The overexpression of FKBP4 in patients with lung adenocarcinoma predicts poor prognosis and tumor progression

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The overexpression of *FKBP4* in patients with lung adenocarcinoma predicts poor prognosis and tumor progression

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Keywords: lung adenocarcinoma, *FKBP4*, TCGA, bioinformatics analysis, GSEA, prognosis.

Abstract

Background: The FK506-binding protein 4 (*FKBP4*), a tumor-related gene, plays a vital role in tumorigenesis and cancer progression. The study is aimed to clarify the effect of *FKBP4* in lung adenocarcinoma (LUAD).

Methods: Relying on The Cancer Genome Atlas (TCGA) cohort, the *FKBP4* expression difference between LUAD tissues and non-tumor tissues was first detected, and verified with public tissue microarrays (TMAs), clinical LUAD specimen cohort and Gene Expression Omnibus (GEO) cohort. Then, logistic regression analysis and chi-square test were applied to detect the correlation between *FKBP4* expression and clinicopathological parameters. Kaplan-Meier survival analysis and Cox regression model were utilized to evaluate the effect of *FKBP4* expression on survival. Signaling pathways related to LUAD were obtained via employing Gene Set Enrichment Analysis (GSEA).

Results: The *FKBP4* expression level in LUAD samples was dramatically higher than that in non-tumor samples. High *FKBP4* expression in LUAD is associated with gender, pathological stage, T classification, lymph node metastasis and distant metastasis. The Kaplan-Meier curve indicated a poor prognosis for LUAD patients with high *FKBP4* expression. Multivariate analysis suggested that the high *FKBP4* expression was a vital independent predictor of poor overall survival (OS). GSEA showed that a total of 15 signaling pathways were enriched in samples with high *FKBP4* expression phenotype.

Conclusions: *FKBP4* may be an oncogene in LUAD, and is promised to become a prognostic indicator and therapeutic target for LUAD.

1 Background

Lung cancer is the cancer with the highest cancer incidence and mortality in the world [1]. The 5-year survival rate is less than 20% [2]. Among non-small cell lung cancer (NSCLC), lung adenocarcinoma (LUAD) is the most common histological subtype, accounting for about 40% of all types of lung malignancies [3]. During the last decade, researchers across the world have carried out large-scale genomic studies to reveal some driver genes of LUAD. It has been reported that the most common somatic mutations in lung cancer include *TP53*, *KRAS*, *KEAP1*, *STK11*, and *EGFR* [4-6]. Although some progress has been made in lung cancer research, there are still a large number of LUAD patients who do not have effective targeted treatment options, either because of the lack of known gene mutations in key oncogenic signaling pathways, or because it is difficult to target oncogenic mutations. The purpose of this study is to reveal a potential diagnostic and therapeutic target for patients with LUAD.

The FK506-binding protein 4 (*FKBP4*), a member of immunophilin family, has been proved to play a key role in immune regulation, protein folding, and transportation [7]. Recent studies have revealed that *FKBP4* might play an important role in tumorigenesis and progression of various cancers, and is expected to become an effective biomarker [8]. For instance, Alain et al. [9] found that the abnormal expression of *FKBP4* has a huge effect on the breast cancer progression and prognosis. In particular, *FKBP4* depletion can decrease cell growth and proliferation in triple-negative breast cancer cell models and xenograft tumor models. Meng et al. [10] proved that *FKBP4* is highly expressed in NSCLC, accelerating the malignant progression of NSCLC by activating the Akt/mTOR signaling pathway.

However, few studies have explored the association between *FKBP4* and LUAD, especially in terms of prognostic biomarkers. Based on public databases and our own fresh frozen tissue specimen cohort, this study has investigated the associations between the *FKBP4* expression level and clinicopathological characteristics of LUAD, as well as the prognostic significance of *FKBP4*, in order to provide more evidences for the potential role of *FKBP4* in LUAD. In addition, GSEA was implemented to deepen the understandings of the signal pathways involved in *FKBP4* regulatory networks related to LUAD.

2 Materials and Methods

2.1 TCGA cohort and fresh frozen tissue specimen cohort

The TCGA cohort including the raw gene expression data for 497 LUAD tissues and 54 adjacent non-tumor tissues were obtained from the TCGA database (https://portal.gdc.cancer.gov). Similarly, the corresponding clinical data of LUAD patients containing the information of age, gender, pathological stage, T stage, N stage, M stage, and vital status **(Table 1)** were also received. This study is in full compliance with the guidelines of the National Institute of Health (NIH) TCGA human subject protection and data access policies. Besides, we have collected 51 pairs of fresh frozen LUAD tissues and adjacent non-tumor tissues at the Affiliated Hospital of Xuzhou Medical University. These samples were preserved at -80°C for quantitative real-time PCR (qRT-PCR). This project was granted approval by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University.

TABLE 1 | Statistics of the TCGA cohort related to patients with LUAD

Characteristics	Variable	Patients (486)	Percentages (%)
Age	<65 years	209	43.00
	≥65 years	258	53.09
	unknown	19	3.91
Gender	Male	222	45.68
	Female	264	54.32
Pathological stage	I	262	53.91
	II	112	23.05
	III	79	16.25
	IV	25	5.14
	unknown	8	1.65
T classification	T1	163	33.54
	T2	260	53.50
	T3	41	8.44
	T4	19	3.91
	TX	3	0.61
N classification	N0	312	64.20
	N1	90	18.52
	N2	70	14.40
	N3	2	0.41
	NX	12	2.47
M classification	M0	333	68.52
	M1	24	4.94
	MX	129	26.54
Vital status	Alive	324	66.67
	Death	162	33.33

2.2 *FKBP4* expression and survival analysis

First, the online public UALCAN (http://ualcan.path.uab.edu/) was utilized to observe the mRNA and protein levels of *FKBP4* in human pan-cancer. Then, the raw mRNA expression data from the TCGA cohort was preprocessed via employing the Perl programming including data sorting, data merging and identification conversion. Based on the limma package and beeswarm package, we visualize *FKBP4* expression data through drawing the scatter difference chart and paired difference chart. Following, we extracted survival data of LUAD patients matching *FKBP4* expression data from the clinical data, and delete samples without survival time or survival status information. As a result, we got data on 455 LUAD patients who met the requirements of the research. More, depending on the median value of *FKBP4* expression, the 455 LUAD patients were divided into two groups (high *FKBP4* expression group and low *FKBP4* expression group). Relying on data in the two groups, we utilized survival package to draw the Kaplan-Meier survival curve.

2.3 RNA extraction and qRT-PCR

According to instructions, we utilized Trizol reagent (Invitrogen) to extract total RNA from fresh frozen LUAD tissues and adjacent non-tumor tissues. Then, Trans Script one-step guide DNA removal and complementary DNA synthesis super mix were used for the reverse transcription reaction. The primer sequences for PCR amplification were as follows: *FKBP4*, forward: 5'-ATTGCCATAGCCACCATGAAG-3', reverse: 5'- CCTGCTGAACCGTAGGCATATT-3'.

2.4 The verification of *FKBP4* by GEO and human protein atlas

In GEO database (https://www.ncbi.nlm.nih.gov/geo), "lung adenocarcinoma" was considered as the search term, and "Homo sapiens" was set as the qualifier to filter out available microarrays for the

verification of *FKBP4* expression in LUAD. By filtering out the datasets with small sample size (N<50), we obtained 6 reliable datasets (GSE101929, GSE11969, GSE18842, GSE27262, GSE32863 and GSE75037), including 338 LUAD tissue samples and 250 non-tumor tissue samples, as well as the *FKBP4* expression value of each sample **(Table 2)**. Relying on Review Manage 5.3 software, we implemented the meta-analysis to validate the differences of *FKBP4* expression level between LUAD samples and non-tumor samples. Based on standard mean difference (SMD) with a 95% confidence interval (CI), we calculated the combined value. Chi-square (χ^2) and I^2 statistical tests were utilized to evaluate the heterogeneity between the six screened datasets. If $p>0.05$ or $I^2<50\%$, a fixed effect model was chosen to calculate the combined effect, otherwise a random effect model was selected ($p<0.05$ or $I^2>50\%$). Moreover, taking "*FKBP4*" and "lung adenocarcinoma" as search terms, we also obtained immunohistochemical results of patients with LUAD from the human protein atlas database (http://www.proteinatlas.org).

TABLE 2 | Information of screened GEO microarrays related to LUAD

GEO datasets	Year	Country	Platform	Sample	N
GSE101929	2019	USA	GPL570	LUAD	32
				Non-LUAD	34
GSE11969	2013	Japan	GPL7015	LUAD	94
				Non-LUAD	5
GSE18842	2019	Spain	GPL570	LUAD	46
				Non-LUAD	45
GSE27262	2019	China	GPL570	LUAD	25
				Non-LUAD	25
GSE32863	2019	USA	GPL6884	LUAD	58
				Non-LUAD	58
GSE75037	2019	USA	GPL6884	LUAD	83
				Non-LUAD	83

2.5 Univariate and multivariate cox regression analyses

In order to quantitatively assess the independent predictive effect of different clinical pathological factors and *FKBP4* expression on survival, the hazard ratio (HR) and 95% CI were calculated, and the Cox regression model was used for univariate and multivariate analyses. The independent prognostic effect of *FKBP4* on survival was assessed by adjusting for confounding factors. Specifically, Perl was applied to preprocess the clinical data in the TCGA cohort, and remove samples with incomplete clinical information. After matching the processed complete clinical data with the *FKBP4* expression data, the LUAD patients were divided into the high *FKBP4* expression group or the low *FKBP4* expression group according to the median *FKBP4* expression value. In the end, depending on complete data of 316 patients with LUAD, we carried out univariate and multivariate cox regression analyses.

2.6 Gene Set Enrichment Analysis (GSEA)

Here, GSEA was applied to confirm whether a given set of genes displays statistically significant and consistent differences between two biological states. We identified signaling pathways related to *FKBP4* between datasets (low or high *FKBP4* expression) through utilizing the GSEA software. In the GSEA software, the main parameters were set as follows: reference gene set: c2.cp.kegg.v6.2.symbols.gmt, the number of genes identifying distinct pathways in each analysis: 1,000, The number of gene set rearrangements in each analysis: 1,000. What's more, the normalized

enrichment score (NES), nominal p-value and false discovery rate (FDR) q-value were given to denote the importance of associations between gene sets and pathways.

2.7 Statistical analysis

Mann-Whitney U test was implemented to inspect the *FKBP4* expression difference between LUAD tissues and non-tumor tissues. Kruskal-Wallis test was utilized to analyse *FKBP4* expression differences among groups. χ^2 test was applied to assess the correlation between *FKBP4* expression and each clinicopathological parameter. Logrank test was employed to compare the survival rate between the high *FKBP4* expression group and the low *FKBP4* expression group. On basis of the Cox regression model, we carried out univariate and multivariate survival analysis. Among statistical methods in this study, we took $p<0.05$ as the criterion for determining the significance level.

3 Results

3.1 *FKBP4* is upregulated in LUAD and Pan-Cancer according to online databases

Through analyzing *FKBP4* expression data from various cancers in UALCAN, it was watched that *FKBP4* expression level was obviously higher in most human cancers compared with the corresponding normal tissues **(Figure 1A)**. Besides, via analyzing *FKBP4* mRNA expression from 551 samples (497 LUAD samples and 54 non-tumor samples) in the TCGA cohort, we found that LUAD tissues showed significantly higher *FKBP4* expression level than normal tissues **(Figure 1B)**. Meanwhile, this result was validated by performing the differential expression analysis for 54 LUAD samples and paired normal samples in the TCGA cohort **(Figure 1C)**, and a CPTAC cohort (111 LUAD samples and 111 normal samples) also proved this result **(Figure 1D)**.

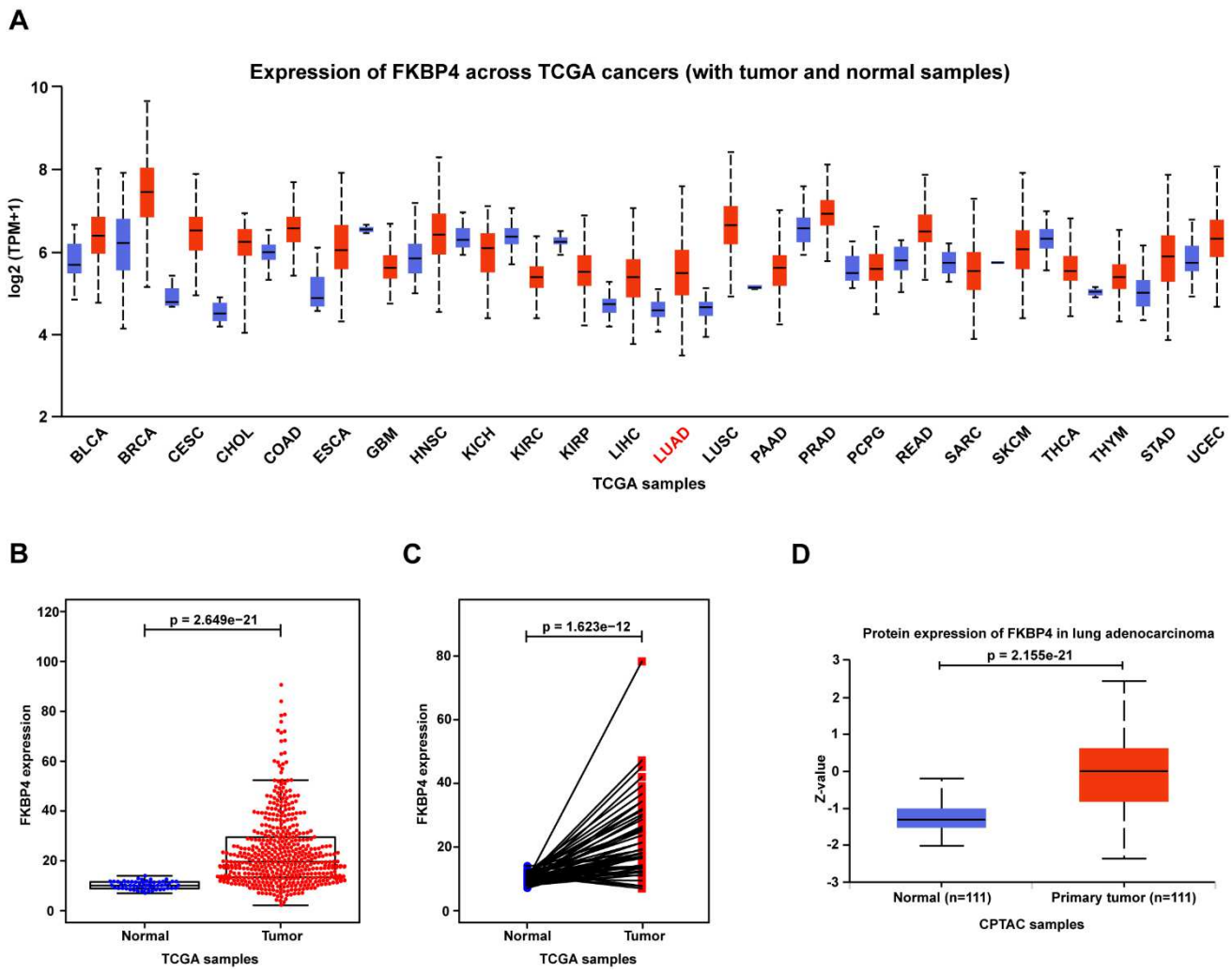

FIGURE 1 | *FKBP4* expression in normal human tissues, human tumors, and in LUAD. **(A)** *FKBP4* mRNA expression between multiple human cancers and corresponding normal samples in UALCAN database. **(B)** *FKBP4* mRNA expression between LUAD samples and normal samples from the TCGA cohort. **(C)** *FKBP4* mRNA expression between LUAD samples and paired normal samples from the TCGA cohort. **(D)** *FKBP4* protein expression between LUAD samples and normal samples from the CPTAC cohort.

3.2 Verification of *FKBP4* upregulation in LUAD by TMAs, qRT-PCR and SMD

With the aim of characterizing *FKBP4* expression status in LUAD, we first detected *FKBP4* protein expression between LUAD samples and normal samples in the human protein atlas database (http://www.proteinatlas.org) **(Figure 2A)**. Then, *FKBP4* mRNA expression was validated in a clinical *FKBP4* cohort containing 51 pairs of fresh frozen tissue specimens, indicating that *FKBP4* was upregulated in LUAD tissues **($p<0.001$, Figure 2B)**. Furthermore, relying on the *FKBP4* expression data for LUAD patients from the GEO dataset **(Table 2)**, we performed a comprehensive meta-analysis. As shown in **(Figure 2C)**, according to the random effect model (95% CI: 0.43-1.20), the combined SMD of *FKBP4* was 0.82. the *I*-square value was 76% ($p<0.001$). The above results manifested that *FKBP4* was significantly expressed in LUAD.

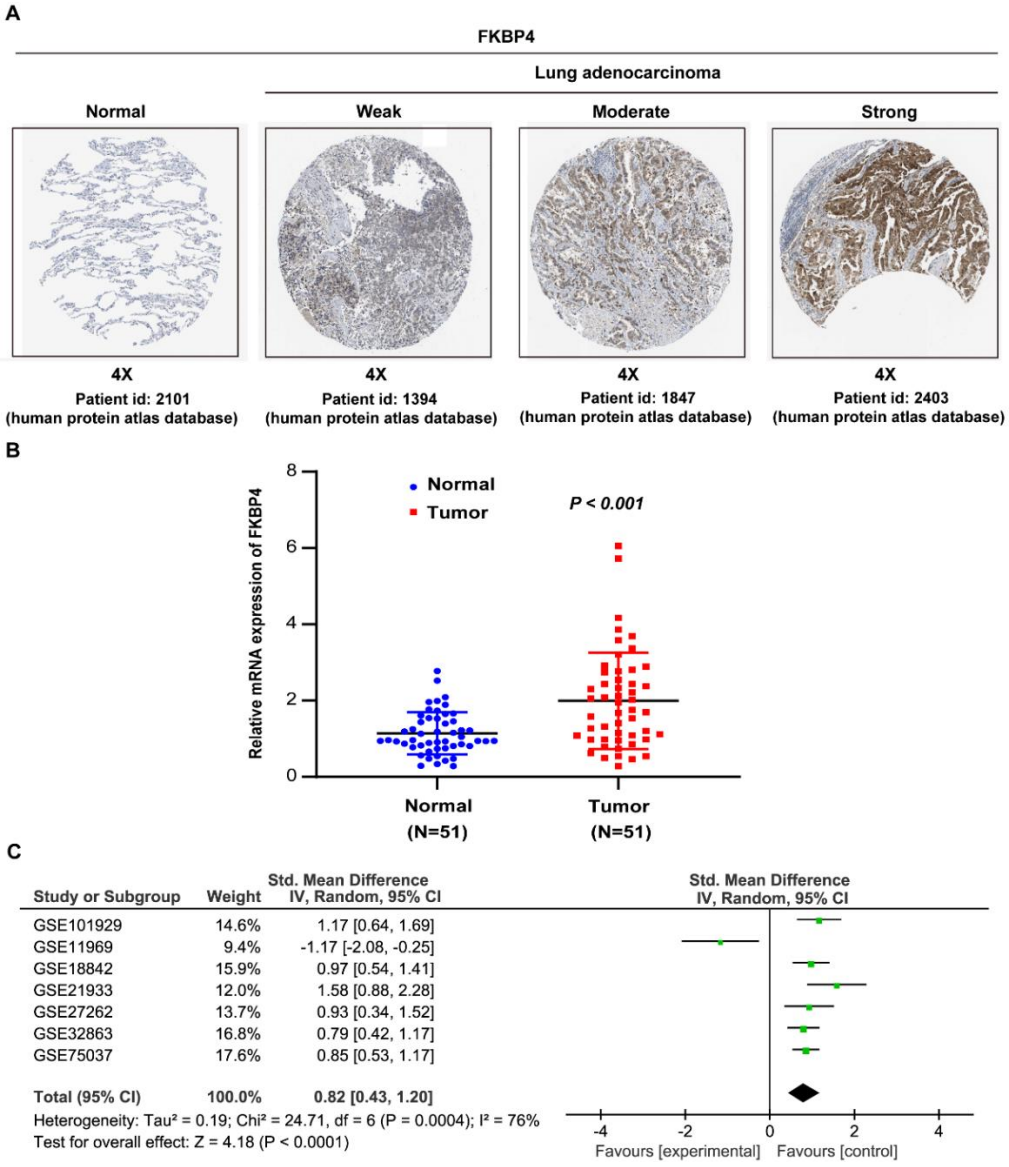

FIGURE 2 | *FKBP4* is upregulated in LUAD patient specimens. **(A)** Representative images of *FKBP4* expression from the human protein atlas (http://www.proteinatlas.org) online database. **(B)** Expression of *FKBP4* between 51 LUAD tissues and corresponding non-tumor tissues were detected by qRT-PCR (p<0.001). **(C)** Forest plot of *FKBP4* expression data from GEO microarrays. Gene Expression Omnibus (GEO), standard mean difference (SMD), confidence interval (CI).

3.3 *FKBP4* is associated with malignant progression in patients with LUAD

Based on *FKBP4* expression and corresponding clinical data from TCGA database, we conducted the in-depth analysis and watched that the expression of *FKBP4* was different in groups classified according to gender (**p<0.001, Figure 3A**), pathological stage (**p=0.0032, Figure 3C**), T classification (**p<0.001, Figure 3D**), N classification (**p=0.031, Figure 3E**), and M classification (**p=0.011, Figure 3F**). In addition, depending on the clinical data of 316 LUAD patients from TCGA database, we explored associations between the *FKBP4* expression and clinicopathological parameters. As described in **Table 3**, the high *FKBP4* expression level was significantly correlated with gender (p=0.007), pathological stage (p=0.013), T stage (p=0.014), lymph node metastasis (p=0.003) and distant metastasis (p=0.013). In **Table 4**, according to logistic regression analysis, we found that the

upregulated expression of *FKBP4* mRNA in LUAD was significantly related to gender (OR=1.708 for male vs. female, p=0.004), pathological stage (OR=1.645 for stage II vs. stage I and p=0.031, OR=3.329 for stage IV vs. stage I and p=0.009), T classification (OR=1.807 for T2 vs. T1 and p=0.004, OR=3.098 for T3 vs. T1 and p=0.002, OR=4.498 for T4 vs. T1 and p=0.006), lymph node metastasis (OR=1.479 for Positive vs. Negative and p=0.047) and distant metastasis (OR=3.231 for Yes vs. no and p=0.015).

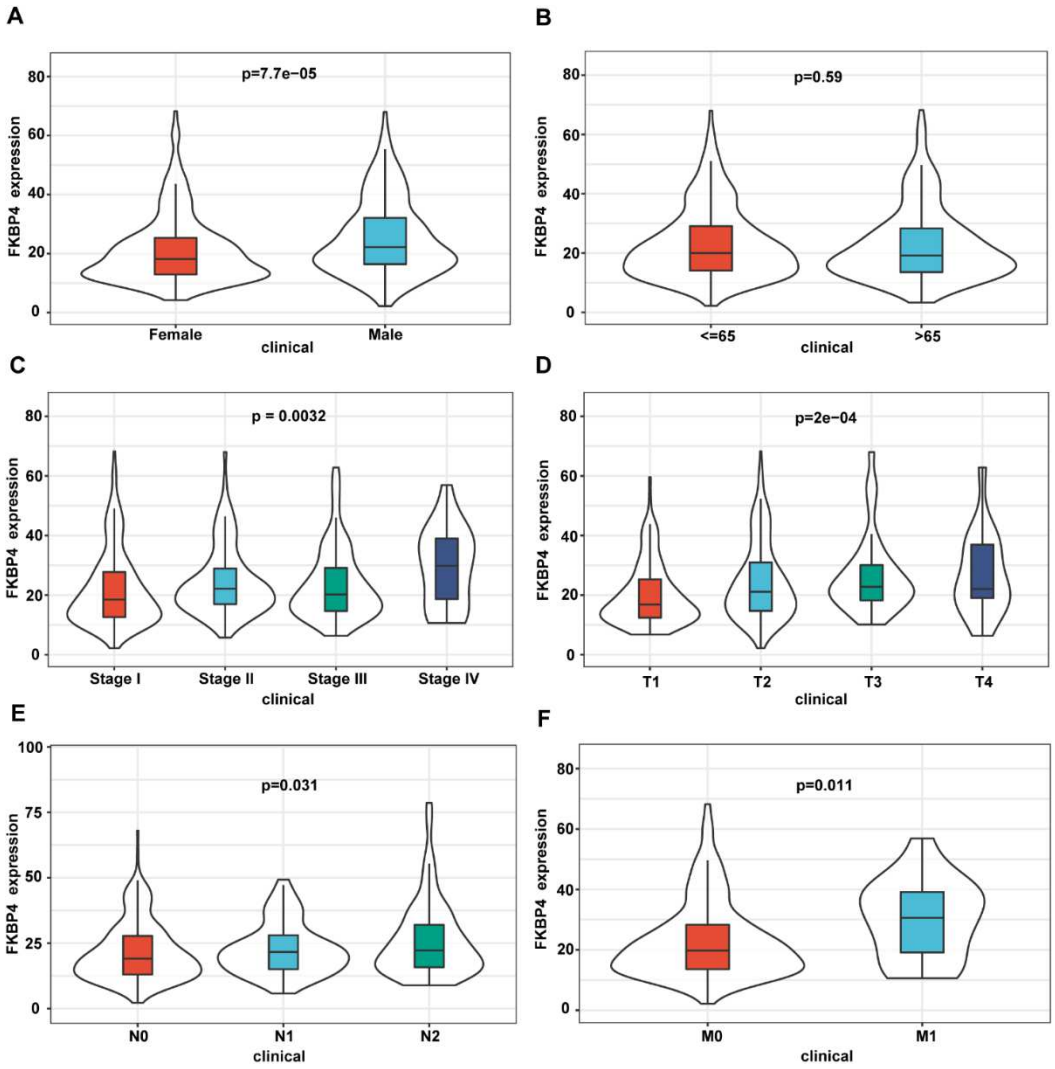

FIGURE 3 | Violin plot to evaluate *FKBP4* mRNA expression in LUAD patients based on clinical characteristics. **(A)** Gender. **(B)** Age. **(C)** Pathological stage. **(D)** T classification. **(E)** N classification. **(F)** M classification.

TABLE 3 | Relationships between *FKBP4* expression and clinicopathological parameters in LUAD

Clinicopathological parameters	*FKBP4* expression		Total	*p*-value
	High (n=158)	Low (n=158)		
Age				
<65 years	82 (53.2)	72 (46.8)	154	0.260
⩾65 years	76 (46.9)	86 (53.1)	162	
Gender				
Male	69 (42.6)	93 (57.4)	162	**0.007**

Female	89 (57.8)	65 (42.2)	154	
Pathological stage				
I-II	110 (46.0)	129 (54.0)	239	**0.013**
III-IV	48 (62.3)	29 (37.7)	77	
T classification				
T1-T2	129 (47.3)	144 (52.7)	273	**0.014**
T3-T4	29 (67.4)	14 (32.6)	43	
Lymph node metastasis				
Negative	88 (43.8)	113 (56.2)	201	**0.003**
Positive	70 (60.9)	45 (39.1)	115	
Distant metastasis				
No	142 (48.1)	153 (51.9)	295	**0.013**
Yes	16 (76.2)	5 (23.8)	21	

Bold values indicate $p<0.05$.

TABLE 4 | *FKBP4* expression correlated with clinicopathological parameters

Clinicopathological parameters	Total (*N*)	Odds ratio in *FKBP4* expression	*p*-value
Age			
<65 vs. ≥65	458	1.036 (0.717-1.498)	0.851
Gender			
Male vs. female	477	1.708 (1.188-2.461)	**0.004**
Pathological stage			
Stage II vs. stage I	366	1.645 (1.049-2.592)	**0.031**
Stage III vs. stage I	335	1.591 (0.957-2.659)	0.074
Stage IV vs. stage I	282	3.329 (1.398-8.818)	**0.009**
T classification			
T2 vs. T1	414	1.807 (1.210-2.714)	**0.004**
T3 vs. T1	200	3.098 (1.529-6.516)	**0.002**
T4 vs. T1	178	4.498 (1.632-14.491)	**0.006**
Lymph node metastasis			
Positive vs. Negative	465	1.479 (1.007-2.181)	**0.047**
Distant metastasis			
Yes vs. no	348	3.231 (1.317-9.099)	**0.015**

Bold values indicate $p<0.05$.

3.4 High expression of *FKBP4* in LUAD patients is related to poor OS

In this study, Kaplan-Meier risk estimate was employed to evaluate the prognostic role of *FKBP4* in LUAD. Comparing low *FKBP4* expression group with high *FKBP4* expression group, we observed that high *FKBP4* expression group has more associations with a poor OS **(Figure 4B)**. When taking gender and race into account, we have reached the same result (**Figure 4C, Figure 4D**). Besides, univariate and multivariate analyses were implemented on 316 LUAD samples in TCGA database to study the impact of *FKBP4* expression and clinicopathological factors on survival. Univariate analysis indicated four important predictors of survival including pathological stage (HR: 1.654, 95% CI: 1.401-1.951, p=0.000), T stage (HR: 1.632, 95% CI: 1.315-2.024, p=0.000), N stage (HR: 1.790, 95% CI: 1.459-2.196, p=0.000) and *FKBP4* expression (HR: 1.012, 95% CI: 1.005-1.018, p=0.001) **(Table 5)**. Multivariate analysis demonstrated that the high *FKBP4* expression was a vital independent predictor of a poor OS in LUAD (HR: 1.008, 95% CI: 0.999-1.015, p=0.012) **(Figure 4A, Table 5)**.

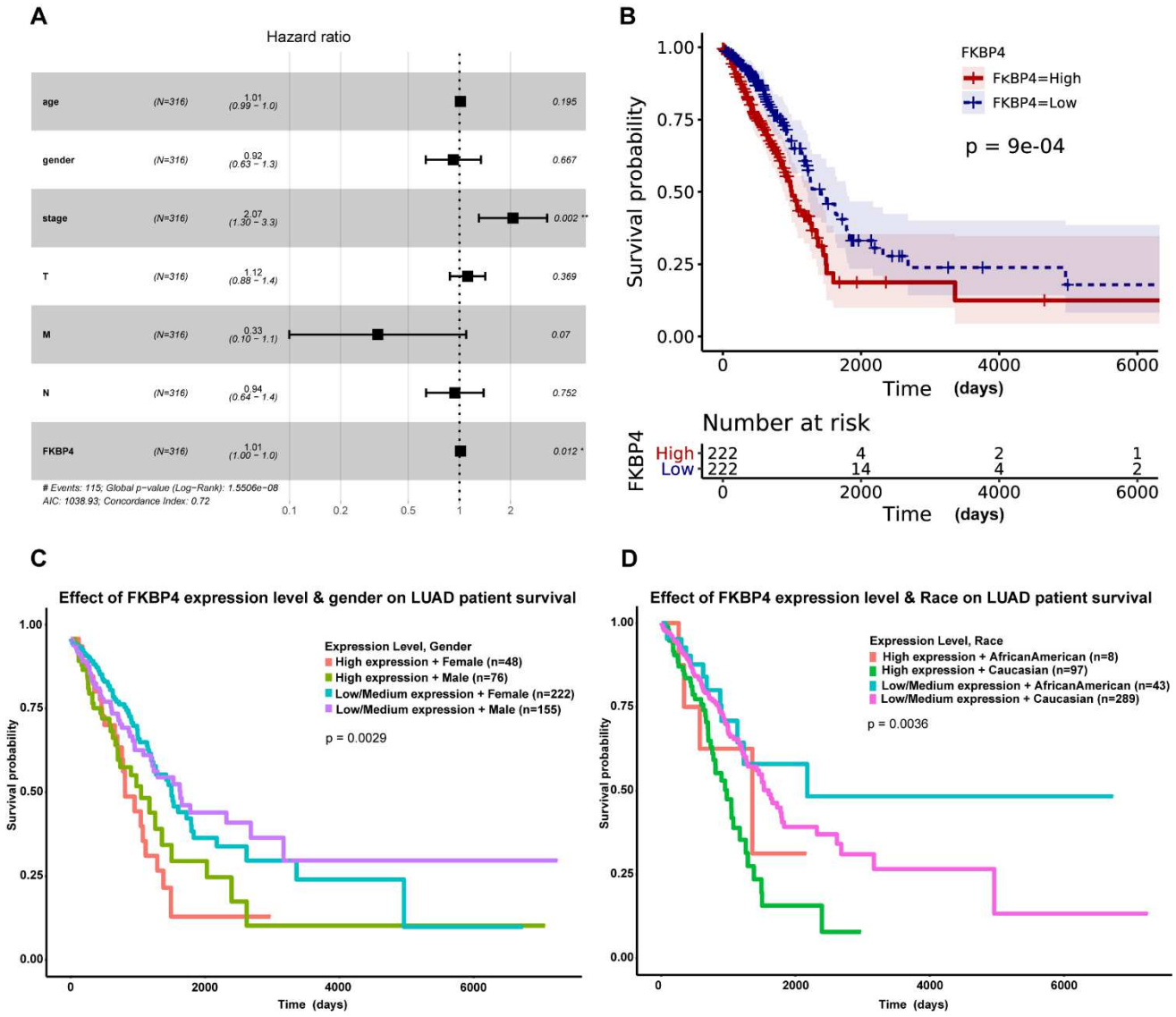

FIGURE 4 | Prognostic role of *FKBP4* in patients with LUAD. **(A)** Forest plot indicated that *FKBP4* was an independent predictor of poor survival rate (HR: 1.008, 95% CI: 0.999-1.015, *p*=0.012). **(B)** Kaplan-Meier curve of the association between *FKBP4* mRNA expression and the prognosis of LUAD patients. **(C)** Effect of *FKBP4* expression level and gender on LUAD patient survival. **(D)** Effect of *FKBP4* expression level and race on LUAD patient survival. hazard ratio (HR), confidence interval (CI). (*p<0.05, **p<0.01, ***p<0.001).

TABLE 5 | Univariate and multivariate analysis of *FKBP4* expression correlations among LUAD patients

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age	1.002	0.983-1.021	0.843	1.011	0.991-1.031	0.195
Gender	1.035	0.717-1.494	0.852	0.908	0.623-1.321	0.667
Pathological stage	1.654	1.401-1.951	**0.000**	1.987	1.255-3.144	**0.002**
T	1.632	1.315-2.024	**0.000**	1.160	0.912-1.476	0.369
N	1.790	1.459-2.196	**0.000**	0.975	0.661-1.439	0.752
M	1.757	0.964-3.203	0.066	0.374	0.116-1.208	0.070
FKBP4	1.012	1.005-1.018	**0.001**	1.008	0.999-1.015	**0.012**

Bold values indicate $p<0.05$.

3.5 Identification of *FKBP4*-related signaling pathways by GSEA

Here, we adopted the GSEA to further investigate possible mechanism of *FKBP4* in promoting LUAD progression. Specifically, the patients with LUAD were first divided into high or low *FKBP4* expression groups. Then, according to normalized enrichment score (NES), false discovery rate (FDR) *q*-value and nominal (NOM) *p*-value, fifteen significantly enriched signaling pathways with the high *FKBP4* expression phenotype were identified and listed as pyrimidine metabolism, pentose phosphate pathway, cell cycle, RNA polymerase, proteasome, spliceosome, DNA replication, citrate cycle tca cycle, oocyte meiosis, aminoacyl tRNA biosynthesis, fructose and mannose metabolism, base excision repair, RNA degradation, basal transcription factors and protein export **(Figure 5A, Table 6)**. We also gave details of four important signal pathways **(Figure 5B-5E)**.

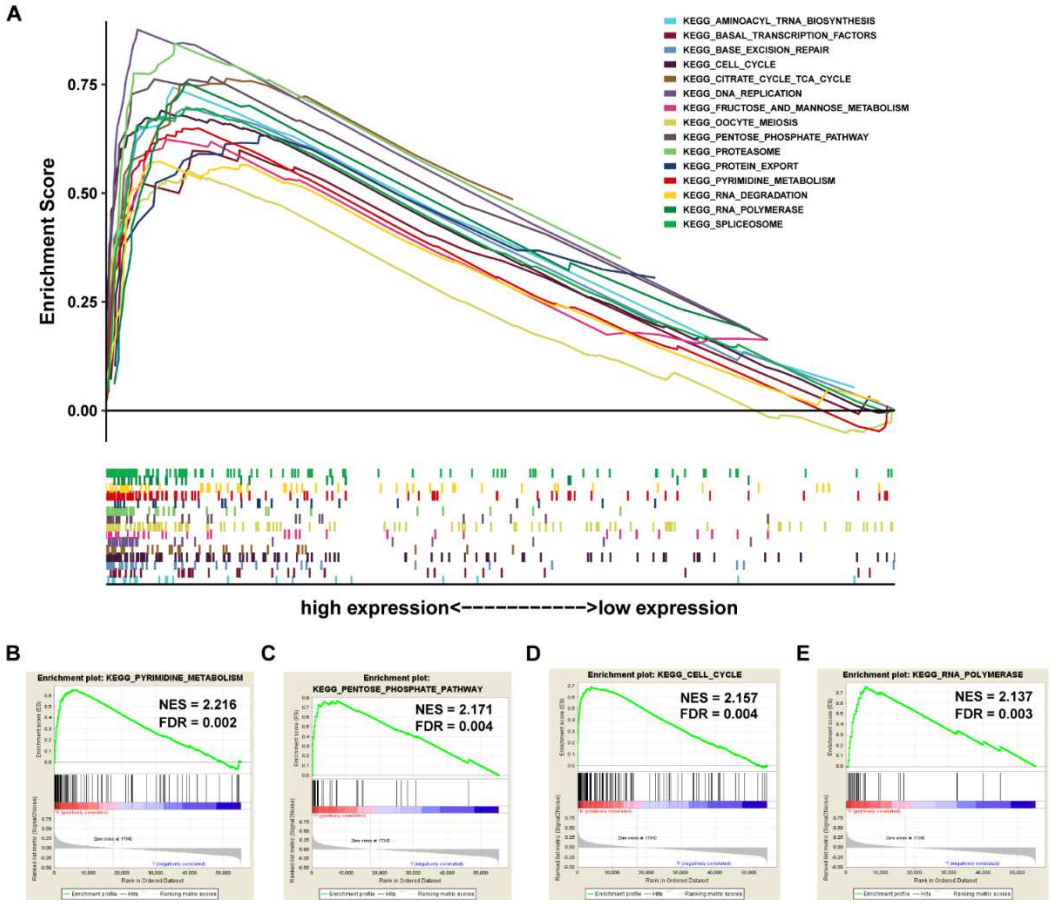

FIGURE 5 | The research on the potential mechanism of *FKBP4* in promoting LUAD progression. (A) The merged enrichment plot related to 15 signal pathways. **(B)** The details of KEGG_PYRIMIDINE_METABOLISM enrichment plot. **(C)** The details of KEGG_PENTOSE_PHOSPHATE_PATHWAY enrichment plot. **(D)** The details of KEGG_CELL_CYCLE enrichment plot. **(E)** The details of KEGG_RNA_POLYMERASE enrichment plot.

TABLE 6 | Gene sets enriched in the high *FKBP4* expression phenotype

Gene set name	NES	NOM *p*-value	FDR *q*-value
KEGG_PYRIMIDINE_METABOLISM	2.216	0.000	0.002
KEGG_PENTOSE_PHOSPHATE_PATHWAY	2.171	0.000	0.004
KEGG_CELL_CYCLE	2.157	0.000	0.004
KEGG_RNA_POLYMERASE	2.137	0.000	0.003
KEGG_PROTEASOME	2.099	0.000	0.005

KEGG_SPLICEOSOME	2.079	0.002	0.006
KEGG_DNA_REPLICATION	2.024	0.000	0.012
KEGG_CITRATE_CYCLE_TCA_CYCLE	2.022	0.002	0.009
KEGG_OOCYTE_MEIOSIS	2.022	0.002	0.009
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	1.996	0.000	0.010
KEGG_FRUCTOSE_AND_MANNOSE_ METABOLISM	1.977	0.000	0.010
KEGG_BASE_EXCISION_REPAIR	1.909	0.004	0.016
KEGG_RNA_DEGRADATION	1.881	0.008	0.020
KEGG_BASAL_TRANSCRIPTION_FACTORS	1.878	0.007	0.019
KEGG_PROTEIN_EXPORT	1.721	0.034	0.050

Normalized enrichment score (NES), nominal (NOM), false discovery rate (FDR).

4 Discussion

To our best knowledge, more and more researchers are engaged in the research of diagnostic and prognostic markers related to LUAD. As we know, some researchers have claimed that *FKBP4* is abnormally expressed in types of cancers. With rapid development of the comprehensive sequencing technology, massive genomic data has been easily available, which has become more feasible for the discovery and verification of biomarkers. In the study, we first analyzed the *FKBP4* expression level among various human tumors via utilizing database UALCAN.

In the research, we first adopted a comprehensive analysis method to study the role of *FKBP4* expression in LUAD, particularly as a prognostic biomarker for LUAD. Besides, by identifying the signaling pathways associated with *FKBP4* in LUAD, we revealed the underlying mechanisms that affect LUAD occurrence and progression. In detail, based on the TCGA cohort, we watched that *FKBP4* expression level in LUAD was significantly higher than that in normal tissues. The result was proved by qRT-PCR and public TMAs from the mRNA and protein level, respectively. We also performed meta-analysis on the GEO cohort to detect *FKBP4* expression level in LUAD and drew a conclusion consistent with the previous experiments. The result turned out that *FKBP4* might play as an oncogene and have a huge impact on LUAD occurrence and progression. Additionally, statistical analysis showed that *FKBP4* expression level was different in groups classified by pathological stage, T stage, N stage and M stage. Further analysis indicated that the high *FKBP4* expression level was significantly correlated with pathological stage, T classification, lymph node metastasis and distant metastasis. In summarize, the above evidences manifested that *FKBP4* expression at the mRNA level is related to some important clinicopathological parameters.

According to Kaplan-Meier survival analysis, we observed that high *FKBP4* expression group was worse than that of low *FKBP4* expression group. The univariate analysis revealed that pathological stage, T stage and N stage were related to the prognosis of LUAD patients, and the high *FKBP4* expression was associated with poor OS. To sum up, *FKBP4* was expected to be an independent prognostic factor for the OS in LUAD patients.

GSEA method was applied to analyze signaling pathways related to *FKBP4* in LUAD. As a result, pyrimidine metabolism, pentose phosphate pathway, cell cycle, RNA polymerase, proteasome, spliceosome, DNA replication, citrate cycle tca cycle, oocyte meiosis, aminoacyl tRNA biosynthesis, fructose and mannose metabolism, base excision repair, RNA degradation, basal transcription factors and protein export were found to be correlated with LUAD progression. Abnormal pyrimidine metabolism can affect tumor invasion and metastasis [11, 12]. Pentose phosphate pathway plays a critical role in regulating cancer cell growth via providing cells with NADPH for detoxification of

reactive oxygen species, reductive biosynthesis and ribose biosynthesis [13, 14]. Besides, the pentose phosphate pathway is regulated by lots of factors, including tumor suppressors, oncoproteins and intracellular metabolites. Dysregulation of the pentose phosphate pathway flux dramatically impacts cancer growth and survival [15, 16]. What's more, unscheduled proteolysis of cell cycle regulators leads to tumorigenesis [17, 18]. Basic transcription factors are necessary for the initiation of RNA polymerase II transcription and can maintain the basic level of transcription. Once the basic level of transcription is imbalanced, it will affect the function of RNAs, and then induce the occurrence and progression of tumors [19, 20]. Proteasome is a multi-protein organelle that participates in cellular proteostasis through destroying damaged or short-lived proteins. Proteasome is involved in all cell processes including decisions on cell survival or death, cell cycle, and differentiation. These processes are also important in cancer. [21, 22]. Moreover, Novel functions of cytoplasmic aminoacyl-tRNA synthetases may shape the hallmarks of cancer [23, 24].

There is no denying that this research has some limitations. On one hand, the clinical data are incomplete and lack some specific information including surgical treatments and surgical details. On the other hand, due to the limitations of the available data, we can not offer a *FKBP4*-related specific axis that regulates LUAD occurrence and progression.

5 Conclusion

In summary, taking full use of *FKBP4* expression data related to LUAD patients, we found that the *FKBP4* expression in LUAD tissues is higher than that in non-tumor tissues. We also watched that the up-regulation of *FKBP4* is dramatically correlated with some clinicopathological features of LUAD. Thus, we believed that the up-regulation of *FKBP4* promotes the occurrence and progression of LUAD According to univariate and multivariate survival analyses, the increased *FKBP4* expression in LUAD was identified as an independent risk factor for shorter OS. To sum up, we hold the opinion that *FKBP4* may become a promising biomarker for diagnosis and prognosis of LUAD.

6 Acknowledgments

Not applicable.

7 Authors' contributions

ST and XT designed the overall idea of this study, conceived the experiments, analyzed the data, prepared the figures and tables and authored the drafts of the paper. SW, PZ, XZ, HH, XH and JL collected the data from the TCGA and GEO dataset, performed the experiments. XT supervised this study and reviewed the drafts of the paper. All the authors read and approved the final draft.

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9 Availability of data and materials

Available datasets in this study were analyzed and can be downloaded from The Cancer Genome Atlas (https://portal.gdc.cancer.gov/). The NCBI Gene Expression Omnibus (GSE101929, GSE11969, GSE18842, GSE27262, GSE32863 and GSE75037).

10 Ethics approval and consent to participate

The project was granted approval by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University.

11 Consent for publication

All authors consent to publication.

12 Competing interests

The authors declare no conflict of interest.

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