the manuscript review: FL and TR; Development of methodology: TL and LC; Collection of data and manuscript writing: SD and LC; Data analysis and interpretation: XY.H; Final approval of manuscript: All authors.

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References

1. Denisenko TV, Budkevich IN, Zhivotovsky B. (2018) Cell death-based treatment of lung adenocarcinoma. *Cell Death Dis.* 9:117. DOI:10.1038/s41419-017-0063-y
2. Zhao Y, Varn FS, Cai G, Xiao F, Amos CI, Cheng C. (2018) A P53-Deficiency Gene Signature Predicts Recurrence Risk of Patients with Early-Stage Lung Adenocarcinoma. *Cancer Epidemiol Biomarkers Prev.* 27:86-95. DOI:10.1158/1055-9965.EPI-17-0478
3. Zhou X, Sun SC. (2021) Targeting ubiquitin signaling for cancer immunotherapy. *Signal Transduct Target Ther.* 6:16. DOI:10.1038/s41392-020-00421-2

4. Kwon YT, Ciechanover A. (2017) The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. *Trends Biochem Sci.* 42:873-86. DOI:10.1016/j.tibs.2017.09.002
5. Budroni V, Versteeg GA. (2021) Negative Regulation of the Innate Immune Response through Proteasomal Degradation and Deubiquitination. *Viruses.* 13:584. DOI:10.3390/v13040584
6. Liu Q, Aminu B, Roscow O, Zhang W. (2021) Targeting the Ubiquitin Signaling Cascade in Tumor Microenvironment for Cancer Therapy. *International journal of molecular sciences.* 22. DOI:10.3390/ijms22020791
7. Kohli S, Bhardwaj A, Kumari R, Das S. (2018) SIRT6 Is a Target of Regulation by UBE3A That Contributes to Liver Tumorigenesis in an ANXA2-Dependent Manner. *Cancer Res.* 78:645-58. DOI:10.1158/0008-5472.CAN-17-1692
8. Gen Y, Yasui K, Kitaichi T, Iwai N, Terasaki K, Dohi O, et al. (2017) ASPP2 suppresses invasion and TGF-beta1-induced epithelial-mesenchymal transition by inhibiting Smad7 degradation mediated by E3 ubiquitin ligase ITCH in gastric cancer. *Cancer Lett.* 398:52-61. DOI:10.1016/j.canlet.2017.04.002
9. Li L, Guturi KKN, Gautreau B, Patel PS, Saad A, Morii M, et al. (2018a) Ubiquitin ligase RNF8 suppresses Notch signaling to regulate mammary development and tumorigenesis. *J Clin Invest.* 128:4525-42. DOI:10.1172/JCI120401
10. Shao G, Wang R, Sun A, Wei J, Peng K, Dai Q, et al. (2018) The E3 ubiquitin ligase NEDD4 mediates cell migration signaling of EGFR in lung cancer cells. *Mol Cancer.* 17:24. DOI:10.1186/s12943-018-0784-2
11. Wu Y, Shao A, Wang L, Hu K, Yu C, Pan C, et al. (2019) The Role of lncRNAs in the Distant Metastasis of Breast Cancer. *Front Oncol.* 9:407. DOI:10.3389/fonc.2019.00407
12. Su L, Han D, Wu J, Huo X. (2016) Skp2 regulates non-small cell lung cancer cell growth by Meg3 and miR-3163. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine.* 37:3925-31. DOI:10.1007/s13277-015-4151-2
13. Zhu T, An S, Choy MT, Zhou J, Wu S, Liu S, et al. (2019) LncRNA DUXAP9-206 directly binds with Cbl-b to augment EGFR signaling and promotes non-small cell lung cancer progression. *Journal of cellular and molecular medicine.* 23:1852-64. DOI:10.1111/jcmm.14085
14. Li F, Li H, Li S, Lv B, Shi J, Yan H, et al. (2020) Long Non-coding RNA MIAT Mediates Non-small Cell Lung Cancer Development Through Regulating the miR-128-3p/PELI3 Axis. *Biochemical genetics.* 58:867-82. DOI:10.1007/s10528-020-09979-6
15. Li Y, Xie P, Lu L, Wang J, Diao L, Liu Z, et al. (2017) An integrated bioinformatics platform for investigating the human E3 ubiquitin ligase-substrate interaction network. *Nat Commun.* 8:347. DOI:10.1038/s41467-017-00299-9
16. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. (2013) Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* 39:782-95. DOI:10.1016/j.immuni.2013.10.003
17. Hänzelmann S, Castelo R, Guinney J. (2013) GSEA: gene set variation analysis for microarray and RNA-seq data. *BMC bioinformatics.* 14:7. DOI:10.1186/1471-2105-14-7

18. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. (2013) Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature communications*. 4:2612. DOI:10.1038/ncomms3612
19. Yu G, Wang LG, Han Y, He QY. (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics : a journal of integrative biology*. 16:284-7. DOI:10.1089/omi.2011.0118
20. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. (2012) Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol*. 12:253-68. DOI:10.1038/nri3175
21. Apetoh L, Végran F, Ladoire S, Ghiringhelli F. (2011) Restoration of antitumor immunity through selective inhibition of myeloid derived suppressor cells by anticancer therapies. *Current molecular medicine*. 11:365-72. DOI:10.2174/156652411795976574
22. Pan T, Yu Z, Jin Z, Wu X, Wu A, Hou J, et al. (2021) Tumor suppressor lnc-CTSLP4 inhibits EMT and metastasis of gastric cancer by attenuating HNRNPAB-dependent Snail transcription. *Molecular therapy Nucleic acids*. 23:1288-303. DOI:10.1016/j.omtn.2021.02.003
23. Xue H, Wu Z, Rao D, Zhuo B, Chen Q. (2020) Long non-coding RNA LINC00858 aggravates the oncogenic phenotypes of ovarian cancer cells through miR-134-5p/RAD18 signaling. *Archives of gynecology and obstetrics*. 302:1243-54. DOI:10.1007/s00404-020-05722-z
24. Tong J, Xu X, Zhang Z, Ma C, Xiang R, Liu J, et al. (2020) Hypoxia-induced long non-coding RNA DARS-AS1 regulates RBM39 stability to promote myeloma malignancy. *Haematologica*. 105:1630-40. DOI:10.3324/haematol.2019.218289
25. Gao S, Zhang F, Sun H, Yang X. (2020a) LncRNA GA-Binding Protein Transcription Factor Subunit Beta-1 Antisense RNA 1 Inhibits Renal Carcinoma Growth Through an MiR-1246/Phosphoenolpyruvate Carboxykinase 1 Pathway. *OncoTargets and therapy*. 13:6827-36. DOI:10.2147/ott.s257275
26. Li YY, Yang C, Zhou P, Zhang S, Yao Y, Li D. (2018b) Genome-scale analysis to identify prognostic markers and predict the survival of lung adenocarcinoma. *Journal of cellular biochemistry*. 119:8909-21. DOI:10.1002/jcb.27144
27. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. (2015) Nomograms in oncology: more than meets the eye. *The Lancet Oncology*. 16:e173-80. DOI:10.1016/s1470-2045(14)71116-7
28. Wang H, Meng Q, Ma B. (2021) Characterization of the Prognostic m6A-Related lncRNA Signature in Gastric Cancer. *Frontiers in oncology*. 11:630260. DOI:10.3389/fonc.2021.630260
29. Geng W, Lv Z, Fan J, Xu J, Mao K, Yin Z, et al. (2021) Identification of the Prognostic Significance of Somatic Mutation-Derived lncRNA Signatures of Genomic Instability in Lung Adenocarcinoma. *Frontiers in cell and developmental biology*. 9:657667. DOI:10.3389/fcell.2021.657667
30. Gao Q, Li Z, Meng L, Ma J, Xi Y, Wang T. (2020b) Transcriptome profiling reveals an integrated mRNA-lncRNA signature with predictive value for long-term survival in diffuse large B-cell lymphoma. *Aging*. 12:23275-95. DOI:10.18632/aging.104100
31. Wu Q, Li Q, Zhu W, Zhang X, Li H. (2021) Identification of autophagy-related long non-coding RNA prognostic signature for breast cancer. *Journal of cellular and molecular medicine*. 25:4088-98.

Figures

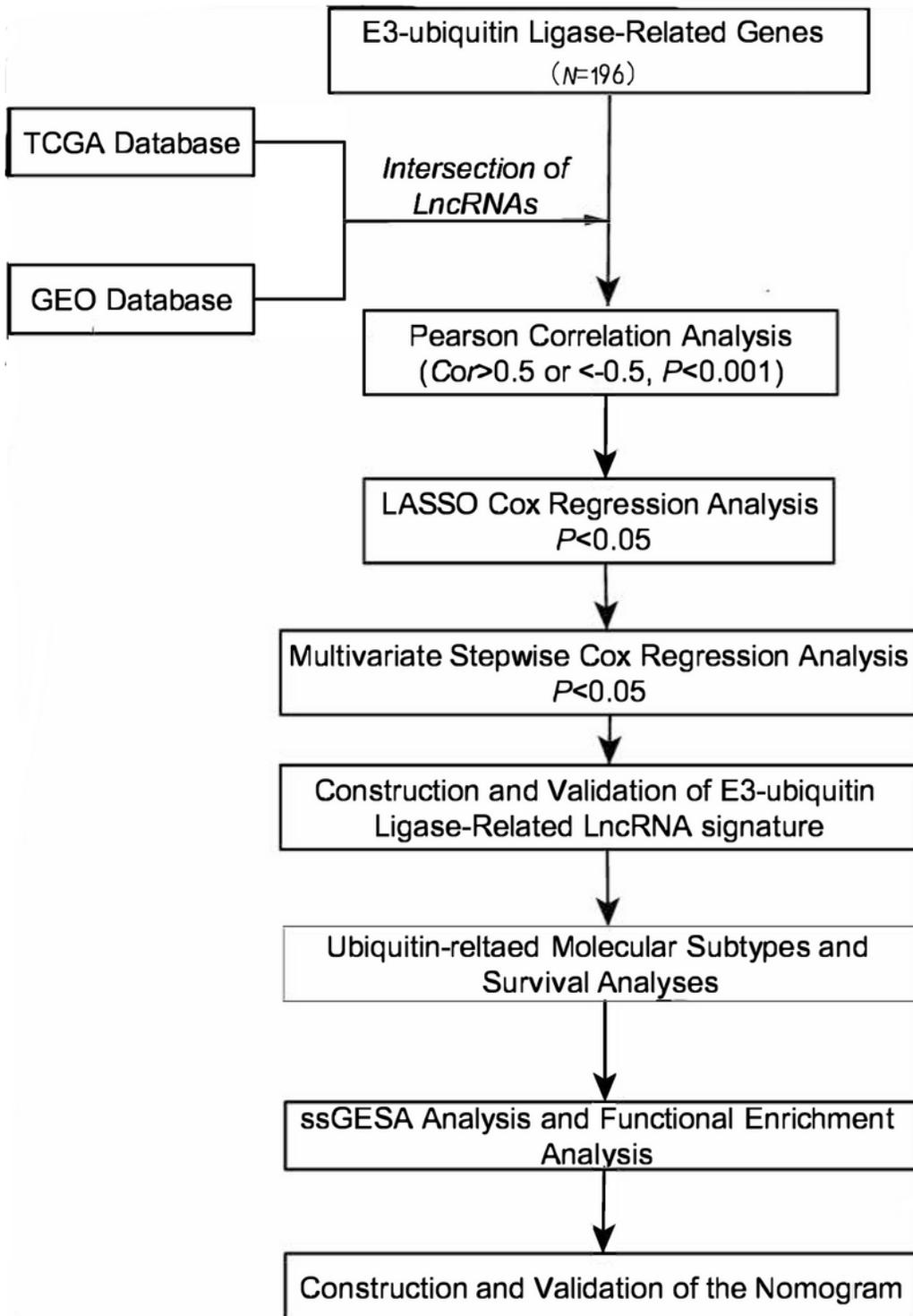


Figure 1

Study Flowchart.

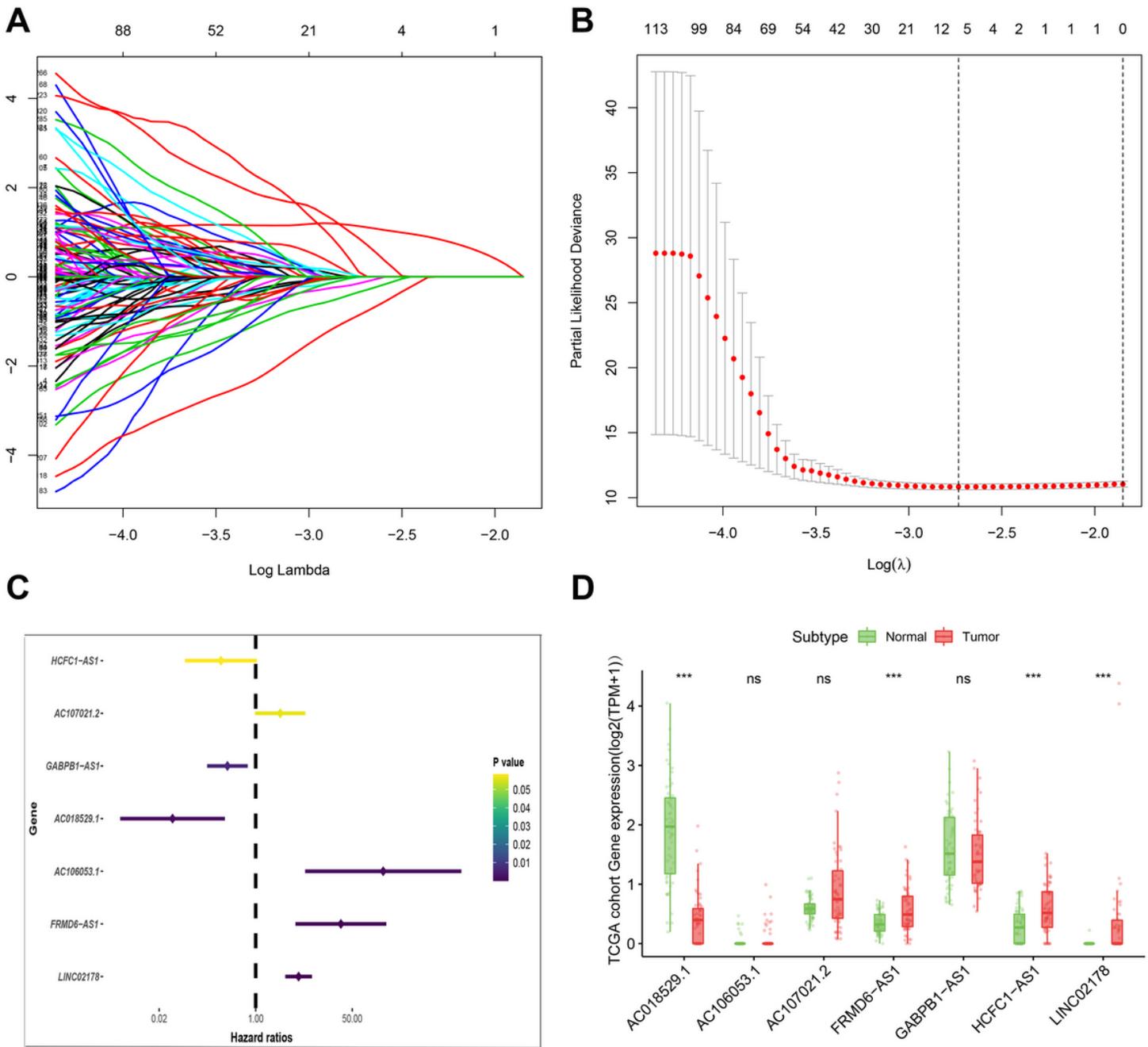


Figure 2

Lasso regression analysis (A, B) and multivariate stepwise Cox regression analysis (C) for construction of E3-ubiquitin ligase-related lncRNA signature. Relative expression of 7 lncRNAs between high- and low-risk groups in TCGA LUAD patients (D).

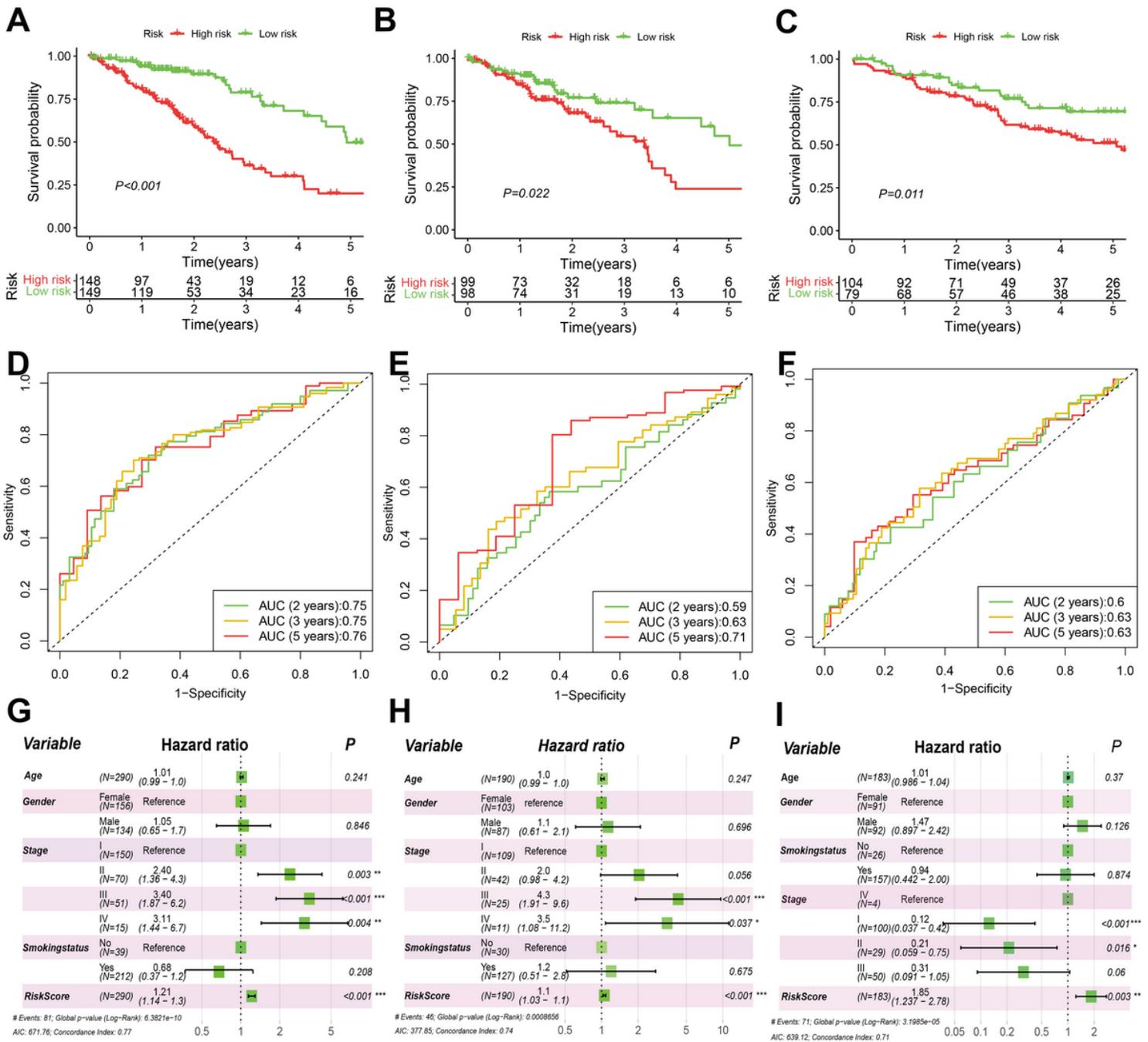


Figure 3

Gene risk signature identified was tightly associated with LUAD survival. Kaplan-Meier curves of overall survival (OS) showed the survival time of low- and high-risk groups in the TCGA training cohort (A), TCGA testing cohort (B), and GEO cohort (C); Receiver operating characteristic (ROC) curves with AUC value revealed predictive performance of gene signature in the three datasets (D-E); Forest plot of the multivariate Cox regression analysis showed the association between gene signature and LUAD survival in the three cohorts compared with clinical variables (G-I), respectively.

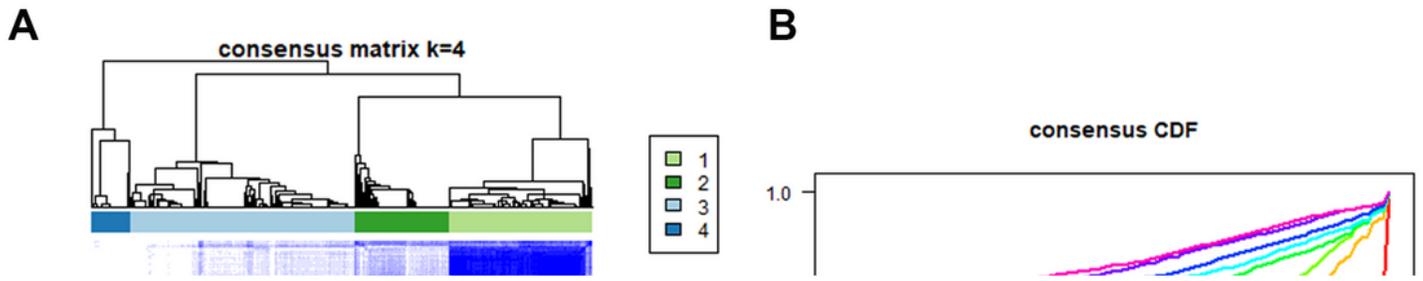


Figure 4

Subtype analysis based on seven E3-ubiquitin ligase-related lncRNAs in TCGA and GSE412717 LUAD patients. (A-C) Unsupervised consensus clustering analysis identified four molecular subtypes ($K = 4$) and (D) Survival analysis based on four subtypes identified.

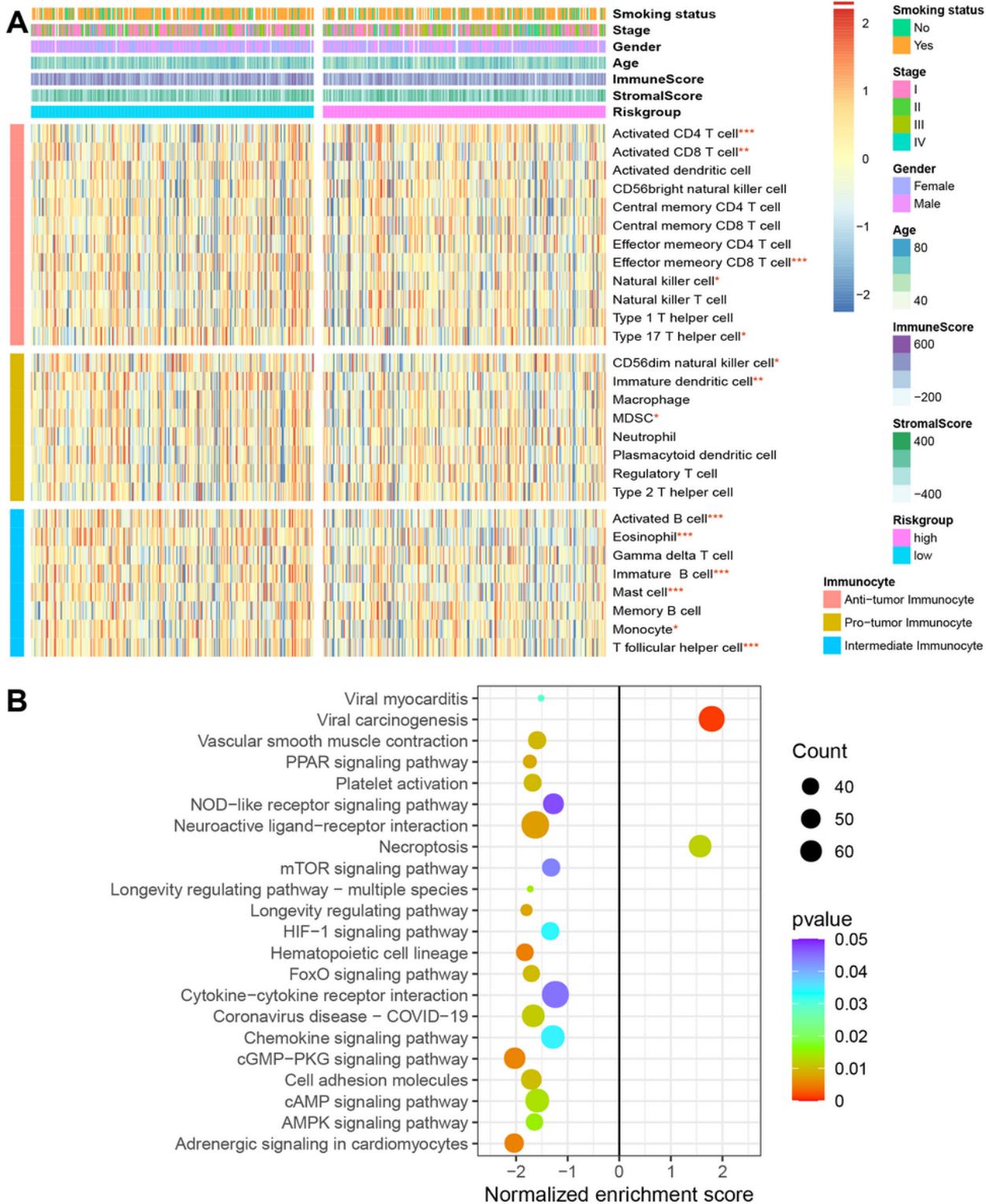


Figure 5

(A) Risk signature constructed can identify the difference of immune cell infiltration (28 types of immune cell subpopulations) between high- and low-risk groups via Single-sample gene set enrichment analysis (ssGESA). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Mann-Whitney-Wilcoxon test). (B) Top enriched signaling pathways in distinct risk signature groups from the TCGA cohort were assessed by using the GSEA algorithm.

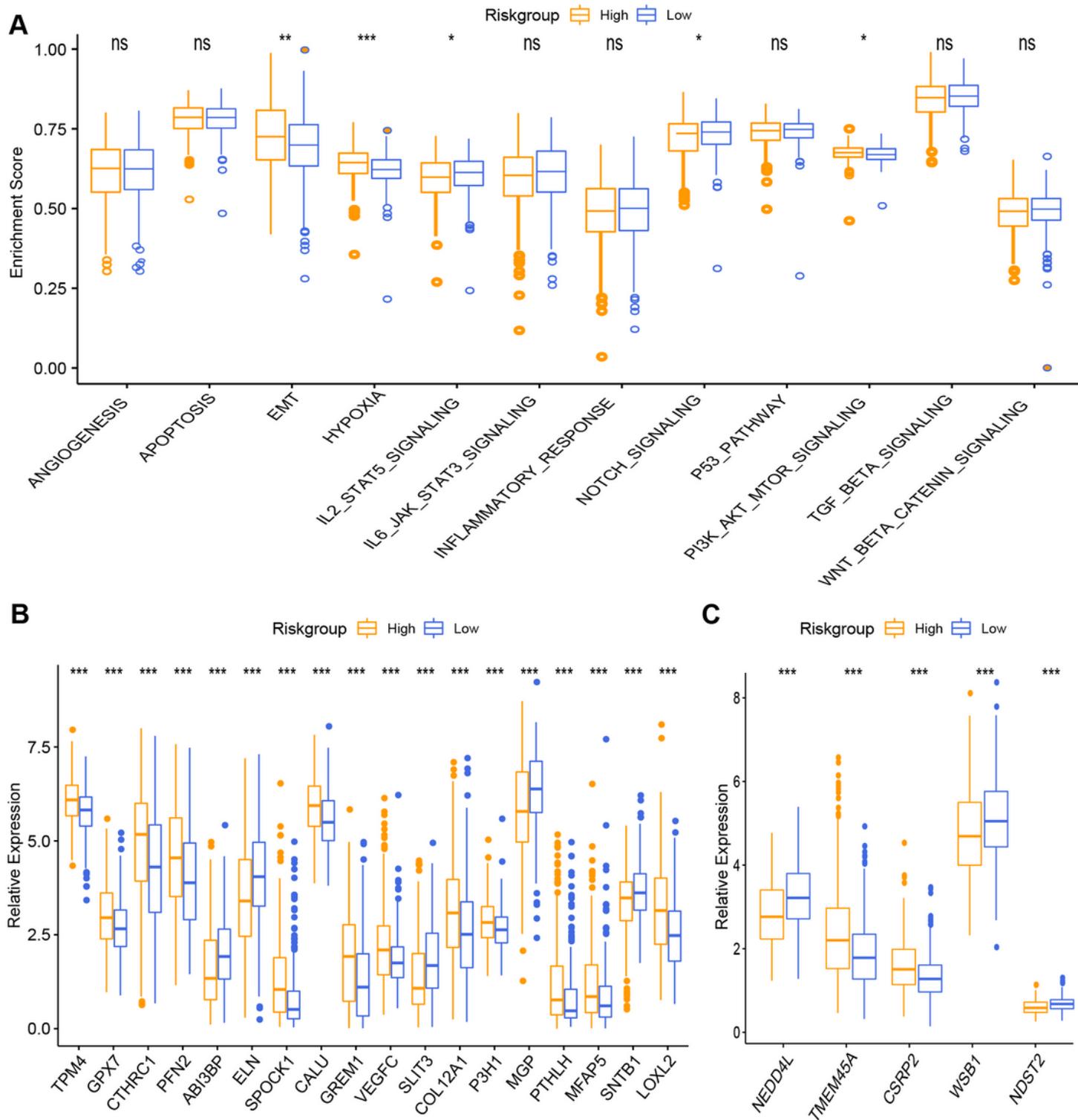


Figure 6

Tumor-related phenotypes in distinct E3-ubiquitin ligase-related lncRNA patterns. (A) Multiple tumor-related pathways in distinct risk signature groups from the TCGA cohort were assessed by using the GSEA algorithm; (B-C) Comparison of expression of multiple genes related to the regulation of EMT and hypoxia in high- and low-risk groups.

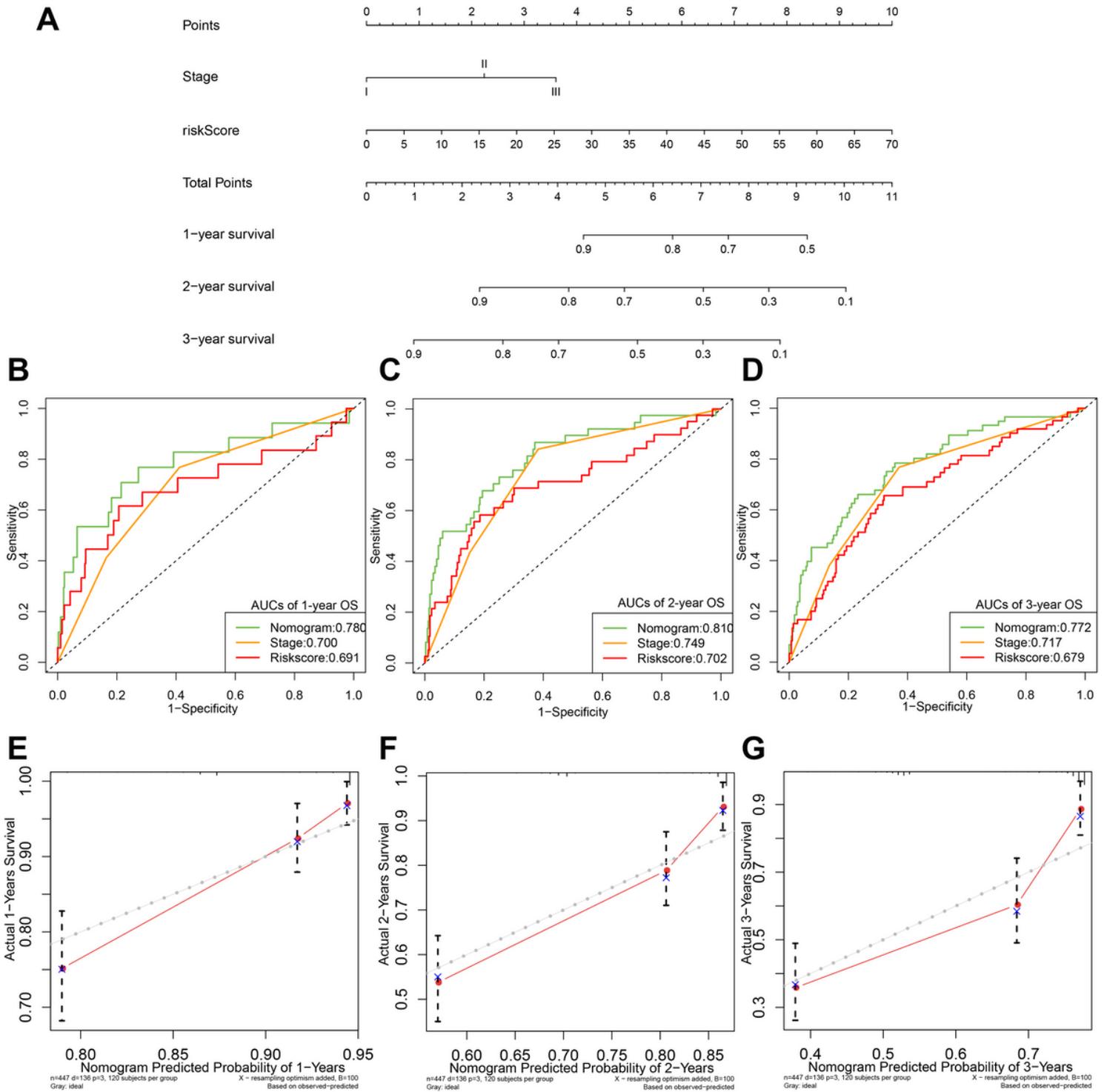


Figure 7

The integrated nomogram consisted of the seven-gene signature and pathological staging for predicting LUAD at 1-, 2-, and 3-year OS in the TCGA database.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementFigure1.tif](#)
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- [SupplementFigure3.tif](#)
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