Observational Study

Medicine

OPEN

High expression of E2F transcription factors 7

An independent predictor of poor prognosis in patients with lung adenocarcinoma

Yu Zhang, MD[a], Lan Lyu, MBBS[b], Wei Wang, MD[c], Liwei Zhang, MD[d,*]

Abstract

Adenocarcinoma is the most common pathological type of lung cancer. The E2F7 transcription factor has been confirmed to be related to the occurrence and development of a variety of solid tumors, but the relationship with the prognosis of lung cancer is still unclear. Therefore, we conducted this study to explore the prognostic value of E2F7 for lung adenocarcinoma (LUAD) patients.

In this study, we analyzed samples from the Cancer Genome Atlas (TCGA) to study the correlation between the expression of E2F7 and clinical features, the difference in expression between tumors and normal tissues, the prognostic and diagnostic value, and Enrichment analysis of related genes. All statistical analysis uses R statistical software (version 3.6.3).

The result shows that the expression level of E2F7 in LUAD was significantly higher than that of normal lung tissue (P=1e-34). High expression of E2F7 was significantly correlated with gender (P=.034), pathologic stage (P=.046) and M stage (P=.025). Multivariate Cox analysis confirmed that E2F7 is an independent risk factor for OS in LUAD patients (P=.027). Genes related to cell cycle checkpoints, DNA damage telomere stress-induced senescence, DNA methylation, chromosome maintenance and mitotic prophase showed differential enrichment in the E2F7 high expression group.

In short, high expression of E2F7 is an independent risk factor for OS in LUAD patients and has a high diagnostic value.

Abbreviations: BP = biological process, CC = cellular component, CI = confidence interval, CR = complete response, DSS = disease free survival, E2F7 = E2F transcription factor 7, GEO = gene expression omnibus, GO = gene ontology, KEGG = Kyoto encyclopedia of gene and gen omes, LUAD = lung adenocarcinoma, MF = molecular function, OS = overall survival, PD = progressive disease, PR = partial response, SD = stable disease, TCGA = the cancer genome atlas program.

Keywords: diagnosis, E2F7, lung adenocarcinoma

1. Introduction

Lung cancer is the second most common malignant tumor in the world. In 2020, there will be approximately 2.2 million new cases of lung cancer worldwide. Lung cancer has become the leading cause of cancer deaths, accounting for about 18% of the total cancer deaths (about 1.8 million cases).[1] Among them, lung adenocarcinoma is the most common pathological type.[2] Early lung cancer has no obvious symptoms, so most patients are already in the advanced stage when they are diagnosed, which leads to a generally low survival rate of lung cancer patients.

The E2F transcription factor family play a key role in the occurrence and development of tumors due to its important cell functions related to cell cycle regulation and apoptosis.[3] As a newly discovered member of the E2F family in recent years, unlike other family members, E2F7 has two special DNA-binding domains (DBD) in structure, lacks the binding domain to the RB protein, and does not need to bind to dimerizing proteins to enter the nucleus.[4,5]

E2F7 is a priming factor involved in cell cycle regulation, apoptosis and differentiation, involved in the late stage of mitosis, embryonic development, DNA stress response, and is likely to participate in the occurrence of tumors.[6–9] As an epithelial transcription inhibitor, amplification, overexpression or deletion of E2F7 can be observed in many malignant tumors, and it can affect tumor differentiation, proliferation and metastasis by interacting with different downstream targets. E2F7 is abnormally expressed in glioma,[10,11] colon cancer[12–14] and breast cancer,[15,16] and has an important influence on the occurrence and development of a variety of tumors.

In view of this, we conducted this study to explore the expression of E2F7 in lung adenocarcinoma (LUAD) and analyze its correlation with clinical parameters, diagnostic and prognostic value of LUAD patients.

2. Materials and Method

2.1. Patient data set

E2F7 mRNA expression data (including 594 samples, data format: FPKM) and clinical characteristics data are downloaded from the TCGA database. The data for pan-cancer analysis is from UCSC XENA (https://xenabrowser.net/datapages/). It is the

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

[a] Xinjiang Medical University, Department of thoracic surgery, Feicheng Hospital Affiliated to Shandong First Medical University, China, [b] Department of Plastic Surgery, Feicheng Hospital Affiliated to Shandong First Medical University, China, [c] Department of Expert's Outpatient, Feicheng Hospital Affiliated to Shandong First Medical University, China, [d] Xinjiang Medical University, China.

**Correspondence: Liwei Zhang, Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China, 830054, China (e-mail: zhangliweixj@163.com).*

How to cite this article: Zhang Y, Lyu L, Wang W, Zhang L. High expression of E2F transcription factors 7: an independent predictor of poor prognosis in patients with lung adenocarcinoma. Medicine. 2022;101:33(e29253).

Received: 17 September 2021 / Received in final form: 14 March 2022 / Accepted: 18 March 2022

http://dx.doi.org/10.1097/MD.0000000000029253

Table 1

Main characteristics of LUAD patients.

Characteristic	levels	Low expression of E2F7	High expression of E2F7	*P*
n		267	268	
Age, n (%)	<=65	121 (23.4%)	134 (26%)	.291
	>65	137 (26.6%)	124 (24%)	
Gender, n (%)	Female	155 (29%)	131 (24.5%)	.041
	Male	112 (20.9%)	137 (25.6%)	
Race, n (%)	Asian	2 (0.4%)	5 (1.1%)	.359
	Black or African American	25 (5.3%)	30 (6.4%)	
	White	208 (44.4%)	198 (42.3%)	
Smoker, n (%)	No	43 (8.3%)	32 (6.1%)	0.169
	Yes	214 (41.1%)	232 (44.5%)	
number_pack_years_smoked, n (%)	<40	102 (27.6%)	86 (23.3%)	0.018
	>=40	75 (20.3%)	106 (28.7%)	
Pathologic stage, n (%)	Stage I	152 (28.8%)	142 (26.9%)	0.210
	Stage II	61 (11.6%)	62 (11.8%)	
	Stage III	39 (7.4%)	45 (8.5%)	
	Stage IV	8 (1.5%)	18 (3.4%)	
T stage, n (%)	T1	97 (18.2%)	78 (14.7%)	0.336
	T2	135 (25.4%)	154 (28.9%)	
	T3	24 (4.5%)	25 (4.7%)	
	T4	10 (1.9%)	9 (1.7%)	
N stage, n (%)	N0	178 (34.3%)	170 (32.8%)	0.787
	N1	49 (9.4%)	46 (8.9%)	
	N2	33 (6.4%)	41 (7.9%)	
	N3	1 (0.2%)	1 (0.2%)	
M stage, n (%)	M0	188 (48.7%)	173 (44.8%)	0.034
	M1	7 (1.8%)	18 (4.7%)	
Anatomic neoplasm subdivision, n (%)	Left	99 (19%)	106 (20.4%)	0.640
	Right	160 (30.8%)	155 (29.8%)	
Anatomic neoplasm subdivision2, n (%)	Central Lung	32 (16.9%)	30 (15.9%)	0.540
	Peripheral Lung	58 (30.7%)	69 (36.5%)	
Residual tumor, n (%)	R0	176 (47.3%)	179 (48.1%)	0.186
	R1	6 (1.6%)	7 (1.9%)	
	R2	0 (0%)	4 (1.1%)	
Primary therapy outcome, n (%)	PD	27 (6.1%)	44 (9.9%)	0.109
	SD	21 (4.7%)	16 (3.6%)	
	PR	3 (0.7%)	3 (0.7%)	
	CR	177 (39.7%)	155 (34.8%)	
OS event, n (%)	Alive	183 (34.2%)	160 (29.9%)	0.041
	Dead	84 (15.7%)	108 (20.2%)	
DSS event, n (%)	Alive	198 (39.7%)	181 (36.3%)	0.079
	Dead	51 (10.2%)	69 (13.8%)	
PFI event, n (%)	Alive	162 (30.3%)	147 (27.5%)	0.202
	Dead	105 (19.6%)	121 (22.6%)	
Age, meidan (IQR)		67 (59, 72)	65 (59, 72)	0.346

CR=complete response, DSS=Disease Free Survival, OS=overall survival, PD=progressive disease, PFI=Progression Free Interva, PR=partial response, SD=stable disease.

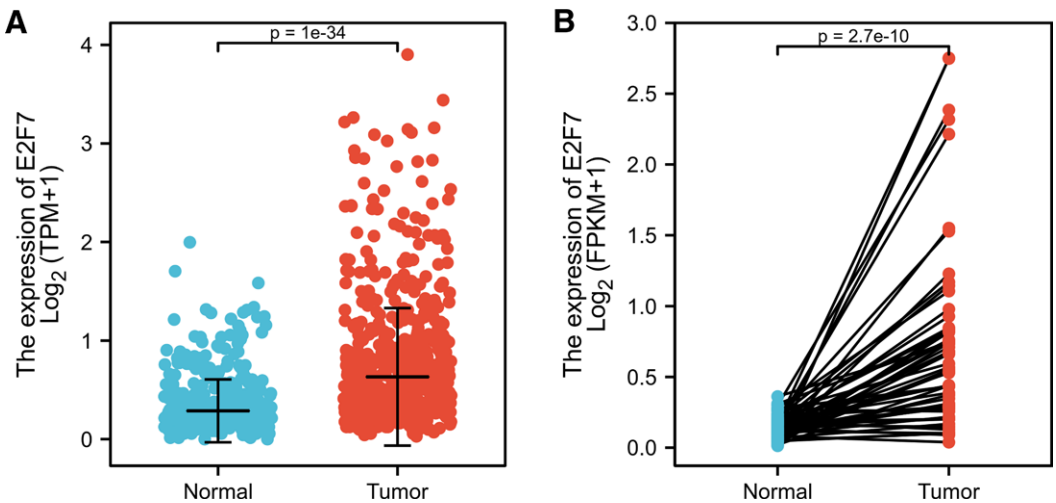

Figure 1. E2F7 expression in LUAD tissues. A. E2F7expression in normal and tumor tissue. B. E2F7 expression in paired tissue.

RNAseq data in TPM format of TCGA and GTEx that has been uniformly converted by the Toil process. Inclusion criteria: 1. Sufficient survival information; 2. Definite gene expression value.

All our data come from public databases such as GEO and TCGA. The patients involved in the database have obtained ethical approval. Our research is based on open-source data and therefore does not require ethics committee approval for the study.

2.2. Statistical analysis

The median of E2F7 expression was selected as the critical value, and the Wilcoxon signed rank test was used to test the differential expression of E2F7 in LUAD and normal tissues, and the results were displayed by box plots. Wilcoxon rank sum test and Dunn's test were used to testing whether the expression of E2F7 is related to clinical features in LUAD.

Table 2

Logistic analysis of the association between E2F7 expression and clinical characteristics.

Characteristics	Total (N)	OR (95%CI)	*P* value
Age (>65 vs <=65)	516	0.817 (0.578–1.154)	.253
Gender (Male vs Female)	535	1.447 (1.030–2.038)	.034
Race (Asian&White vs Black or African American)	468	0.806 (0.455–1.415)	.453
Smoker (Yes vs No)	521	1.457 (0.892–2.402)	.135
Pathologic stage (Stage IV vs Stage I)	320	2.408 (1.047–6.029)	.046
T stage (T2&T3&T4 vs T1)	532	1.383 (0.963–1.993)	.080
N stage (N1 vs N0)	443	0.983 (0.623–1.548)	.941
M stage (M1 vs M0)	386	2.794 (1.186–7.337)	.025
Anatomic neoplasm subdivision (Left vs Right)	520	1.105 (0.777–1.572)	.577
Anatomic neoplasm subdivision2 (Central Lung vs Peripheral Lung)	189	0.788 (0.428–1.448)	.443
Residual tumor (R1&R2 vs R0)	372	1.803 (0.671–5.331)	.256
Primary therapy outcome (PD&SD vs CR&PR)	446	1.424 (0.922–2.208)	.112

CR=complete response, PD=progressive disease, PR=partial response, SD=stable disease.

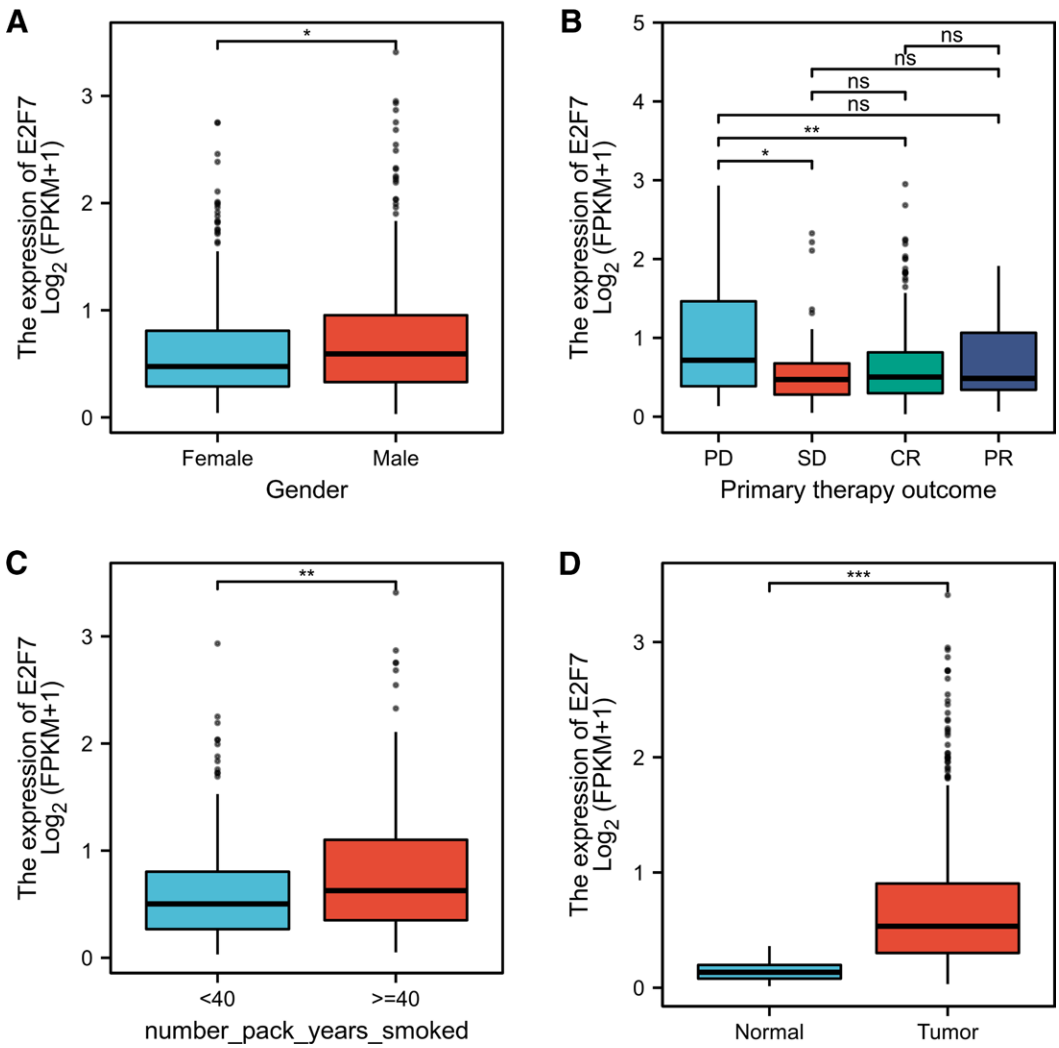

Figure 2. Box plot of E2F7 expression of LUAD patients according to different clinical characteristics. A. Gender. B. Primary therapy outcome. C. Number pack years smoked. D. Cancer status. ns, $P \geq .05$; $^{*}P<.05$; $^{**}P<.01$; $^{***}P<.001$.

Kaplan-Meier analysis was performed to compare the differences in OS and DSS between E2F7 high and low expression groups, and to draw survival curves.[17] The pROC package and the ggplot2 package are used to study the role of E2F7 prognosis and draw the ROC curve, where AUC represents the diagnostic value. Univariate Cox regression analysis was used to screen potential prognostic factors, and multivariate Cox regression was used to verify the independent predictive value of multiple indicators including E2F7 for prognosis. The rms package and survival package are used to draw nomograms to show the relationship between various variables and survival rates. The clusterProfiler package and the org.Hs.eg.db package are used for the enrichment analysis of GO and KEGG.[18] The clusterProfiler package and the ggplot2 package are used to perform GSEA enrichment analysis and plotting. In addition, we used an independent GEO data set (GSE50081) for external verification. The difference in the expression of E2F7 in pan-tumor and normal tissues is verified in UCSC XENA (https://xenabrowser.net/datapages/)[19] and Timmer database (https://cistrome.shinyapps.io/timmer/). All statistical analysis uses R statistical software (version 3.6.3).

3. Result

3.1. Baseline characteristics of included patients

A total of 535 patients diagnosed with lung adenocarcinoma were included in this study, and the data of these patients were all obtained through the TCGA data portal. The detailed clinical characteristics are shown in Table 1. Among the included

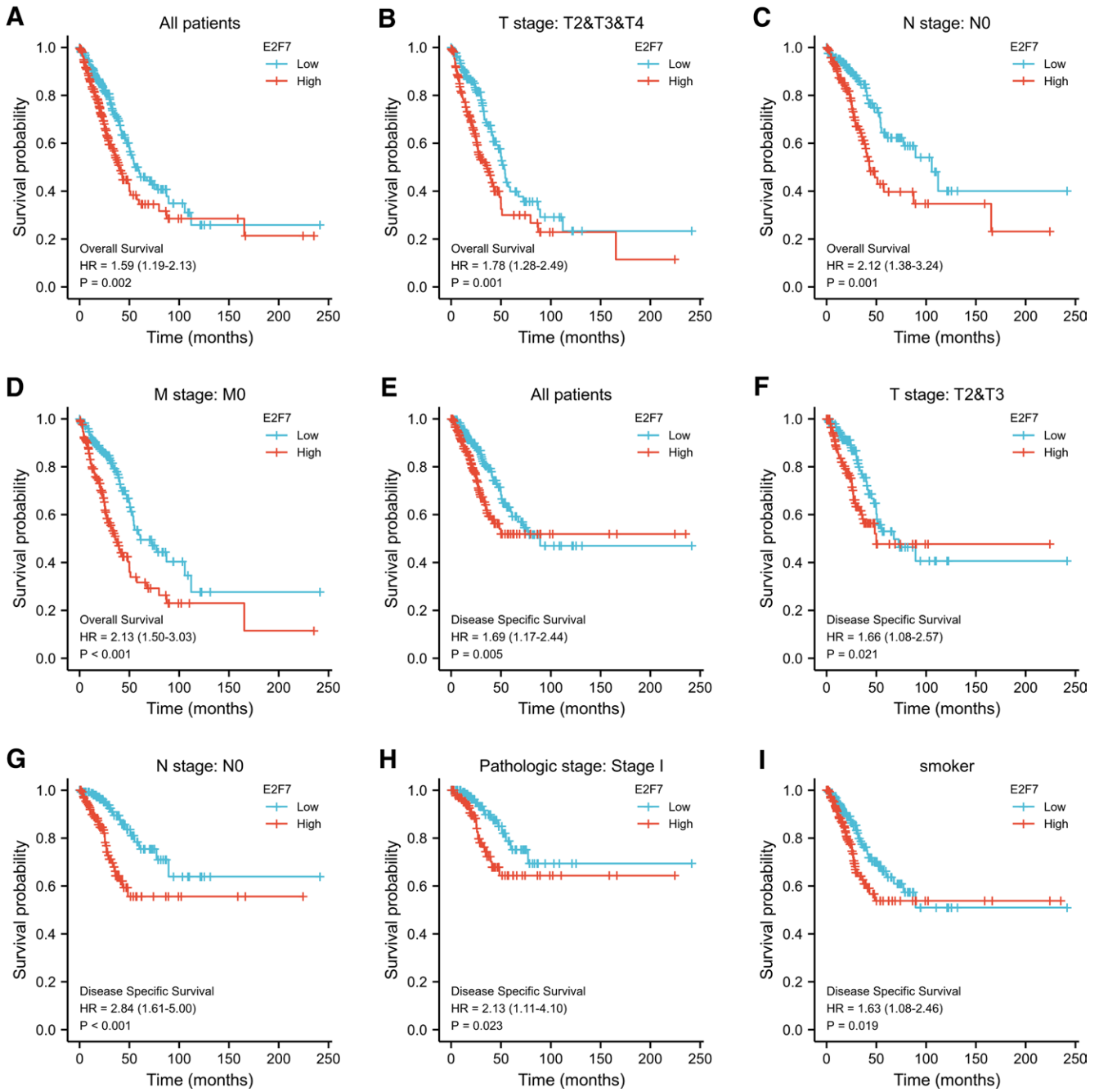

Figure 3. Kaplan-Meier curve for survival in LUAD. A. OS of all patients. B. OS of T2/T3/T4. C. OS of N0. D. OS of M0. E. DSS of all patients. F. DSS of T2/T3. G. DSS of N0. H. DSS of stage I. DSS of smoker.

patients, 249 were males (46.5%) and 286 were females (53.5%). Regarding the TNM staging of lung cancer: 294 patients were stage I, 123 patients were stage II, 84 patients were stage III, and 26 patients were stage IV. The median age of patients in the E2F7 high and low expression group was 65 and 67years, respectively, and the results were not statistically different (P=.346). Regarding surgical treatment: the number of patients undergoing R0, R1, and R2 resection was 355, 13, and 4, respectively, and there was no significant difference between the groups (P=.186). In gender (P=.041), number pack year smoked (P=.018), M stage (P=.034) and OS event (P=.041), there are significant differences between the 2 groups.

3.2. High expression of E2F7 in LUAD

We compared the expression levels of E2F7 in LUAD and normal lung tissues. Taking the median of the gene expression level of CCNA2 as the cutoff value, the patients were divided into high expression group and low expression group. The results of the study on unpaired samples showed that the expression of E2F7 in LUAD was higher than that of normal lung tissue (P=1e-34) (Fig. 1A). In the paired samples of LUAD and normal lung tissue, this conclusion was verified. (P=2.7e-10) (Fig. 1B).

3.3. E2F7 expression and clinical characteristics

The logistic regression analysis results of the correlation between E2F7 expression level and clinical characteristics are summarized in Table 2. The high expression of E2F7 was significantly correlated with gender (P=.034), pathologic stage (P=.046) and M stage (P=.025). As shown in Figure 2, the Mann-Whitney U test results verify the correlation between E2F7 expression and gender (P=.029) and the number pack-years smoked (P=.002). The results of multiple hypothesis test (Dunn's test) using Bonferroni method to correct the significance level show that the difference between SD and PD (P.adj=.037), CR and PD (P.adj=.001) was statistically significant. The same result appeared in the comparison of tumor and normal tissue (P<.001).

3.4. E2F7 high expression is an independent prognostic risk factor

Kaplan-Miere survival analysis of all adenocarcinoma patients showed that high expression of E2F7 was associated with shorter OS (P=.002) (Fig. 3A). The results of subgroup analysis showed that in patients with T2/T3/T4 (P=.001) (Fig. 3B), N0 (P=.001) (Fig. 3C), M0 (P<0.001) (Fig. 3D), E2F7 was highly expressed Significantly related to shorter OS. In terms

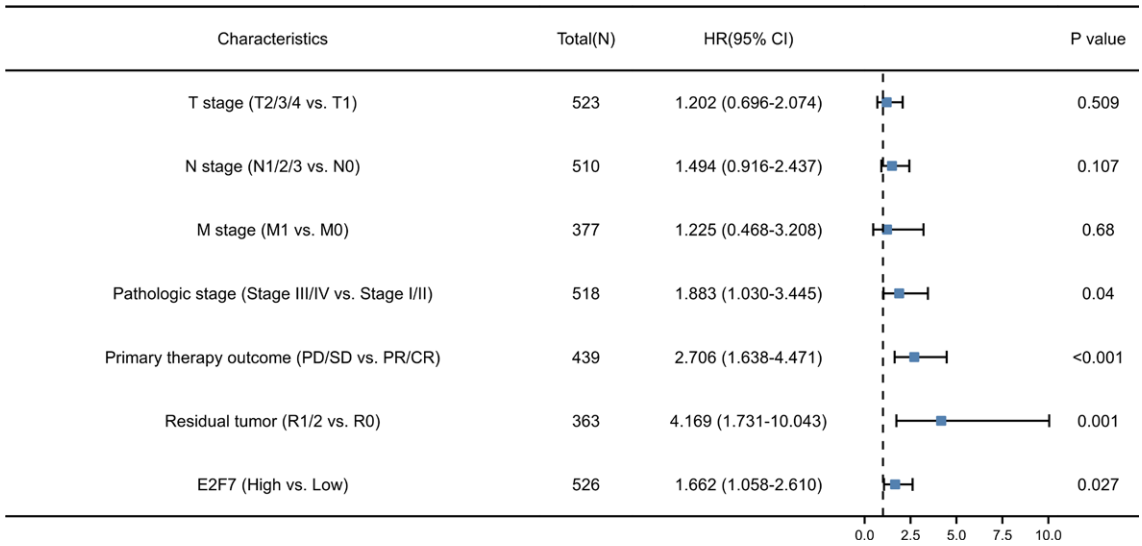

Figure 4. Forest plot of the multivariate Cox regression analysis of OS in LUAD.

Table 3

Univariate and multivariate Cox regression analysis of the relationship between clinical characteristics and overall survival.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
T stage (T2&T3&T4 vs T1)	523	1.728 (1.229–2.431)	**.002**	1.202 (0.696–2.074)	.509
N stage (N1&N2&N3 vs N0)	510	2.601 (1.944–3.480)	**<.001**	1.494 (0.916–2.437)	.107
M stage (M1 vs M0)	377	2.136 (1.248–3.653)	**.006**	1.225 (0.468–3.208)	.680
Age (>65 vs <=65)	516	1.223 (0.916–1.635)	.172		
Gender (Male vs Female)	526	1.070 (0.803–1.426)	.642		
Pathologic stage (Stage III&Stage IV vs Stage I&Stage II)	518	2.664 (1.960–3.621)	**<.001**	1.883 (1.030–3.445)	**.040**
Primary therapy outcome (PD&SD vs PR&CR)	439	2.653 (1.888–3.726)	**<.001**	2.706 (1.638–4.471)	**<.001**
Residual tumor (R1&R2 vs R0)	363	3.879 (2.169–6.936)	**<.001**	4.169 (1.731–10.043)	**.001**
Anatomic neoplasm subdivision (Right vs Left)	512	1.037 (0.770–1.397)	.810		
Smoker (Yes vs No)	512	0.894 (0.592–1.348)	.591		
Race (Black or African American vs White&Asian)	468	0.698 (0.422–1.157)	.163		
E2F7 (High vs Low)	526	1.594 (1.193–2.129)	**.002**	1.662 (1.058–2.610)	**.027**
number_pack_years_smoked (<40 vs >=40)	363	0.932 (0.654–1.328)	.697		

CR=complete response, PD=progressive disease, PR=partial response, SD=stable disease.

of DSS (*P*=.005), E2F7 showed similar results (Fig. 3E), at T2/T3 (*P*=.021) (Fig. 3F) The prognostic value of E2F7 in the subgroups of, N0 (*P*<.001) (Fig. 3G), M0 (*P*=.023) (Fig. 3H) and smoker (*P*=.019) (Fig. 3I) was also verified.

Univariate Cox regression results show T stage (*P*=.002),N stage (*P*<.001),M stage (*P*=.006), pathologic stage (*P*<.001), primary therapy outcome (*P*<.001), residual tumor (*P*<.001) and E2F7 (*P*=.002) were significantly related to poor prognosis.

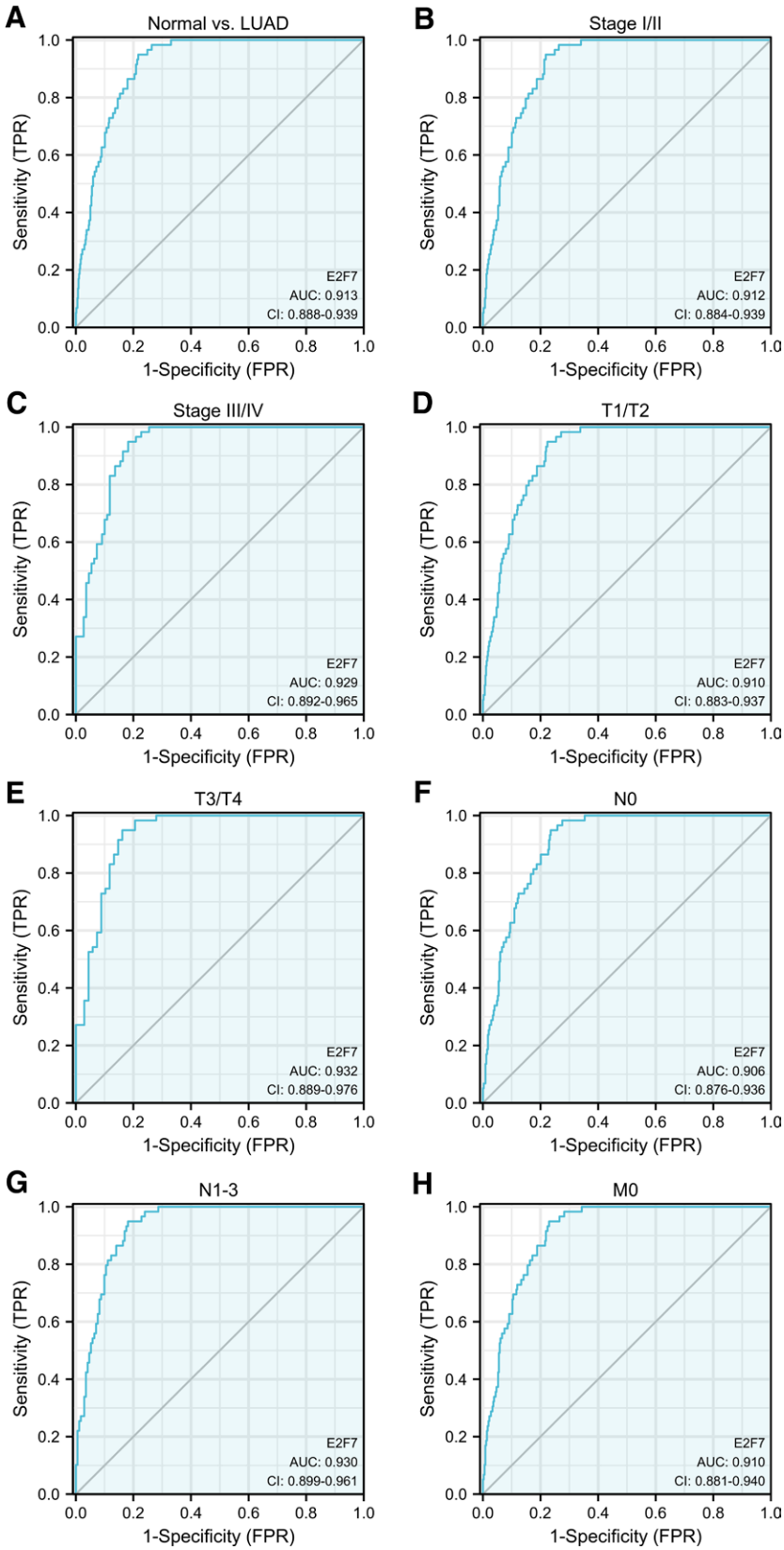

Figure 5. ROC curve of E2F7 expression in LUAD. A. Normal vs LUAD. B. Stage I/II. C. Stage III/IV. D. T1/T2. E. T3/T4. F. N0. G. N1-3. H. M0.

Further multivariate Cox analysis confirmed that pathologic stage (*P*=.040), primary therapy outcome (*P*<.001), residual tumor (*P*=.001) and E2F7 (*P*=.027) are independent factors affecting the prognosis of LUAD patients (Fig. 4, Table 3).

3.5. The diagnostic value of E2F7

We performed ROC curve analysis on the expression data of E2F7, and the results showed that this index has a high diagnostic value for patients with LUAD (AUC = 0.913, 95%CI: 0.888–0.939) (Fig. 5A). Further subgroup analysis verified its diagnostic value in stage I/II (AUC = 0.912) (Fig. 5B), stage III/IV (AUC = 0.929) (Fig. 5C), T1/T2 (AUC = 0.910) (Fig. 5D), T3/T4 (AUC = 0.932) (Fig. 5E), N0 (AUC = 0.906) (Fig. 5F), N1–3 (AUC = 0.930) (Fig. 5G) and M0 (AUC = 0.910) (Fig. 5H).

Due to its high diagnostic value, we combined E2F7 with clinical variables widely considered to be related to prognosis to construct a nomogram to predict the 1-, 3-, and 5-year survival probability (Fig. 6).

3.6. E2F7 related signal pathways

We performed GO/KEGG enrichment analysis on E2F7. Under the conditions of *P*.adj<0.1 and *q* value<0.2, there are 6 BPs, 12 CCs, 1 MF, and KEGG 2 signal pathways (Table 4).

We performed GSEA on the data set of high and low expression of E2F7 to determine the differentially activated signaling pathways in LUAD. A total of 39 data sets satisfy FDR (*q* value) <0.25 and *P*.adjust < 0.05. Cell cycle checkpoints, DNA damage telomere stress-induced senescence, DNA methylation, chromosome maintenance and mitotic prophase and other pathway-related genes showed enrichment in the high E2F7 expression group (Fig. 7).

3.7. Verification through other independent external databases

We used an independent GEO dataset (GSE50081) containing 127 LUAD patients to further verify the above results. The

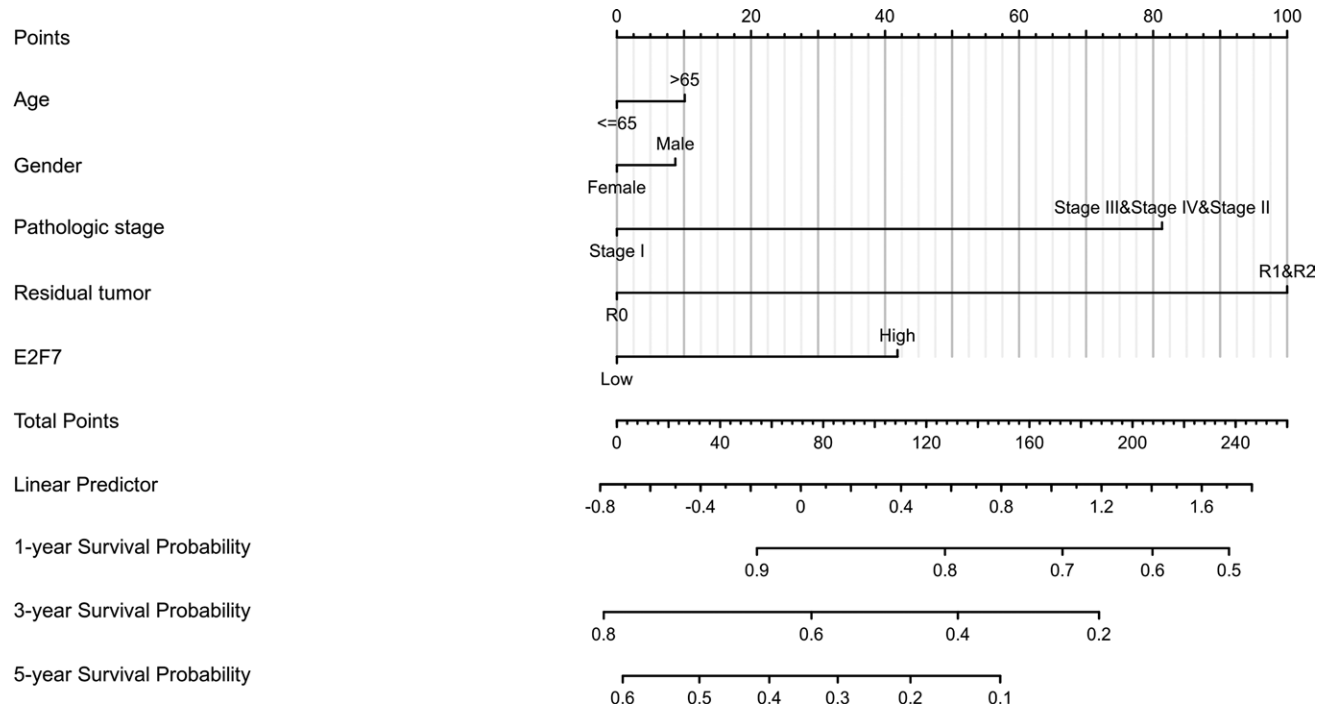

Figure 6. Nomogram for predicting the probability of LUAD patients with 1-, 3-, 5-year OS.

Table 4

Gene sets enriched in the high E2F7 expression phenotype.

ONTOLOGY	ID	Description	Gene Ratio	Bg Ratio	*P* value	*P*.adjust	FDR q-value
BP	GO:0000353	formation of quadruple SL/U4/U5/U6 snRNP	4/300	12/18670	2.92e-05	0.020	0.020
BP	GO:0000365	mRNA trans splicing, via spliceosome	4/300	12/18670	2.92e-05	0.020	0.020
BP	GO:0045291	mRNA trans splicing, SL addition	4/300	12/18670	2.92e-05	0.020	0.020
BP	GO:0007389	pattern specification process	20/300	446/18670	3.85e-05	0.020	0.020
BP	GO:0000244	spliceosomal tri-snRNP complex assembly	5/300	26/18670	5.17e-05	0.022	0.022
CC	GO:0015030	Cajal body	10/309	77/19717	3.35e-07	7.95e-05	7.83e-05
CC	GO:0072588	box H/ACA RNP complex	4/309	10/19717	1.15e-05	0.001	0.001
CC	GO:0005732	small nucleolar ribonucleoprotein complex	5/309	28/19717	6.69e-05	0.005	0.005
CC	GO:0097525	spliceosomal snRNP complex	7/309	99/19717	9.43e-04	0.050	0.050
CC	GO:0030532	small nuclear ribonucleoprotein complex	7/309	105/19717	0.001	0.050	0.050
MF	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	17/223	439/17697	4.37e-05	0.012	0.012
KEGG	hsa05034	Alcoholism	8/75	187/8076	3.19e-04	0.037	0.036
KEGG	hsa05322	Systemic lupus erythematosus	6/75	136/8076	0.002	0.092	0.089

BP=biological process, CC=cellular component, KEGG=Kyoto Encyclopedia of Genes and Genomes, MF=molecular function.

P.adj<.05 and FDR q-value<.2 were considered as significantly enriched.

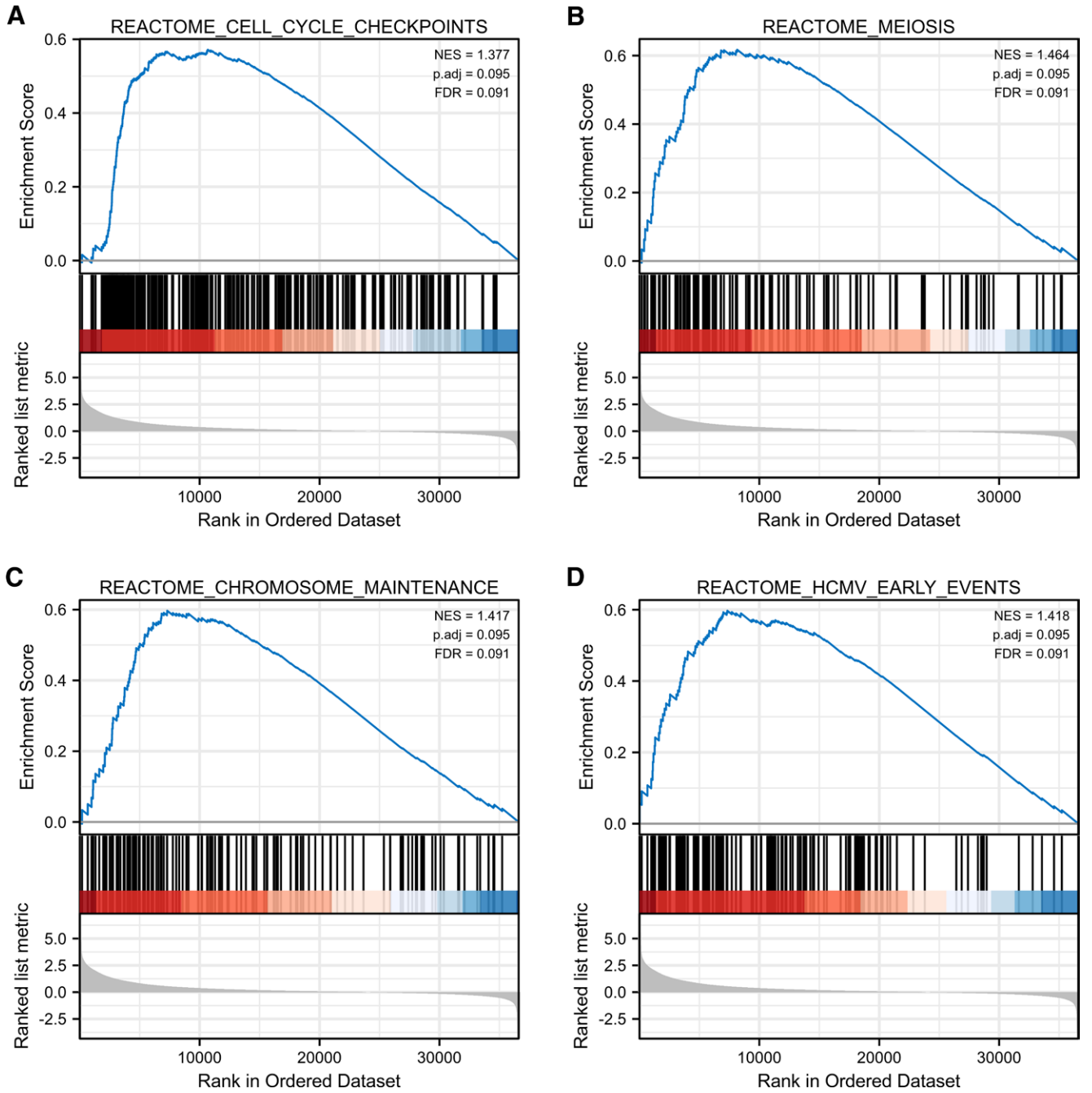

Figure 7. Enrich plots from GSEA. The possible biological processes or signaling pathways of E2F7.

results of the Kaplan-Meier survival analysis confirmed the prognostic value of E2F7 for LUAD patients (Fig. 8A–C).

We used the Timmer database to perform pan-tumor E2F7 expression analysis and showed that E2F7 is highly expressed in a variety of solid tumors including LUAD (Fig. 9A). We also integrated the pan-tumor analysis of the two databases of TCGA and GTEx and reached similar conclusions (Fig. 9B).

4. Discussion

In our study, the expression of E2F7 in many tumors including LUAD was higher than normal, and its expression level was higher in men and patients greater than 40 number pack-year, and it was related to the primary therapy outcome of disease, that is It is said that patients with the progressive disease have higher expression of E2F7. Subsequent survival analysis also showed that high expression of E2F7 is an independent risk factor for OS, and it has a high diagnostic value. This provides a basis for E2F7 to judge the prognosis of LUAD patients in future clinical work. Genes related to cell cycle checkpoints, DNA damage, telomere stress-induced senescence, DNA methylation, chromosome maintenance, and mitogenic pathways showed significant enrichment in the E2F7 high expression group, suggesting that E2F7 affects lung adenocarcinoma The potential mechanism of occurrence and development provides an important reference for further exploration of its mechanism through experiments in the future.

The occurrence and development of malignant tumors is a complex process involving multiple genes and their expressed proteins. Transcription is the beginning of gene expression and is strictly regulated by transcription factors (TFs) and its cofactors, RNA polymerase, and chromatin-modifying proteins.[6]

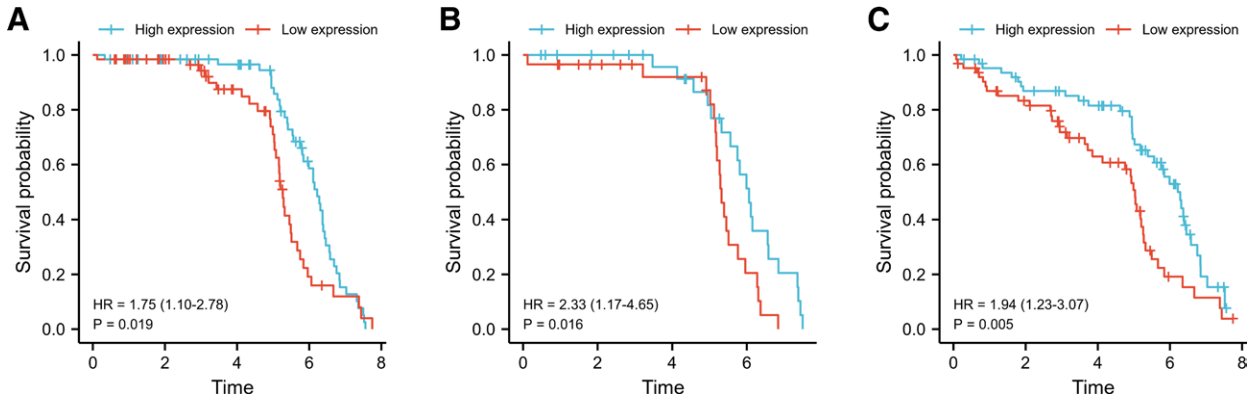

Figure 8. Kaplan-Meier curve for survival in LUAD patients in the validation datasets GSE50081. A. OS of all patients. B. OS of ex-smoker. C. Disease-free survival (DFS) of all patients.

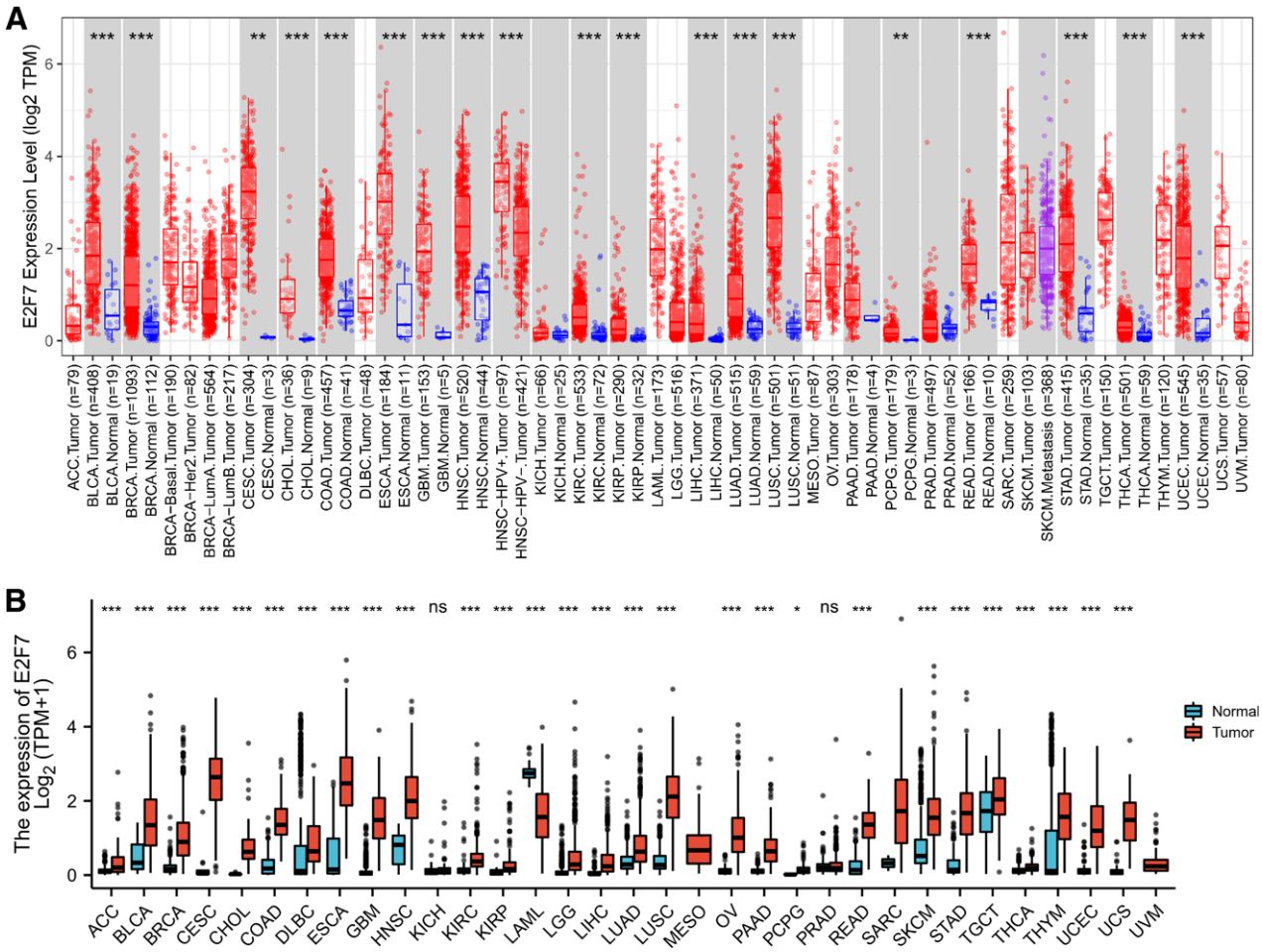

Figure 9. Expression analysis of E2F7 in different types of human tumors. A. Timmer databases. B. TCGA+ GTEx.

E2Fs are an important family of transcription factors, which have been confirmed to be involved in the process of cell proliferation,[20–23] differentiation,[24–26] apoptosis,[27–30] cycle regulation[31,32] and DNA damage response.[33,34] So far, a total of 8 family members have been discovered (E2F1-E2F8). According to their different functions, E2Fs are divided into transcription activators (E2F1–3) and transcription repressors (E2F4–8), and according to their structure, they are divided into typical E2Fs (E2F1–6) and atypical E2Fs (E2F7–8). The clinical value of many E2Fs members in the diagnosis and treatment of many solid tumors has been affirmed.[35–38]

E2F7 is different from the typical E2Fs members in that it binds to DNA in a non-DP protein way to play a transcriptional inhibitory effect.[4,39] Studies have shown that E2F7 can inhibit cell proliferation by inhibiting the transcription of proliferation-related miRNAs.[40] However, in recent years, more and more studies have shown that E2F7 plays a role in promoting tumor occurrence and development in tumors. Chu et al. reported that the overexpression of E2F7 in breast cancer can inhibit miR-15a/16 transcription, cause Cyclin E1 and Bcl-2 to participate in tumor invasion and metastasis, and increase the resistance of breast cancer cells to tamoxifen.[15]

In our study, the expression of E2F7 in a variety of solid tumors was analyzed through the Timmer database and UCSC XENA. The results showed that E2F7 is highly expressed in LUAD, lung squamous cell carcinoma (LUSC), esophageal squamous cell carcinoma (ESCA) and other solid tumors.

In previous existing studies, there is no content about the prognostic value of E2F7 expression in LUAD patients. In this study, the diagnostic value of E2F7 was analyzed on the TCGA database by means of bioinformatics analysis. Kaplan-Meier survival analysis showed that high expression of E2F7 was associated with shorter OS and DSS, and this conclusion was verified in the GEO dataset. Multivariate Cox analysis further confirmed that the expression of E2F7 is independently related to OS of patients with LUAD. Other clinical features, such as local advanced stage, lymph node metastasis, distant metastasis, later TNM staging, and the degree of surgical resection are closely related, and are also related to poor prognosis. We further constructed a nomogram of the prognosis of LUAD patients based on clinical variables and the expression of E2F7, which provided a basis for clinicians to predict the survival rate of individual patients.

The mechanism by which E2F7 mediates the development of tumors is not completely clear. It may promote tumor proliferation, differentiation, infiltration and metastasis through the following methods: (1) E2F7 up-regulates Beclin-1 and mediates autophagy induced by miR-129 Trigger autophagy flux[10]; (2) E2F7 increases the expression level of vimentin, reduces the expression of E-cadherin protein, and promotes the EMT process[41–43]; (3) As the transcriptional activators of VEGFA, E2F7 cooperates with HIF-1α to induce the transcription of VEGFA and promote blood vessel Generation[44]; (4) Induce the transcription of collagen and calcium-binding domains and Flt to promote the generation of lymphatic vessels.[35,45] Our study found that the expression of E2f7 is related to pathways such as cell cycle checkpoints, DNA damage telomere stress-induced senescence, DNA methylation, chromosome maintenance and mitotic prophase. Our research results are related to the above-mentioned mechanisms, but these mechanisms need further research to confirm.

Although our study provides a new method to explore the relationship between E2F7 and the prognosis of lung adenocarcinoma, it still has many limitations. First of all, although we have adopted the GEO database to verify the results of the TCGA database analysis, the study object is still only patients in the public database, which will lead to bias. Secondly, due to the limited sample size and clinical indicator, our research conclusions need to be further confirmed by a large sample of research. Finally, we need further experiments to explore the role of E2F7 in tumor progression and its mechanism of affecting tumors.

In short, high expression of E2F7 is an independent risk factor for OS in LUAD patients, and has a high diagnostic value. cell cycle checkpoints, DNA damage telomere stress-induced senescence, DNA methylation, chromosome maintenance and mitotic prophase may be the key pathways through which LUAD is regulated by E2F7.

References

[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.

[2] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

[3] Thurlings I, de Bruin A. E2F transcription factors control the roller coaster ride of cell cycle gene expression. Methods Mol Biol (Clifton, NJ) 2016;1342:71–88.

[4] Lammens T, Li J, Leone G, De Veylder L. Atypical E2Fs: new players in the E2F transcription factor family. Trends Cell Biol 2009;19:111–8.

[5] Logan N, Delavaine L, Graham A, et al. E2F-7: a distinctive E2F family member with an unusual organization of DNA-binding domains. Oncogene 2004;23:5138–50.

[6] Wasserman WW, Sandelin A. Applied bioinformatics for the identification of regulatory elements. Nat Rev Genet 2004;5:276–87.

[7] Lammens T, Boudolf V, Kheibarshekan L, et al. Atypical E2F activity restrains APC/CCCS52A2 function obligatory for endocycle onset. Proc Natl Acad Sci U S AV 105 2008;14721–6.

[8] Thurlings I, Martínez-López LM, Westendorp B, et al. Synergistic functions of E2F7 and E2F8 are critical to suppress stress-induced skin cancer. Oncogene 2017;36:829–39.

[9] Reimer D, Sadr S, Wiedemair A, et al. Clinical relevance of E2F family members in ovarian cancer--an evaluation in a training set of 77 patients. Clinical cancer research: an official journal of the American Association for Cancer Research 2007;13:144–51.

[10] Yin W, Wang B, Ding M, et al. Elevated E2F7 expression predicts poor prognosis in human patients with gliomas. J Clin Neurosci 2016;33:187–93.

[11] Chen X, Zhang Y, Shi Y, et al. MiR-129 triggers autophagic flux by regulating a novel Notch-1/E2F7/Beclin-1 axis to impair the viability of human malignant glioma cells. Oncotarget 2016;7:9222–35.

[12] Kurokawa K, Akaike Y, Masuda K, et al. Downregulation of serine/arginine-rich splicing factor 3 induces G1 cell cycle arrest and apoptosis in colon cancer cells. Oncogene 2014;33:1407–17.

[13] Liu W, Song Y, Zhang C, Gao P, Huang B, Yang J. The protective role of all-transretinoic acid (ATRA) against colorectal cancer development is achieved via increasing miR-3666 expression and decreasing E2F7 expression. Biomed Pharmacother V 104 2018;94–101.

[14] Guo AY, Zhai K, Xu JL, Hu JL, Gao L. Identification of a Low-Frequency Missense Variant in E2F Transcription Factor 7 Associated with Colorectal Cancer Risk In A Chinese Population. APJCP 2017;18:271–5.

[15] Chu J, Zhu Y, Liu Y, et al. E2F7 overexpression leads to tamoxifen resistance in breast cancer cells by competing with E2F1 at miR-15a/16 promoter. Oncotarget 2015;6:31944–57.

[16] Browne AL, Charmsaz S, Varešlija D, et al. Network analysis of SRC-1 reveals a novel transcription factor hub which regulates endocrine resistant breast cancer. Oncogene 2018;37:2008–21.

[17] Liu J, Lichtenberg T, Hoadley KA, et al. An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. Cell 2018;173: 400–416.e411.

[18] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16:284–7.

[19] Vivian J, Rao AA, Nothaft FA, et al. Toil enables reproducible, open source, big biomedical data analyses. Nat Biotechnol 2017;35:314–6.

[20] Dupont E, Sansal I, Evrard C, Rouget P. Developmental pattern of expression of NPDC-1 and its interaction with E2F-1 suggest a role in the control of proliferation and differentiation of neural cells. J Neurosci Res 1998;51:257–67.

[21] Jeong YW, Kim KS, Oh JY, et al. Exogenous wild-type p16INK4A gene induces delayed cell proliferation and promotes chemosensitivity through decreased pRB and increased E2F-1 expressions. Int J Mol Med 2003;12:61–5.

[22] Jin YJ, Lee JH, Kim YM, Oh GT, Lee H. Macrophage inhibitory cytokine-1 stimulates proliferation of human umbilical vein endothelial cells by up-regulating cyclins D1 and E through the PI3K/Akt-, ERK-, and JNK-dependent AP-1 and E2F activation signaling pathways. Cell Signal 2012;24:1485–95.

[23] Sansal I, Dupont E, Toru D, Evrard C, Rouget P. NPDC-1, a regulator of neural cell proliferation and differentiation, interacts with E2F-1, reduces its binding to DNA and modulates its transcriptional activity. Oncogene 2000;19:5000–9.

[24] Flink IL, Oana S, Maitra N, Bahl JJ, Morkin E. Changes in E2F complexes containing retinoblastoma protein family members and increased cyclin-dependent kinase inhibitor activities during terminal differentiation of cardiomyocytes. J Mol Cell Cardiol 1998;30:563–78.

[25] Amanullah A, Hoffman B, Liebermann DA. Deregulated E2F-1 blocks terminal differentiation and loss of leukemogenicity of M1 myeloblastic leukemia cells without abrogating induction of p15(INK4B) and p16(INK4A). Blood 2000;96:475–82.

[26] White J, Stead E, Faast R, Conn S, Cartwright P, Dalton S. Developmental activation of the Rb-E2F pathway and establishment of cell cycle-regulated cyclin-dependent kinase activity during embryonic stem cell differentiation. Mol Biol Cell 2005;16:2018–27.

[27] Yamazaki K, Hasegawa M, Ohoka I, et al. Increased E2F-1 expression via tumour cell proliferation and decreased apoptosis are correlated with adverse prognosis in patients with squamous cell carcinoma of the oesophagus. J Clin Pathol 2005;58:904–10.

[28] Rieber M, Rieber MS. N-Acetylcysteine enhances UV-mediated caspase-3 activation, fragmentation of E2F-4, and apoptosis in human C8161 melanoma: inhibition by ectopic Bcl-2 expression. Biochem Pharmacol V 65 2003;1593–601.

[29] Alvira D, Yeste-Velasco M, Folch J, et al. Neuroprotective effects of caffeine against complex I inhibition-induced apoptosis are mediated by inhibition of the Atm/p53/E2F-1 path in cerebellar granule neurons. J Neurosci Res 2007;85:3079–88.
[30] Sola S, Ma X, Castro RE, Kren BT, Steer CJ, Rodrigues CM. Ursodeoxycholic acid modulates E2F-1 and p53 expression through a caspase-independent mechanism in transforming growth factor beta1-induced apoptosis of rat hepatocytes. J Biol Chem 2003;278:48831–8.
[31] Lario LD, Ramirez-Parra E, Gutierrez C, Spampinato CP, Casati P. ANTI-SILENCING FUNCTION1 proteins are involved in ultraviolet-induced DNA damage repair and are cell cycle regulated by E2F transcription factors in Arabidopsis. Plant Physiol 2013;162:1164–77.
[32] Nohara K, Ao K, Miyamoto Y, et al. Arsenite-induced thymus atrophy is mediated by cell cycle arrest: a characteristic downregulation of E2F-related genes revealed by a microarray approach. Toxicol Sci 2008;101:226–38.
[33] Nichols AF, Itoh T, Zolezzi F, Hutsell S, Linn S. Basal transcriptional regulation of human damage-specific DNA-binding protein genes DDB1 and DDB2 by Sp1, E2F, N-myc and NF1 elements. Nucleic Acids Res 2003;31:562–9.
[34] Verschuren EW, Ban KH, Masek MA, Lehman NL, Jackson PK. Loss of Emi1-dependent anaphase-promoting complex/cyclosome inhibition deregulates E2F target expression and elicits DNA damage-induced senescence. Mol Cell Biol 2007;27:7955–65.
[35] Chen HZ, Tsai SY, Leone G. Emerging roles of E2Fs in cancer: an exit from cell cycle control. Nat Rev Cancer 2009;9:785–97.
[36] Yan Y, Xu Y, Zhao Y, et al. Combination of E2F-1 promoter-regulated oncolytic adenovirus and cytokine-induced killer cells enhances the antitumor effects in an orthotopic rectal cancer model. Tumour Biol 2014;35:1113–22.
[37] Kurayoshi K, Shiromoto A, Ozono E, et al. Ectopic expression of the CDK inhibitor p21(Cip1) enhances deregulated E2F activity and increases cancer cell-specific cytotoxic gene expression mediated by the ARF tumor suppressor promoter. Biochem Biophys Res Commun 2017;4831:107–14.
[38] Itoshima T, Fujiwara T, Waku T, et al. Induction of apoptosis in human esophageal cancer cells by sequential transfer of the wild-type p53 and E2F-1 genes: involvement of p53 accumulation via ARF-mediated MDM2 down-regulation. Clin Cancer Res 2000;6:2851–9.
[39] Di Stefano L, Jensen MR, Helin K. E2F7, a novel E2F featuring DP-independent repression of a subset of E2F-regulated genes. EMBO J 2003;22:6289–98.
[40] Mitxelena J, Apraiz A, Vallejo-Rodríguez J, Malumbres M, Zubiaga AM. E2F7 regulates transcription and maturation of multiple microRNAs to restrain cell proliferation. Nucleic Acids Res 2016;44:5557–70.
[41] Ye YY, Mei JW, Xiang SS, et al. MicroRNA-30a-5p inhibits gallbladder cancer cell proliferation, migration and metastasis by targeting E2F7. Cell Death Dis 2018;9:410.
[42] Kraljevic Pavelic S, Sedic M, Bosnjak H, Spaventi S, Pavelic K. Metastasis: new perspectives on an old problem. Mol Cancer 2011;10:22.
[43] de Bruin A, Maiti B, Jakoi L, Timmers C, Buerki R, Leone G. Identification and characterization of E2F7, a novel mammalian E2F family member capable of blocking cellular proliferation. J Biol Chem 2003;278:42041–9.
[44] Roberts N, Kloos B, Cassella M, et al. Inhibition of VEGFR-3 activation with the antagonistic antibody more potently suppresses lymph node and distant metastases than inactivation of VEGFR-2. Cancer Res 2006;66:2650–7.
[45] Djordjevic S, Driscoll PC. Targeting VEGF signalling via the neuropilin co-receptor. Drug Discov Today 2013;18:447–55.