Original Article

Comprehensive bioinformatics analysis on exportins in lung adenocarcinoma and lung squamous cell carcinoma

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Background: Lung cancer is one of the most common malignant tumors in the world. Exportins are closely associated with the cellular activity and disease progression in a variety of different tumors. However, the expression level, genetic variation, immune infiltration, and biological function of different exportins in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), as well as their relationship with the prognosis of patients with LUAD and LUSC have not been fully clarified.

Methods: To analyze the differential expression, prognostic value, genetic variation, biological function, and immune cell infiltration of exportins in patients with LUAD and LUSC, the ONCOMINE; UALCAN; Human Protein Atlas (HPA); Kaplan-Meier plotter; cBioPortal; Search Tool for the Retrieval of Interacting Genes/Proteins (STRING); Database for Annotation, Visualization, and Integrated Discovery (DAVID); Tumor Immune Estimation Resource (TIMER); and LinkedOmics databases were used in this study.

Results: The transcriptional and protein expression levels of *CSE1L* and *XPO1/5/6/7* were increased in patients with LUAD and LUSC, and the increased transcriptional levels of *CSE1L* and *XPO5/6/7* were related to worse prognosis. An increased transcriptional level of *XPO1* was associated with a better prognosis. These results indicated that *CSE1L* and *XPO1/5/6/7* may be potential prognostic biomarkers for the survival of patients with LUAD and LUSC. Moreover, the high mutation rate of exportins in non-small cell lung cancer was 50.48%, and the largest proportion of mutations included high messenger RNA expression. The expression of exportins was significantly correlated with the infiltration of various immune cells. Differentially expressed exportins could regulate the occurrence and development of LUAD and LUSC by involving a variety of microRNAs and transcription factor *E2F1*.

Conclusions: Our study provides novel insights into the selection of prognostic biomarkers of exportins in LUAD and LUSC.

Keywords: Bioinformatics analysis; exportins; lung cancer; biomarkers; prognostic value

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Introduction

As one of the most common malignant tumors in the world and the most common cause of global cancer-related mortality, lung cancer results in more than a million deaths each year (1). There are two main types of lung cancer, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with the most common subtypes of NSCLC being lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), accounting for about 85% of new lung cancer cases (2). Despite the progress of molecular targeted and immune-based checkpoint therapies, the 5-year overall survival (OS) of patients with NSCLC remains less than 15% (3), and cancer therapy still faces great challenges, including delayed diagnosis, recurrence, metastasis, and cancer-related mutations. Therefore, it is crucial to determine more reliable tumor molecular markers for early the screening, diagnosis, individualized treatment, and prognosis of NSCLC.

Exportins are a group of nucleocytoplasmic transport receptor proteins that are widely distributed in eukaryotes. At present, 7 exportins have been identified in the mammalian cell, namely *XPO1* (also called *CRM1*), *CSE1L* (also called *XPO2* or *CAS*), *XPOT* (also called *XPO3*), *XPO4*, *XPO5*, *XPO6*, and *XPO7* (4,5). These exportins belong to the Karyopherin-β (Kap-β; also known as *importin* and *exportin*) family and are responsible for transporting most proteins and RNA in the nucleus across the nuclear membrane to the cytoplasm (5,6). Kaps have low sequence identity (10–20%), share similar molecular weights (90–150 kDa) and isoelectric points (PI =4.0–5.0), and all have helical HEAT repeats. Through these special structures, biological macromolecules can be exported to the nucleus (7). Kaps are also closely linked to many cellular processes, including gene differential expression, cell signal transduction, tumor immune response, and tumorigenesis because they interact with a large number of proteins with different functions and play a role in controlling a variety of protein localizations (8). Some studies have found that exportins are associated with cell activity and progression of various cancers, such as liver cancer (9), breast cancer (10), gastric cancer (11), lung cancer (12), and prostate cancer (13). A study showed that *CRM1* is frequently over-expressed in NSCLC, especially in LUAD and LUSC. Furthermore, the study also found that after tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (*NNK*) exposure or transfection with *CRM1* vector, the overexpression of *CRM1* in the lung epithelial cell line *BEAS-2B* led to cellular transformation, which suggests that the up-regulation of *CRM1* may be an important pathway for malignant transformation of lung epithelial cells (14). *CSE1L* interacts with p65 and activates nuclear factor-κB (*NF-κB*), and Mitogen-activated protein kinase (*MAPK*) signaling pathway promotes NSCLC proliferation and inhibits apoptosis (15). However, the expression level, genetic variation, immune infiltration and biological function of different exportins in LUAD and LUSC was well as their relationship with prognosis of patients with LUAD and LUSC have not been fully clarified.

With the rapid development of RNA-sequencing technology and microarrays, RNA and DNA exploration have become a significant constituent of biomedical and biological research (16). For example, Guo *et al.* (17) analyzed the DEGs and hub genes that affect the development of LUAD through bioinformatics technology, providing potential diagnosis and treatment strategies for the treatment of LUAD. In our study, we used various large public databases to expand the relevant knowledge of LUAD and LUSC, and conducted a comprehensive bioinformatics analysis on the relationship between 7 different exportins and the prognosis of LUAD and LUSC. We present the following article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-228/rc).

Highlight box

Key findings

- The transcriptional and protein expression levels of *CSE1L* and *XPO1/5/6/7* were increased in patients with LUAD and LUSC, and the increased transcriptional levels of *CSE1L* and *XPO5/6/7* were related to worse prognosis. The increased transcriptional level of *XPO1* suggested a better prognosis. These results indicated that *CSE1L* and *XPO1/5/6/7* potential prognostic biomarkers for the survival of patients with LUAD and LUSC.

What is known and what is new?

- Exportins are associated with cell activity and progression of various cancers.
- To analyze the differential expression, prognostic value, genetic variation, biological function, and immune cell infiltration of exportins in patients with LUAD and LUSC, the ONCOMINE; UALCAN; HPA, Kaplan-Meier plotter; cBioPortal; STRING; DAVID; TIMER; and LinkedOmics databases were used in this study.

What is the implication, and what should change now?

- Our study provides novel insights into the selection of prognostic biomarkers of exportins in LUAD and LUSC.

Methods

ONCOMINE analysis

ONCOMINE (www.ONCOMINE.org) is currently the world's largest publicly accessible oncogene chip database and comprehensive data mining platform, including 715 data sets and 86,733 samples (18). The ONCOMINE database was used to determine the transcriptional levels of exportins in different types of cancer. In our study, a P value <1E-4, a fold change of 2, and a gene rank in the top 10% were set as the significance thresholds. A *t*-test was used to analyze the differences in the expression of exportins in LUAD and LUSC, and statically significant differences were considered present with a P value <0.05. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

UALCAN analysis

UALCAN (http://ualcan.path.uab.edu/) mainly uses The Cancer Genome Atlas (TCGA) transcriptome and clinical patient data to help identify candidate biomarkers of specific cancer subclasses with diagnostic, prognostic, or therapeutic significance, and it can also be used as a computer verification platform for target genes (19). In our study, the "TCGA analysis" module and the "LUAD" and "LUSC" data sets were used to analyze the relationship between exportins transcription levels in LUAD and LUSC and normal tissues in the "expression" link. Statically significant differences were considered present with a P value <0.05.

Human Protein Atlas analysis

The Human Protein Atlas (HPA; https://www.proteinatlas.org/) database is an online free database that provides a large amount of proteomic and transcriptomic data of specific human tissues and cells (20). In our study, the protein expression levels of exportins in LUAD and LUSC and normal tissues were studied.

Kaplan-Meier plotter analysis

As an important prognostic biomarker evaluation tool, the Kaplan-Meier plotter (https://kmplot.com/analysis/index.php?p=background) can evaluate the correlation between the expression of the 54,000 genes on the survival rates in 21 different cancers (21). We used this database to study the prognostic value of exportins mRNA expression in lung cancer, including overall survival (OS) and progression-free survival (PFS). We also evaluated the hazard ratio (HR) with 95% confidence intervals and log rank p value. Statically significant differences were considered present with a P value <0.05.

cBioPortal analysis

cBioPortal (https://www.cbioportal.org/) is a comprehensive network resource that can visualize and analyze multidimensional cancer genomics data (22). In our study, by using the cBioPortal online tool of LUAD (TCGA, Firehose legacy), the genome profiles of 7 exportin members were analyzed, which contained mutations, putative copy number alterations (CNAs) from genomic identification of significant targets in cancer (GISTIC), and mRNA expression Z scores (microarray). Coexpression genes of exportin members were determined using the "co-expression" module of cBioPortal. Pearson correlation coefficient was used to calculate the correlation between exportin members and coexpression genes, and the top 10 coexpression genes of each exportin with the largest Pearson correlation coefficient were identified.

Protein-protein interaction (PPI) network construction and module analysis

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 11.5; https://cn.string-db.org/) is an online database for analyzing the functional interactions between proteins encoded by different genes (23). Cytoscape (version 3.8.2) is an open bioinformatics software platform for visualizing gene interaction networks (24). The STRING database was used to construct the PPI network of 69 genes significantly related to exportins mutations, and then the module analysis was conducted in Cytoscape software.

Database for Annotation, Visualization, and Integrated Discovery analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID; version 6.8; https://david.ncifcrf.gov/tools.jsp) is a comprehensive bioinformatics website that can visually annotate biological functions (25). In our study, 69 coexpression genes significantly related to exportins mutations were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Statically significant differences were considered present at a P value <0.05.

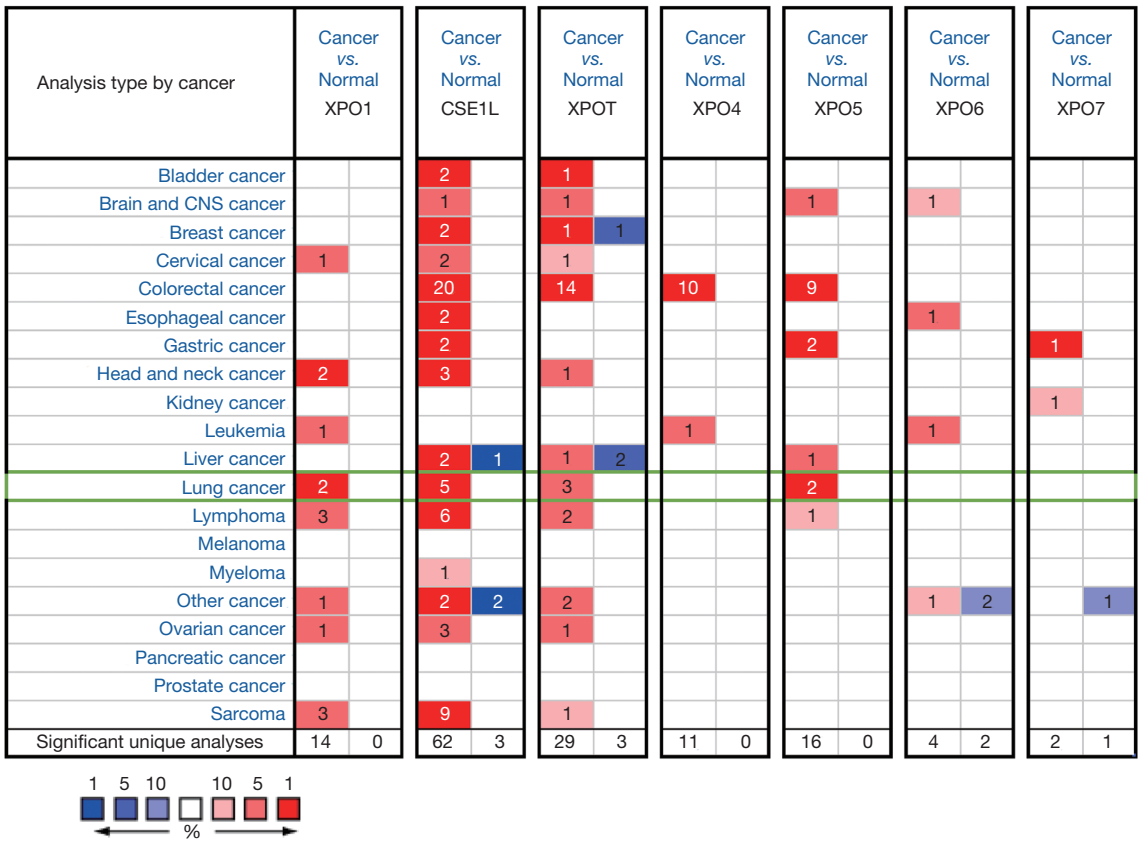


Figure 1 Transcriptional levels of exportins in 20 different types of cancer diseases according to the ONCOMINE database. CNS, central nervous system.

Tumor Immune Estimation Resource analysis

Tumor Immune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer) is a reliable and convenient online analysis tool to analyze immune infiltration systematically in various malignancies. We used the "gene" module of this database to study the correlation between the expression of exportins in LUAD and LUSC and immune cell infiltration. A statically significant difference was considered present with a P value <0.05.

LinkedOmics analysis

LinkedOmics (http://www.linkedomics.org/login.php) is a comprehensive multiomics database for the analysis of 32 TCGA cancer types (26). We studied the microRNA (miRNA) target enrichment and transcription factor target enrichment of exportins through the "LinkInterpriser" module in TCGA_LUAD data set. Statically significant differences were considered present at a P value <0.05.

Results

Transcription levels and protein expression of exportins in patients with LUAD and LUSC

The transcriptional and protein levels of different exportins members between LUAD, LUSC, and normal tissues were compared in the ONCOMINE, UALCAN and HPA databases. ONCOMINE differential expression analysis revealed that the transcriptional levels of *XPO1*, *CSE1L*, *XPOT*, and *XPO5* were upregulated in patients with LUAD and LUSC (*Figure 1* and *Table 1*). The transcription level of *XPO1* was significantly higher in patients with LUAD and

Table 1 Changes of exportins expression in transcription level between LUAD, LUSC, and normal lung tissues from the ONCOMINE database

Gene	Type of lung cancer versus normal lung tissue	Fold change	P value	*t*-test	Source and/or reference
XPO1	LUAD	1.375	1.09E-09	6.704	Hou (27)
	LUSC	2.828	6.44E-09	7.792	Hou (27)
	LUSC	1.179	3.11E-39	14.842	TCGA
	LUAD	1.043	1.78E-12	7.296	TCGA
	LUAD	1.35	1.95E-05	4.689	Stearman (28)
CSE1L	LUSC	2.51	1.54E-17	13.493	Hou (27)
	LUAD	1.487	2.01E-08	6.38	Hou (27)
	LUSC	7.468	7.67E-06	4.994	Bhattacharjee (29)
	LUAD	1.566	0.065	1.588	Bhattacharjee (29)
	LUSC	3.497	5.78E-05	5.227	Garber (30)
	LUAD	2.29	0.001	4.431	Garber (30)
XPOT	LUAD	1.075	0.382	0.304	Bhattacharjee (29)
	LUSC	4.078	9.25E-05	4.166	Bhattacharjee (29)
	LUSC	2.527	5.04E-13	11.371	Hou (27)
	LUAD	1.572	3.86E-11	7.771	Hou (27)
	LUAD	1.768	0.002	3.571	Garber (30)
XPO5	LUSC	2.044	1.72E-11	9.733	Hou (27)
	LUAD	1.684	1.63E-10	7.593	Hou (27)
	LUSC	2.161	0.001	3.977	Garber (30)
	LUAD	2.126	0.002	4.505	Garber (30)

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

LUSC in the 3 data sets. In the Hou Lung data set (27), *XPO1* overexpression was detected in LUAD and LUSC compared with normal tissues, with fold changes of 1.375 (P=1.09e-09) and 2.828 (P=6.44e-09), respectively. However, in the TCGA Lung 2 data set, the *XPO1* transcription level in LUAD and LUSC samples was 1.043 times (P=1.78e-12) and 1.179 times (P=3.11e-39) greater, respectively. Moreover, in the Stearman Lung data set, there was a 1.35-fold increase in *XPO1* mRNA expression in LUAD tissues (P=1.95e-05) (28). The *CSE1L* transcriptional level in patients with LUAD and LUSC was also found to be elevated in the 3 data sets. In the Hou Lung data set (27), *CSE1L* was overexpressed in LUAD and LUSC compared with the normal samples, with a fold change of 1.487 (P=0.01e-08) and 2.51 (P=1.54e-17), respectively. In the Bhattacharjee Lung data set (29), *CSE1L* was overexpressed in LUSC with fold changes of 7.468 (P=0.67e-06), and the transcription level of *CSE1L* in LUAD was slightly higher than that in normal lung tissue, but the P value did not exceed 0.05. Furthermore, *CSE1L* was also overexpressed in LUAD and LUSC in the Garber Lung data set, with fold changes of 2.29 (P=0.001) and 3.497 (P=5.78e-05) (30). Similarly, the *XPOT* transcription level in patients with LUAD and LUSC was also elevated in the 3 data sets. In the Hou Lung data set (27) and Garber Lung data set (30), *XPOT* was significantly overexpressed in LUAD with fold changes of 1.572 (P=3.86E-13) and 1.768 (P=0.002), respectively.

In the Bhattacharjee Lung data set (29), the transcription level of *XPOT* in LUSC had an increased fold change of 4.078 (P=9.25e-05), but the same change was not found in LUAD samples (P=0.382), compared with normal tissues. A

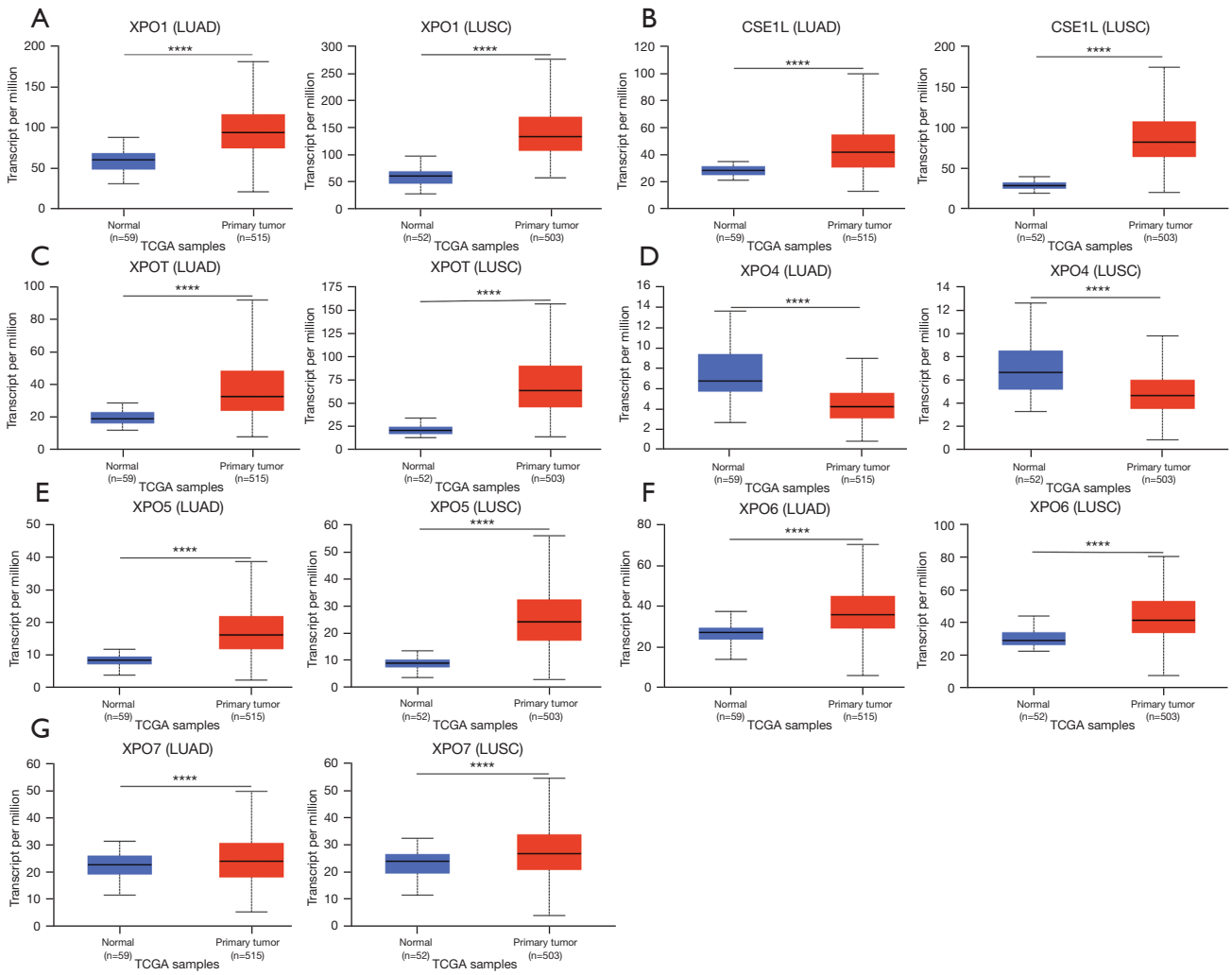


Figure 2 Transcriptional levels of exportins in LUAD and LUSC tissues and adjacent normal lung tissues in the UALCAN database. (A) Transcription level of *XPO1*. (B) Transcription level of *CSE1L*. (C) Transcription level of *XPOT*. (D) Transcription level of *XPO4*. (E) Transcription level of *XPO5*. (F) Transcription level of *XPO6*. (G) Transcription level of *XPO7*. ****, P<0.0001. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

similar pattern for *XPO5* was found in the Hou Lung data set (27). *XPO5* was significantly upregulated in LUAD and LUSC with fold changes of 1.684 (P=1.63e-10) and 2.044 (P=1.72e-11) respectively. Similar results were apparent in the Garber Lung data set (30).

Next, the transcriptional expression of exportins members between LUAD, LUSC and normal tissues was confirmed by using the UALCAN data set (*Figure 2*). The results indicated that the transcription levels of *XPO1*, *CSE1*L, *XPOT*, *XPO5*, *XPO6*, and *XPO7* in LUAD and LUSC tissues were significantly higher than those in normal lung tissues, while the transcription level of *XPO4* in normal lung tissues was higher than that in LUAD and LUSC tissues.

After analyzing the transcription levels of exportins in LUAD and LUSC, we confirmed the protein expression levels of exportins in patients with LUAD and LUSC and normal lung tissues using the HPA database (*Figure 3*). It should be noted that *XPO1* protein was expressed in both normal lung tissues and lung cancer tissues (*Figure 3A*). *CSE1L*, *XPO5*, *XPO6*, and *XPO7* were not expressed in normal lung tissues, but had low and medium expression in lung cancer tissues (*Figure 3B-3E*). Moreover, there was no protein expression of *XPOT* and *XPO4* in normal or lung cancer tissues. In

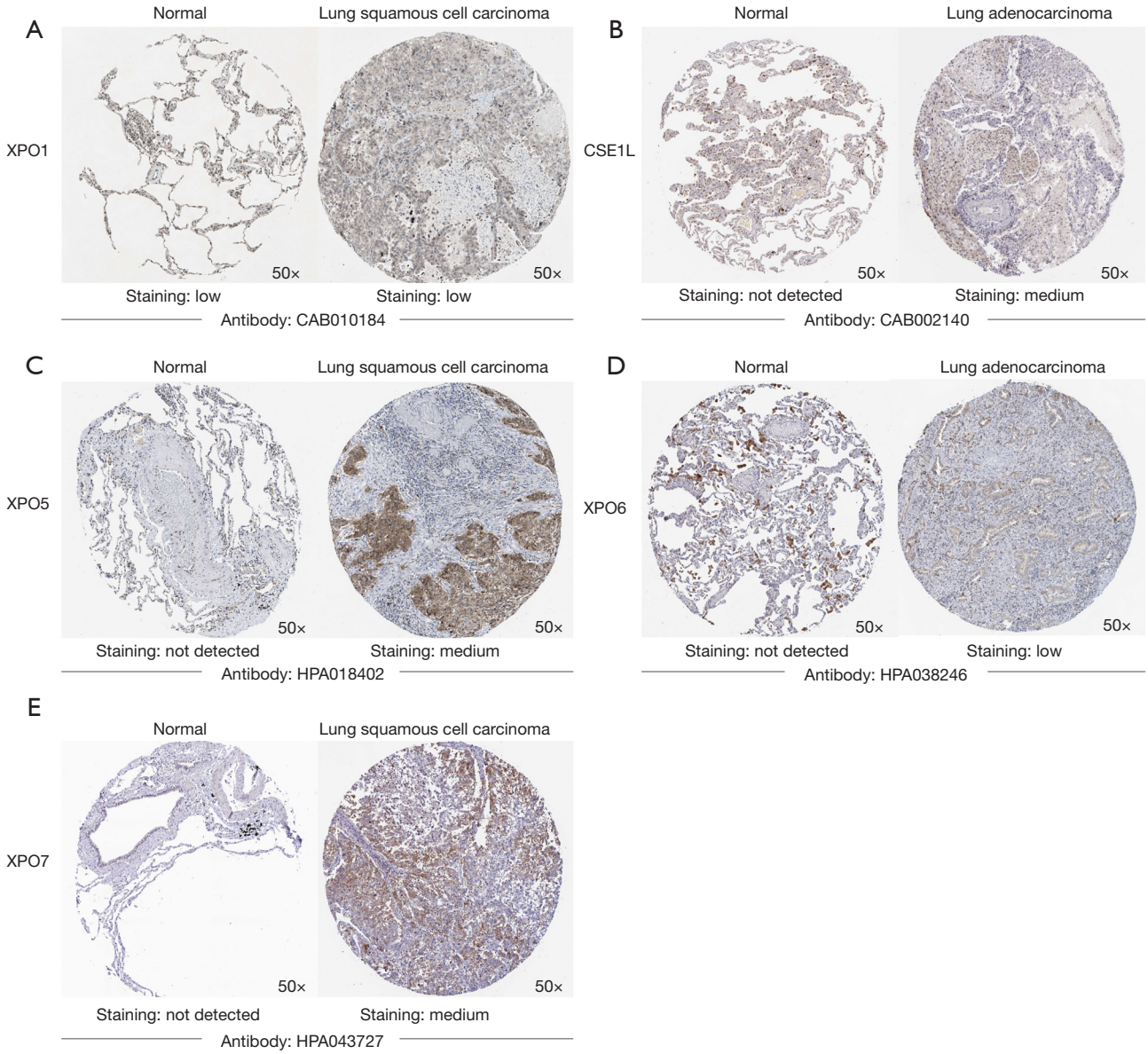


Figure 3 Representative immunohistochemical images of exportins in LUAD and LUSC tissues and normal lung tissues in HPA database. (A) Immunohistochemical images of *XPO1* in normal tissues and LUSC. (B) Immunohistochemical images of *CSE1L* in normal tissues and LUAD. (C) Immunohistochemical images of *XPO5* in normal tissues and LUSC. (D) Immunohistochemical images of *XPO6* in normal tissues and LUAD. (E) Immunohistochemical images of *XPO7* in normal tissues and LUSC. Data was obtained from https://www.proteinatlas.org/ENSG00000082898-XPO1/pathology/lung+cancer#img; https://www.proteinatlas.org/ENSG00000124207-CSE1L/pathology/lung+cancer#img; https://www.proteinatlas.org/ENSG00000124571-XPO5/pathology/lung+cancer#img; https://www.proteinatlas.org/ENSG00000169180-XPO6/pathology/lung+cancer#img; https://www.proteinatlas.org/ENSG00000130227-XPO7/pathology/lung+cancer#img. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma. Magnification: 50 times.

summary, the results of the above 3 databases revealed that the transcriptional and translational expression levels of the *XPO1*, *CSE1L*, *XPO5*, *XPO6*, and *XPO7* genes were increased in patients with LUAD and LUSC.

Prognostic characteristics of exportin members in patients with LUAD and LUSC

In order to evaluate the clinical significance of exportins, the publicly accessible Kaplan-Meier plotter tool was used to determine the correlation between exportin members' transcriptional levels and the survival of patients with LUAD and LUSC. The main parameters of survival analysis included overall survival (OS) and progression-free survival (PFS). According to the Kaplan-Meier survival curves shown in *Figure 4* and *Table 2*, OS was negatively correlated with the transcriptional levels of *CSE1L* and *XPOT/4/5/6/7* but positively correlated with *XPO1* (*Figure 4A-4G*). Moreover, the transcriptional levels of *XPO4/6/7* in LUAD and LUSC were negatively correlated with PFS, while the transcriptional levels of *XPO1* and *XPOT* were positively correlated with PFS, but there was no significant correlation between the transcriptional levels of *CSE1L* and *XPO5* with PFS (*Figure 4A-4G*). In summary, according to the transcriptional and protein expression levels of exportins in the above databases and the results of the Kaplan-Meier plotter tool, the elevated transcriptional levels of *CSE1L* and *XPO5/6/7* were associated with a worse prognosis in patients with LUAD and LUSC. In contrast, increased *XPO1* transcription levels were associated with a better prognosis in patients with LUAD and LUSC. Therefore, *CSE1L* and *XPO1/5/6/7* may be useful biomarkers for predicting the survival rate of patients with LUAD and LUSC.

The genetic alteration and mutation information of exportins

The LUAD (TCGA, Firehose legacy) module was used in cBioPortal online tool to analyze the mutations of exportin members. As shown in *Figure 5A*, 261 of 517 patients had gene mutations (mutation rate 50.48%), of which *CSE1L* and *XPO7* were the genes with the largest mutations, with mutation rates of 17% and 18%, respectively. Furthermore, the mutation rates of the *XPO1*, *XPOT*, *XPO4*, *XPO5*, and *XPO6* genes were 9%, 10%, 1%, 11%, 2.7%, and 12% in the LUAD samples, respectively. Gene mutations in exportins members included mRNA upregulation (120 cases, 23.21%), multiple alterations (56 cases, 10.83%), mRNA downregulation (44 cases, 8.51%), genetic amplification (18 cases, 3.48%), deep deletion (14 case, 2.71%), and mutation (9 cases, 1.74%). Among them, the highest proportion of mutation was mRNA upregulation, especially in *XPO1*, *CSE1L*, *XPOT*, and *XPO6*. However, the overexpression of mRNA was not detected in *XPO5*, but it had high frequency of genetic amplification of 2.7%. we also figured the correlations between exportins family by analyzing their mRNA expression (RNA Seq V2 RSEM) via the cBioPortal online tool for LUAD (TCGA, Firehose Legacy). The results showed that *XPO1* was significantly and positively correlated with *CSE1L*, *XPOT*, *XPO4*, *XPO6*, and *XPO7*; *CSE1L* was positively correlated with *XPOT*, *XPO5*, *XPO6* and *XPO7*; and *XPOT* was significantly and positively correlated with *XPO6* (*Figure 5B*). In addition, the "mRNA expression z-scores relative to diploid samples (RNA Seq V2 RSEM)" option in the genome map was selected to analyze the correlation between exportin members through the "mutual exclusivity" module. The results (*Figure 5C*) indicated that there was a correlation between the transcriptional levels of *XPO1*, *CSE1L*, *XPOT*, and *XPO6*, and that the transcriptional levels of *XPO1* and *CSE1L* were also correlated with *XPO7*. Furthermore, the relationship between the mRNA expression and copy-number alterations of exportins members was analyzed, and it was found that the mRNA expression of exportins members was positively correlated with copy-number alterations (*Figure 6*).

GO and KEGG enrichment analysis of exportins and their coexpression genes in patients with LUAD and LUSC

Subsequently, the "Coexpression" module of cBioPortal was used to list the top ten co-expression genes with the largest Pearson's correlation coefficient among the seven exportins, and a total of 70 genes were listed (Table S1). After the deletion of duplicate genes, a total of 69 genes were selected. Following this, the STRING database was used to construct the PPI network of the 69 coexpression genes that were significantly related to exportins mutations, with the module analysis being conducted using Cytoscape software. As shown in *Figure 5C*, the top 11 genes significantly associated with the exportins mutations were *TOP2A*, *AURKA*, *BUB1*, *EXO1*, *TTK*, *MCM10*, *NCAPG*, *KIF2C*, *NEK2*, *KIF15*, and *TPX2*. Next, the biological functions of the 69 coexpression genes significantly associated with exportins mutations were further analyzed through GO

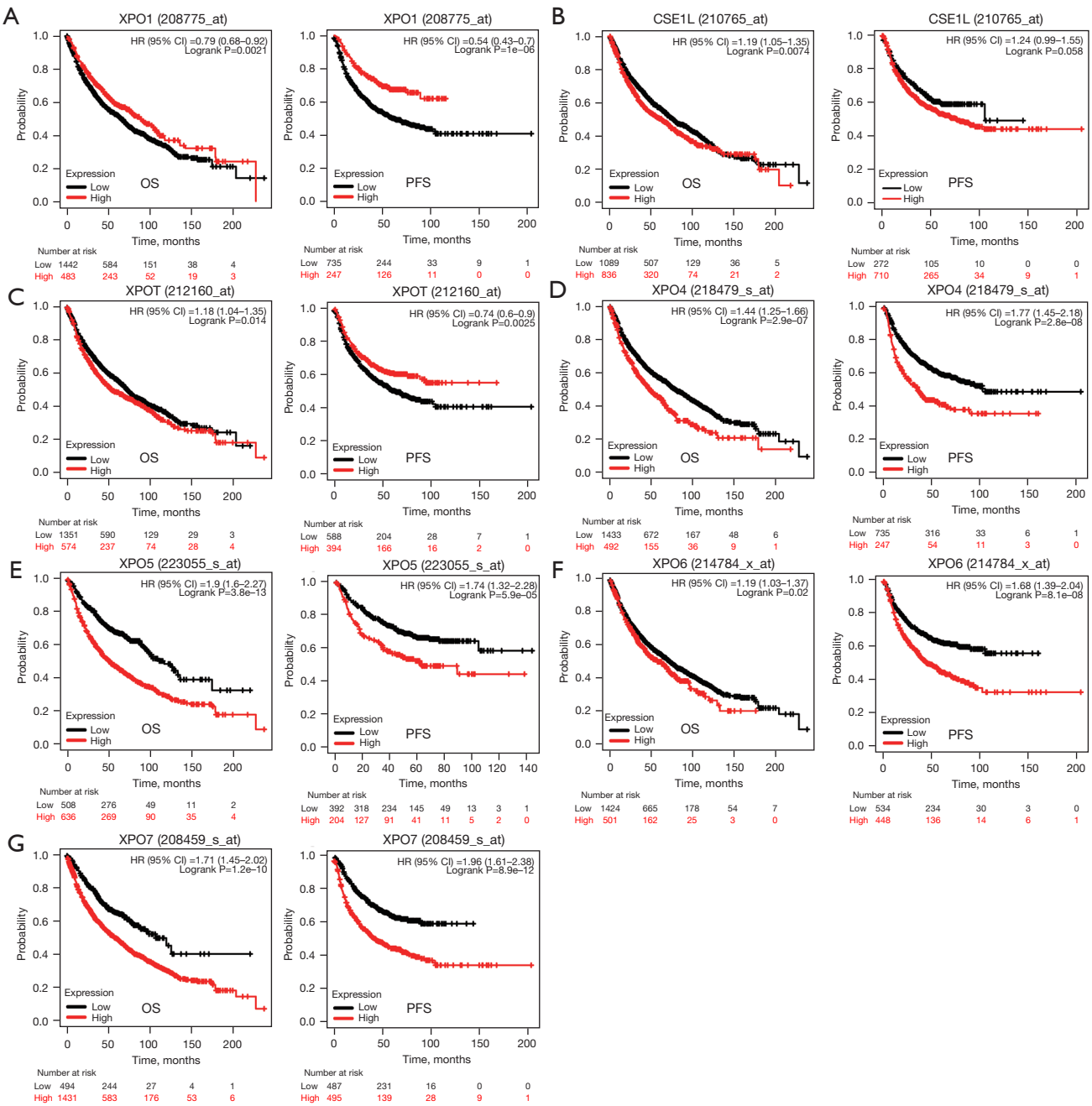


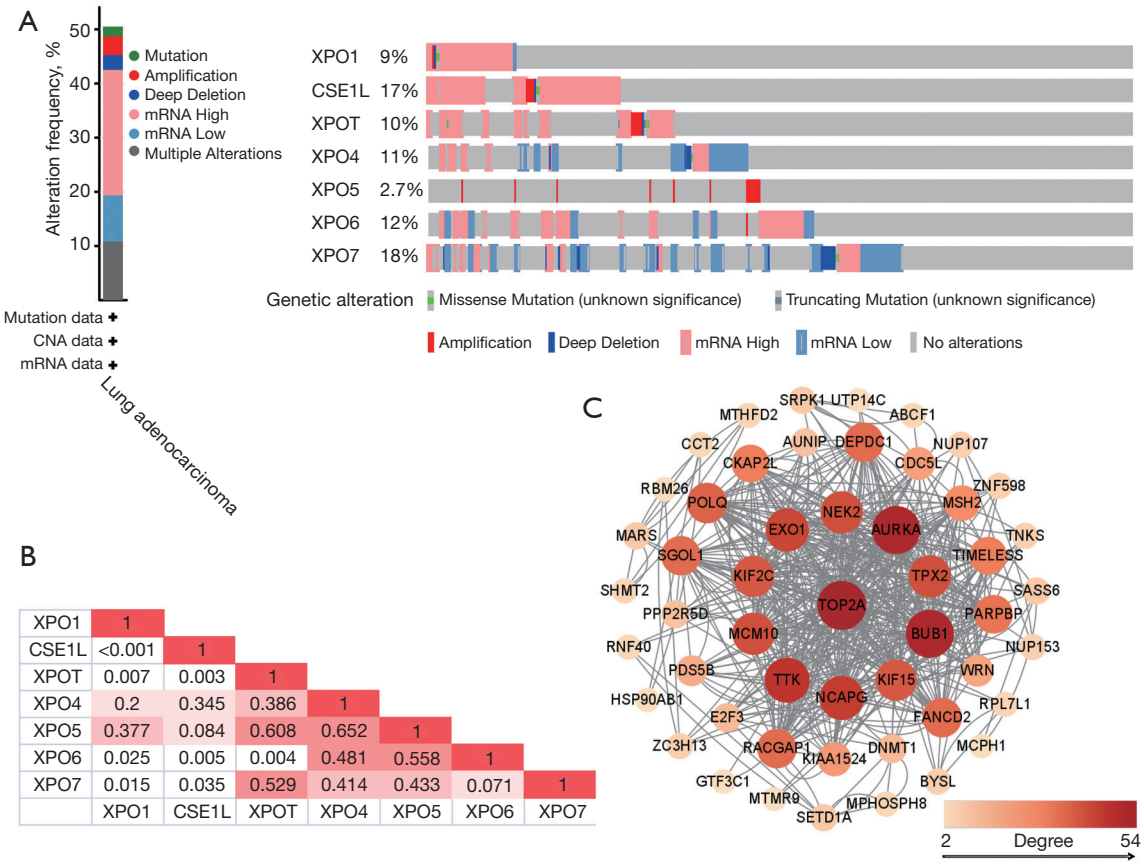
Figure 4 Correlation between exportins and prognosis of patients with LUAD and LUSC in the Kaplan-Meier plotter database. (A) Correlation between *XPO1* and OS, PFS in patients suffering from LUAD and LUSC. (B) Correlation between *CSE1L* and OS, PFS in patients suffering from LUAD and LUSC. (C) Correlation between *XPOT* and OS, PFS in patients suffering from LUAD and LUSC. (D) Correlation between *XPO4* and OS, PFS in patients suffering from LUAD and LUSC. (E) Correlation between *XPO5* and OS, PFS in patients suffering from LUAD and LUSC. (F) Correlation between *XPO6* and OS, PFS in patients suffering from LUAD and LUSC. (G) Correlation between *XPO7* and OS, PFS in patients suffering from LUAD and LUSC. P<0.05 implies a statistically considerable variation. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OS, overall survival; PFS, progression-free survival.

Table 2 The Prognostic values of exportins in patients with LUAD and LUSC (Kaplan-Meier plotter)

Exportin	Histology	OS				PFS			
		Cases	HR	95% CI	P value	Cases	HR	95% CI	P value
XPO1	Overall	1,925	0.79	0.68–0.92	0.0021	982	0.54	0.43–0.71	1.00E-06
208775_at	LUAD	719	0.27	0.19–0.38	1.50E–15	461	0.55	0.4–0.75	0.00017
	LUSC	524	1.07	0.84–1.35	0.6	141	1.89	1.12–3.2	0.016
CSE1L	Overall	1,925	1.19	1.05–1.35	0.0074	982	1.24	0.99–1.55	0.058
210765_at	LUAD	719	1.35	1.04–1.73	0.021	461	0.7	0.51–0.96	0.026
	LUSC	524	0.58	0.66–1.08	0.19	141	1.67	1–2.78	0.048
XPOT	Overall	1,925	1.18	1.04–1.35	0.014	982	0.74	0.6–0.9	0.0025
212160_at	LUAD	719	0.58	0.46–0.74	4.60E–06	461	0.69	0.5–0.95	0.022
	LUSC	524	0.89	0.67–1.18	0.42	141	1.79	1.05–3.05	0.031
XPO4	Overall	1,925	1.44	1.25–1.68	2.90E–07	982	1.77	1.45–2.18	2.80E-08
218479_s_at	LUAD	719	0.86	0.68–1.08	0.19	461	1.59	1.15–2.2	0.0051
	LUSC	524	1.34	1.04–1.72	0.025	141	2.42	1.34–4.36	0.0025
XPO5	Overall	1,144	1.9	1.6–2.27	3.80E–13	596	1.74	1.32–2.28	5.90E-05
223055_s_at	LUAD	672	1.76	1.38–2.24	3.40E–06	443	1.9	1.38–2.63	7.40E-05
	LUSC	271	1.59	1.09–2.32	0.014	141	1.61	0.97–2.69	0.065
XPO6	Overall	1,925	1.19	1.03–1.37	0.02	982	1.68	1.39–2.04	8.10E-08
214784_x_at	LUAD	719	1.47	1.12–1.93	0.0053	461	1.66	1.22–2.28	0.0013
	LUSC	524	0.82	0.63–1.07	0.14	141	1.47	0.84–2.56	0.17
XPO7	Overall	1925	1.71	1.45–2.02	1.20E–10	982	1.96	1.61–2.38	8.90E-12
208459_s_at	LUAD	719	2.24	1.76–2.84	1.10E–11	461	2.04	1.47–2.83	1.30E-05
	LUSC	524	0.84	0.64–1.1	0.21	141	1.54	0.9–2.63	0.11

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

enrichment analysis and KEGG pathway enrichment in DAVID. As shown in *Figure* 7 and Table S2, it was found that biological processes of mitotic nuclear division (GO:0007067), cell population proliferation (GO:0008283), regulation of signal transduction by p53 class mediator (GO:1901796), G2/M transition of mitotic cell cycle (GO:0000086), and G2/M transition of mitotic cell cycle (GO:0000086) were regulated by exportins mutations in NSCLC (*Figure* 7*A*). Moreover, the cellular components of nucleoplasm (GO:0005654), centrosome (GO:0005813), kinetochore (GO:0000776), nucleolus (GO:0005730), and spindle pole (GO:0000922) were significantly associated with the exportin alterations (*Figure* 7*B*). Furthermore, exportins mutations also prominently affected the molecular functions of protein binding (GO:0005515), adenosine triphosphate (ATP) binding (GO:0005524), poly (A) RNA binding (GO:0044822), DNA binding (GO:0003677 and ATP hydrolysis activity (GO:0016887) (*Figure* 7*B*). In KEGG analysis, oocyte meiosis was closely related to the function of exportins in NSCLC, and cell cycle pathway was also found to be related to exportins, although nonsignificantly (P>0.05) (Table S2). The above results suggest that the functions of exportins mutations and their coexpressed genes may be associated with cell proliferation, cell division, regulation of signal transduction by p53 class mediator, and cell cycle, among other processes.



XPO1	1						
CSE1L	<0.001	1					
XPOT	0.007	0.003	1				
XPO4	0.2	0.345	0.386	1			
XPO5	0.377	0.084	0.608	0.652	1		
XPO6	0.025	0.005	0.004	0.481	0.558	1	
XPO7	0.015	0.035	0.529	0.414	0.433	0.071	1
	XPO1	CSE1L	XPOT	XPO4	XPO5	XPO6	XPO7

Figure 5 Mutations, interaction analysis, and the neighbor gene network of the differentially expressed exportins in patients with LUAD and LUSC from cBioPortal, STRING, and Cytoscape.(A) Mutations in exportins of patients with LUAD and LUSC in cBioPortal database. (B) Correlation heat map of exportins of LUAD and LUSC patients in the cBioPortal database. (C) PPI interaction network diagram of 69 co-expressed genes significantly associated with exportins mutations in STRING database. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; PPI, protein-protein interaction.

The relationship between exportins expression levels and immune infiltration levels in LUAD and LUSC

Immunity is closely related to the occurrence and development of tumors. Therefore, the relationship between the transcriptional levels of exportins and the levels of immune infiltration in LUAD and LUSC was evaluated using the TIMER online analysis tool. It was found that exportins were associated with immune cell infiltration. As shown in *Figure 8*, *XPO1* was negatively correlated with B cells infiltration and positively correlated with neutrophils infiltration. *CSE1L* was negatively correlated with B cells infiltration, CD4+ T cells infiltration, macrophages infiltration, neutrophils infiltration, and dendritic cells infiltration. Additionally, *XPO4* and *XPO6* were positively correlated with CD4+ T cells infiltration, macrophages infiltration, neutrophils infiltration, and dendritic cells infiltration; *XPO4* was positively correlated with CD8+ T cells infiltration; *XPO6* was also positively correlated with B cells infiltration; and *XPO7* was positively correlated with CD8+ T cells infiltration, macrophages infiltration, and neutrophils infiltration. However, it was discovered that *XPO5* was only positively correlated with CD8+ T cells infiltration. These studies indicated that the expression levels of exportins were correlated with the levels of immune infiltration in LUAD and LUSC.

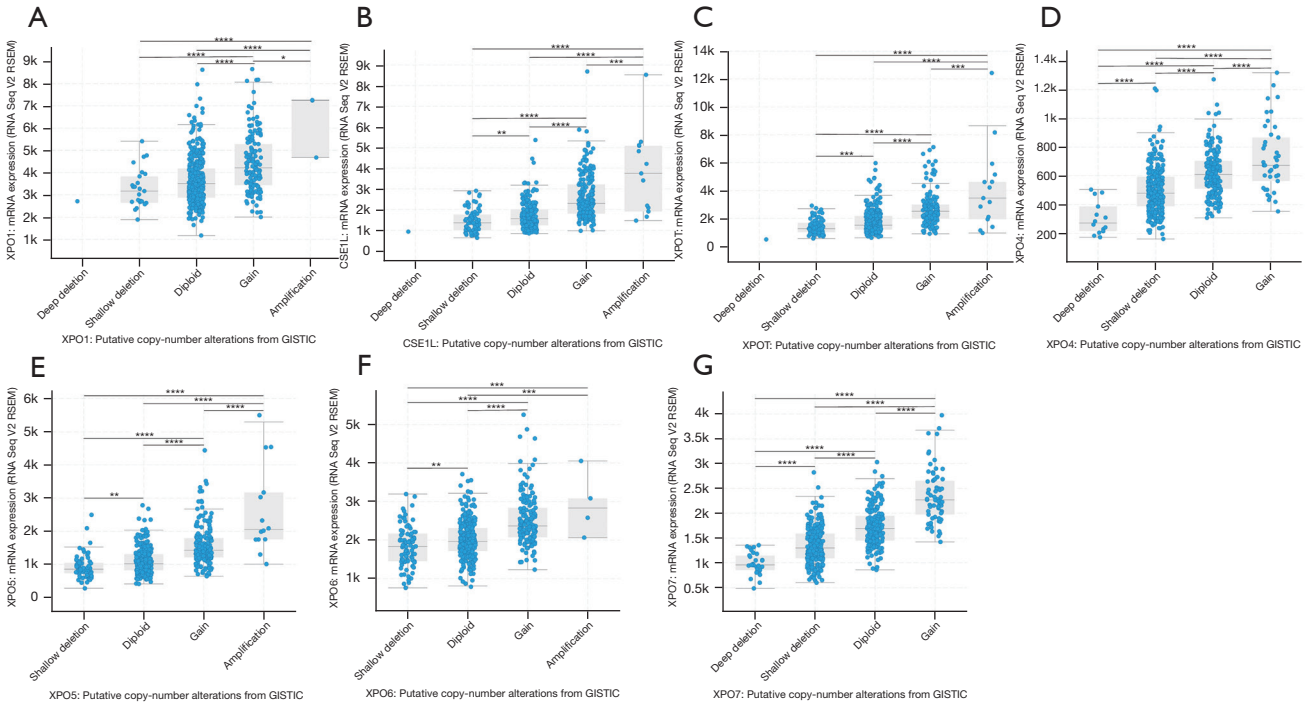


Figure 6 Correlation between mRNA expression and copy number alterations of exportins in LUAD and LUSC in the cBioPortal database. (A) Correlation between mRNA expression and copy number alterations of *XPO1*. (B) Correlation between mRNA expression and copy number alterations of *CSE1L*. (C) Correlation between mRNA expression and copy number alterations of *XPOT*. (D) Correlation between mRNA expression and copy number alterations of *XPO4*. (E) Correlation between mRNA expression and copy number alterations of *XPO5*. (F) Correlation between mRNA expression and copy number alterations of *XPO6*. (G) Correlation between mRNA expression and copy number alterations of *XPO7*. *, P<0.05, **, P<0.01, ***, P<0.001, ****, P<0.0001. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GISTIC, genomic identification of significant targets in cancer.

Analysis of transcription factors and miRNA targets of exportins in patients with LUAD and LUSC

Finally, the transcription factor targets (TFs) and mRNA targets of differentially expressed exportins from the LinkedOmics database were analyzed. The first 3 enriched miRNA targets of each exportins are shown in *Table 3*. It was found that miR-433 (ATCATGA) was the common target of *XPO1* and *CSE1L*, and miR-26A, miR-26B (TACTTGA) were the common targets of *XPOT* and *XPO4*. Similarly, the common targets of *XPO4* and *XPO7* were miR-181A, miR-181B, miR-181C, miR-181D (TGAATGT), while miR-452 (GAGACTG) was the common target of *XPO5* and *XPO7*. Finally, it was found that *E2F1* appears to be the key TF regulated by exportins (*Table 4*).

Discussion

Exportin members have been partially shown to be associated with a variety of different tumors, such as liver cancer (9), breast cancer (10,31), gastric cancer (11), and lung cancer (12). One study found that the stable overexpression of *CRM1* in human bronchial epithelial cells leads to malignant cellular transformation (14). *XPO1* knockout enhanced the sensitivity of SCLC cells to chemotherapy, and *XPO1* inhibition showed synergistic effect with first-line and second-line chemotherapy. Selinur is a small molecule *XPO1* inhibitor, which can significantly inhibit tumor growth in patients with SCLC in combination with cisplatin or ibrutinib (32). In our study, the expression level, genetic variation, immune infiltration, biological function of

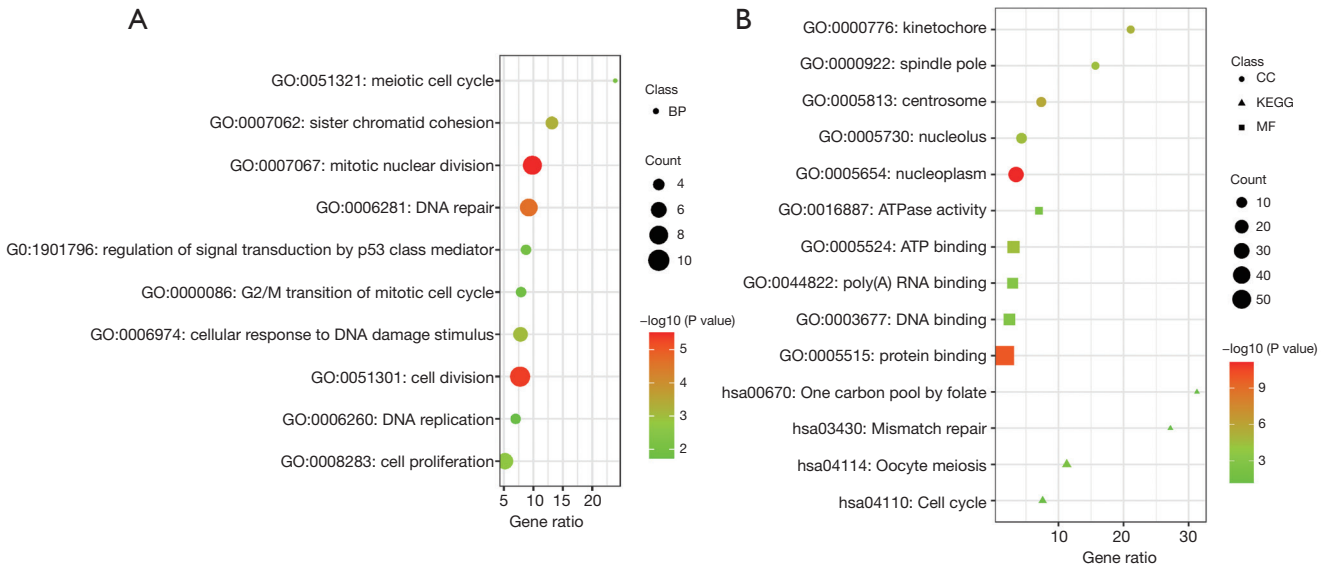


Figure 7 GO functional enrichment and KEGG pathway analysis of 69 co-expressed genes significantly associated with exportins in the BioPortal and DAVID databases. (A) BP analysis results. (B) CC, MF and KEGG analysis results. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cell component; MF, molecular function.

different exportins, and their relationship with the prognosis of patients with LUAD and LUSC were comprehensively analyzed by bioinformatics for the first time.

XPO1 was identified as a gene required to modify chromosome structures or regulate the maintenance of high-order chromosome structures in *Schizosaccharomyces pombe*, so it was originally named *CRM1* (chromosome region maintenance 1). Subsequently, it was found that it had homology with importin beta-like transporter and could specifically interact with NES motif and Ran GTPase to mediate the nuclear export of protein and mRNA in *Saccharomyces cerevisiae*, and it was finally renamed *XPO1* (export 1) (33). As a well-defined nuclear export protein, *XPO1* is responsible for exporting a variety of proteins and RNAs [including ribosomal RNA, small nuclear RNA, mRNA, microRNA, and transfer RNA (tRNA)] (34), and plays an important role in regulating mitosis and chromosome structure (35), which indicates that many carcinogenic mechanisms may involve *XPO1*. In eukaryotic cells, *XPO1* has been considered to be an important exporter of most tumor suppressor proteins, including *p53*, *p21*, *PI3K/Akt*, *p27*, and *BRCA1/2*, all of which are important targets for tumorigenesis (36). *XPO1* has been shown to be highly expressed in many types of malignant tumors and associated with poor prognosis, including ovarian cancer (37), prostate cancer (38), osteosarcoma (39), leukemia (40), multiple myeloma (41,42), and glioma (43). One study demonstrated *CRM1* overexpression in lung cancer, with *CRM1* being frequently overexpressed in NSCLC, especially adenocarcinoma and squamous cell carcinoma, which may be caused by the synergistic effect of *CRM1* overexpression and *p53* phosphorylation in cell malignant transformation. Meanwhile, inhibiting *CRM1* can improve the efficacy of cisplatin in the treatment of lung cancer (14). In our study, we found through database analysis that the transcriptional level of *XPO1* in patients with LUAD and LUSC was higher than that in normal tissues. Nagasaka *et al.* (44) retrospectively analyzed de-identified pathological and molecular information from 18,218 NSCLC samples to describe the prevalence of *XPO1* mutations and amplifications in NSCLC. Their study found that presence of *XPO1* pathogenic mutations was associated with a poor OS in both the entire NSCLC cohort and the adenocarcinoma subgroup. However, Li *et al.* (45) divided patients with NSCLC into a pure *XPO1* mutant, wild-type, and *XPO1-STK11/KEAP1* mutant groups and found that patients with pure *XPO1* mutations had longer survival. So, in order to study the relationship between *XPO1* and the

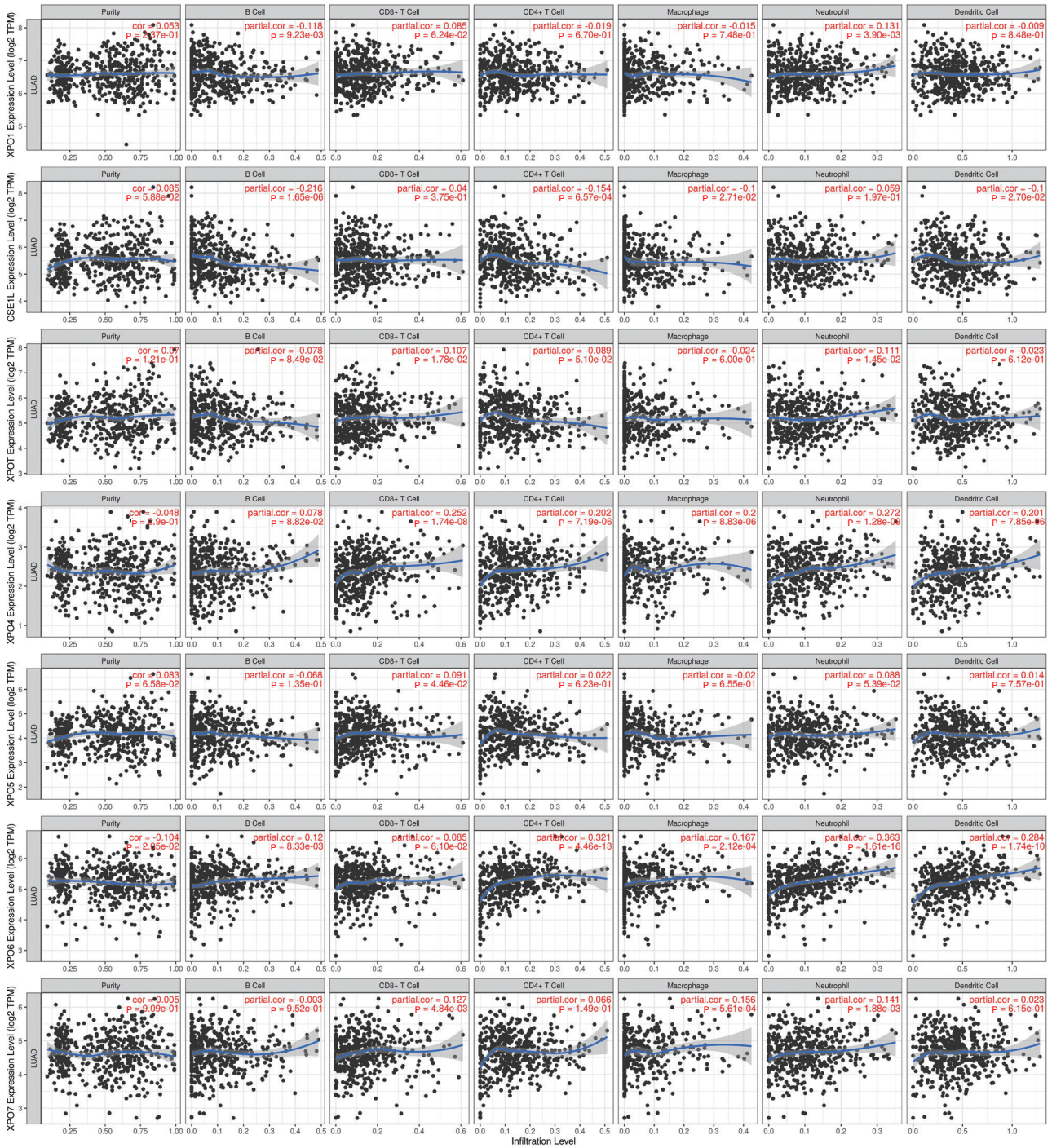


Figure 8 Correlation between exportins and infiltration of different immune cells in the TIMER database. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

Table 3 The miRNA target network of exportins in LUAD and LUSC from the LinkedOmics database

Gene	Enriched miRNA target	Leading edge, n	P value
XPO1	ATCATGA, MIR-433	45	<0.0001
	ATTCTTT, MIR-186	75	<0.0001
	CATGTAA, MIR-496	47	<0.0001
CSE1L	ATAACCT, MIR-154	20	<0.0001
	GAGCCTG, MIR-484	37	<0.0001
	ATCATGA, MIR-433	35	<0.0001
XPOT	AGTCTTA, MIR-499	26	<0.0001
	TACTTGA, MIR-26A, MIR-26B	69	<0.0001
	TAGCTTT, MIR-9	63	0.003
XPO4	TGAATGT, MIR-181A, MIR-181B, MIR-181C, MIR-181D	156	<0.0001
	CAGTGTT, MIR-141, MIR-200A	112	<0.0001
	TACTTGA, MIR-26A, MIR-26B	109	<0.0001
XPO5	GTTTGTT, MIR-495	82	<0.0001
	GTGGTGA, MIR-197	28	<0.0001
	GAGACTG, MIR-452	42	<0.0001
XPO6	CAGCAGG, MIR-370	43	<0.0001
	AGGGCCA, MIR-328	25	<0.0001
	CACTGCC, MIR-34A, MIR-34C, MIR-449	94	<0.0001
XPO7	CTTGTAT, MIR-381	75	<0.0001
	TGAATGT, MIR-181A, MIR-181B, MIR-181C, MIR-181D	180	<0.0001
	GAGACTG, MIR-452	27	<0.0001

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

prognosis of lung cancer, we used Kaplan-Meier plotter to analyze the findings that in patients with NSCLC, increased *XPO1* transcript level was associated with better OS and PFS. We believe that this may be related to the *XPO1* mutation, which renders NSCLC more sensitive to treatment and thus improves prognosis. Our study also confirmed that *XPO1* has a high mutation rate in LUAD, with a mutation rate of 9%. This finding suggests that *XPO1* may be a valuable prognostic marker for NSCLC, but further studies are still needed.

CSE1L, also known as human cellular apoptosis susceptibility and exportin *2* (*XPO2*), has been shown to be involved in the regulation of different cellular mechanisms, including mitotic spindle checkpoint, proliferation, and apoptosis (46). However, the role of *CSE1L* in lung cancer has rarely been studied. A previous study showed that *ACOT11* can promote the proliferation, migration, and invasion of lung cancer cells by binding with *CSE1L* (47). *CSE1L* can regulate the *MAPK* pathway and promote the development of lung cancer by interacting with p65 (15). Another study reported that *CSE1L* silencing impaired the cell proliferation, invasion, and migration of lung cancer cells, and that the expression of *STAT3* and *MET* protein was decreased; the apoptosis-related marker protein *c-PAR*P was induced, which indicated that cell proliferation and antiapoptosis may be regulated by *CSE1L* via the *MET*–*STAT3* pathway (48). In our study, ONCOMINE and UALCAN databases revealed that the transcriptional level of *CSE1L* in LUAD and LUSC was higher than that in normal tissues. HPA database also found that *CSE1L* was not expressed in normal lung tissue, but was moderately expressed in LUAD tissue. Additionally, Kaplan-Meier plotter analysis revealed that the high expression level of *CSE1L* was significantly linked with a worse OS but not

Table 4 The transcription factor target networks of exportins in LUAD and LUSC from the LinkedOmics database

Gene	Enriched transcription factor target	Leading edge, n	P value
XPO1	V$E2F_Q4	86	<0.0001
	V$E2F_Q6	86	<0.0001
	V$E2F1_Q6	84	<0.0001
CSE1L	V$E2F_Q6	99	<0.0001
	V$E2F1DP1_01	93	<0.0001
	V$E2F1DP2_01	93	<0.0001
XPOT	V$E2F_Q4	82	<0.0001
	V$E2F_Q6	82	<0.0001
	V$E2F1_Q6	85	<0.0001
XPO4	V$NFMUE1_Q6	66	<0.0001
	V$HTF_01	17	<0.0001
	V$STAT5A_04	71	<0.0001
XPO5	V$E2F_Q4	107	<0.0001
	V$E2F_Q6	108	<0.0001
	V$E2F1_Q6	108	<0.0001
XPO6	V$E2F_Q6	86	<0.0001
	V$E2F_Q4	86	<0.0001
	V$E2F1DP1_01	86	<0.0001
XPO7	V$HTF_01	17	<0.0001
	V$RREB1_01	45	<0.0001
	V$E2F_Q6	62	<0.0001

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

with PFS in patients with NSCLC.

XPOT and *XPO5* are Kap-β family of tRNA nuclear output receptors. *XPOT* is a specific nuclear export receptor of tRNA, and *XPO5* mainly exports miRNA to the cytoplasm, and thus the role of *XPO5* in tRNA export is considered to be secondary (49). Previous studies have reported the key roles of *XPOT* in the development and progression of various tumors, such as breast cancer (50) and human promyelocytic leukemia (51). The overexpression of *XPOT* was reported to lead to more aggressive and chromosomally unstable breast cancers (50), while the elevated expression of *XPOT* was associated with cell proliferation in human promyelocytic leukemia (51). In addition, Pan *et al.* (52) have found in recent years that knocking out XPOT through small interfering RNA can inhibit the proliferation and migration of neuroblastoma cells. However, the role of *XPOT* in lung cancer has not been previously explored. Regarding XPO5, Özdaş *et al.* (53) found that the mRNA and protein levels of XPO5 were upregulated in metastatic cells of head and neck squamous cell carcinoma (HNSCC), and silencing XPO5 resulted in reduced cell proliferation, delayed wound healing, and increased Caspase-3 enzyme activity in HNSCC cell lines. A previous report indicated that the miR-single-nucleotide polymorphism (*SNP*) of rs11077 in the miRNA processing gene *XPO5* was associated with the recurrence of resected NSCLC, which suggests that the miR-SNP in the miRNA processing mechanism gene of *XPO5* is involved in the prognosis of NSCLC (54). In our study, the ONCOMINE database analysis results in transcriptional levels of *XPOT* and *XPO5* in NSCLC being significantly higher than those in normal lung tissue, and these results were corroborated by the UALCAN data set. Immunohistochemical staining demonstrated that *XPO5* was moderately expressed in lung cancer tissues, but not in normal tissues. However, *XPOT* expression was not found in lung cancer or normal tissues. Furthermore, Kaplan-Meier plotter analysis indicated that the high expression of *XPO5* was associated with worse OS. Therefore, we believe that *XPO5* may be an important indicator for predicting the prognosis of patients with NSCLC.

XPO4 is a bidirectional nuclear transporter involved in the nuclear export of translation initiation factor *EIF5A* and transcriptional regulator *Smad3* (55,56). Smad3, an effector of tumor growth factor (TGF)-β signal transduction, can promote or inhibit the growth of cancer cells (57). *EIF-5A* has been identified as the initiation gene of eukaryotic translation and to be encoded by 2 highly related genes (*EIF5A1* and *EIF5A2*). Although the mechanism of *EIF5A1* causing cancers has not been fully clarified, a study found that the *EIF5A1* and *EIF5A2* proteins are overexpressed in some human tumors (58). At present, there are no more reports on the relationship of *XPO4* with any cancers. We found that the ONCOMINE data set did not record the expression of *XPO4* transcription level in lung cancer and that the HPA data set did not contain the expression of *XPO4* protein in lung cancer or normal tissues. Due to the lack of relevant studies on *XPO4*, its role in the occurrence and development of NSCLC cannot be confirmed.

Similar to *XPO4*, there are relatively few reports on *XPO6/7* in NSCLC. The UALCAN data set in our study revealed that the transcriptional levels of *XPO6* and *XPO7*

in LUAD and LUSC were higher than those in normal tissues. Immunohistochemical staining revealed that *XPO6* and *XPO7* were low and moderately expressed in lung cancer tissues but not in normal tissues. Survival analysis demonstrated that the high expressions of *XPO6* and *XPO7* were associated with worse OS and PFS in patients with LUAD and LUSC. Based on these results, we speculate that *XPO6/7* may be a potential biomarker for patients with NSCLC, but this needs to examined in further study.

Furthermore, it was found that exportins had a high mutation rate (50.48%) in patients with LUAD and LUSC. The highest mutation was mRNA upregulation, and a co-occurring relationship was found between differentially expressed exportins, which indicated that these genes play a synergistic role in the occurrence and development of LUAD and LUSC. A network of coexpression genes significantly associated with exportins mutations was constructed, and then their functions were analyzed through GO enrichment analysis and KEGG pathway enrichment. Our studies have found that the functions of these proteins are mainly related to cell proliferation, cell division, regulation of signal transduction by *p53* class mediator, cell cycle, and other processes. These functions are significantly related to the occurrence and development of tumors. These results provide clues for the rational development of multitarget and exportin-mediated targeted therapy.

Recent data have suggested that as an important determinant of prognosis and response to immunotherapy immune cell (59), infiltration may affect tumor progression and recurrence (60). Tumor-derived microbubbles (TMVs) are extracellular vesicles released from tumor cells, which are now understood to promote the communication between tumor and surrounding microenvironment. A research showed that the interaction between *ARF6-GTP* and *XPO5* transported a pre-miRNA complex to sites of TMV biogenesis for inclusion as TMV cargo. This indicates that *XPO5* is related to tumor microenvironment (61). Our study conducted an in-depth investigation of the infiltration of 6 immune cells (B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells) related to exportins. We found that the expression levels of exportins were found to be associated with the levels of immune infiltration in LUAD and LUSC, which can reflect the immune status indicators of patients with LUAD and LUSC.

We also sought to characterize the transcription miRNA targets of the differentially expressed exportins and found that several miRNAs (miR-433, miR-26, miR-181 and miR-452) were associated with differentially expressed exportins. These small noncoding RNAs mainly promote tumor growth, invasion, angiogenesis, and immune escape by controlling the expression of their target mRNAs. Moreover, tumor miRNA distribution can define related subtypes, patient survival, and treatment response, and it plays an important role in cancer biology (62). It was found that decreased miR-433 expression was strongly associated with the tumor-node-metastasis stage and lymph node metastasis of patients with NSCLC (63). miR-433 reduces cell proliferation and invasive phenotype in patients with NSCLC by mediating *Smad2* and *Id-1* (64). Furthermore, miR-181 and miR-452 also play an important role in epithelial-mesenchymal transition (EMT), proliferation, invasion, and metastasis of lung cancer cells (65,66). Our analysis suggested that differentially expressed exportins may play a role through these miRNAs to regulate the occurrence and development of LUAD and LUSC.

By mining the TF targets of differentially expressed exportins, it was found that E2F1 may the key TF regulated by exportins. *E2F1* is one of the key links in the cell cycle regulation network (67) and is a central player in cell cycle progression, DNA-damage response, and apoptosis (68). A study proven that *E2F1* promotes EMT by regulating *ZEB2* in SCLC (69). In SCLC cells, *ILF2* interacts with E2F1 and regulates the transcriptional activity of *E2F1*, exerting a carcinogenic effect (70). Our analysis revealed that *E2F1* is a promising regulatory target of exportins, as differentially expressed exportins may regulate cell genesis and development by interacting with E2F1 in LUAD and LUSC.

Conclusions

This study is the first to systematically demonstrated the association between exportins and LUAD and LUSC. We further showed that the transcriptional and protein expression levels of *CSE1L* and *XPO1/5/6/7* were increased in patients with LUAD and LUSC, and the increased transcriptional levels of *CSE1L* and *XPO5/6/7* were associated with worse prognosis, with an increased transcriptional level of *XPO1* suggesting a better prognosis. These results point to *CSE1L* and *XPO1/5/6/7* as potential prognostic biomarkers for the survival of patients with LUAD and LUSC. Furthermore, it was found that the expression of exportins was significantly correlated with the infiltration of a variety of immune cells. Differentially expressed exportins regulate the occurrence and development of LUAD and LUSC by involving a variety of

miRNAs along with transcription factor *E2F1*. Our study may provide novel insights into the selection of prognostic biomarkers of exportins in LUAD and LUSC. However, our research still has some limitations. Since all the data in our study are from the public databases, there is no cellular function researches or animal experiments *in vivo* and *in vitro* of exportins, and then we will start to carry out relevant experimental researches to promote the clinical application of exportins as potential biomarkers of LUAD and LUSC.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-228/rc

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-228/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-228/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014;511:543-50.
2. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018;553:446-54.
3. Chen Z, Chen X, Lei T, et al. Integrative Analysis of NSCLC Identifies LINC01234 as an Oncogenic lncRNA that Interacts with HNRNPA2B1 and Regulates miR-106b Biogenesis. Mol Ther 2020;28:1479-93.
4. Muqbil I, Kauffman M, Shacham S, et al. Understanding XPO1 target networks using systems biology and mathematical modeling. Curr Pharm Des 2014;20:56-65.
5. Cautain B, Hill R, de Pedro N, et al. Components and regulation of nuclear transport processes. FEBS J 2015;282:445-62.
6. Xu D, Grishin NV, Chook YM. NESdb: a database of NES-containing CRM1 cargoes. Mol Biol Cell 2012;23:3673-6.
7. Chook YM, Süel KE. Nuclear import by karyopherin-βs: recognition and inhibition. Biochim Biophys Acta 2011;1813:1593-606.
8. Soniat M, Chook YM. Nuclear localization signals for four distinct karyopherin-beta nuclear import systems. Biochem J 2015;468:353-62.
9. Lin J, Hou Y, Huang S, et al. Exportin-T promotes tumor proliferation and invasion in hepatocellular carcinoma. Mol Carcinog 2019;58:293-304.
10. Tai CJ, Shen SC, Lee WR, et al. Increased cellular apoptosis susceptibility (CSE1L/CAS) protein expression promotes protrusion extension and enhances migration of MCF-7 breast cancer cells. Exp Cell Res 2010;316:2969-81.
11. Sun YQ, Xie JW, Xie HT, et al. Expression of CRM1 and CDK5 shows high prognostic accuracy for gastric cancer. World J Gastroenterol 2017;23:2012-22.
12. Geng JQ, Wang XC, Li LF, et al. MicroRNA-related single-nucleotide polymorphism of XPO5 is strongly correlated with the prognosis and chemotherapy response in advanced non-small-cell lung cancer patients. Tumour Biol 2016;37:2257-65.
13. Hao J, Chiang YT, Gout PW, et al. Elevated XPO6 expression as a potential prognostic biomarker for prostate cancer recurrence. Front Biosci (Schol Ed) 2016;8:44-55.
14. Gao W, Lu C, Chen L, et al. Overexpression of CRM1: A Characteristic Feature in a Transformed Phenotype of Lung Carcinogenesis and a Molecular Target for Lung Cancer

Adjuvant Therapy. J Thorac Oncol 2015;10:815-25.
15. Lin HC, Li J, Cheng DD, et al. Nuclear export protein CSE1L interacts with P65 and promotes NSCLC growth via NF-κB/MAPK pathway. Mol Ther Oncolytics 2021;21:23-36.
16. Sealfon SC, Chu TT. RNA and DNA microarrays. Methods Mol Biol 2011;671:3-34.
17. Guo P, Xu T, Jiang Y, et al. Identification of potential key molecular biomarkers in lung adenocarcinoma by bioinformatics analysis. Transl Cancer Res 2022;11:227-41.
18. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 2007;9:166-80.
19. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017;19:649-58.
20. Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. Science 2015;347:1260419.
21. Nagy Á, Lánczky A, Menyhárt O, et al. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Sci Rep 2018;8:9227.
22. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
23. Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013;41:D808-15.
24. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 2003;4:2.
25. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1-13.
26. Vasaikar SV, Straub P, Wang J, et al. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res 2018;46:D956-63.
27. Hou J, Aerts J, den Hamer B, et al. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. PLoS One 2010;5:e10312.
28. Stearman RS, Dwyer-Nield L, Zerbe L, et al. Analysis of orthologous gene expression between human pulmonary adenocarcinoma and a carcinogen-induced murine model. Am J Pathol 2005;167:1763-75.
29. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci U S A 2001;98:13790-5.
30. Garber ME, Troyanskaya OG, Schluens K, et al. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci U S A 2001;98:13784-9.
31. Zhu C, Kim SJ, Mooradian A, et al. Cancer-associated exportin-6 upregulation inhibits the transcriptionally repressive and anticancer effects of nuclear profilin-1. Cell Rep 2021;34:108749.
32. Quintanal-Villalonga A, Taniguchi H, Hao Y, et al. Inhibition of XPO1 Sensitizes Small Cell Lung Cancer to First- and Second-Line Chemotherapy. Cancer Res 2022;82:472-83.
33. Adachi Y, Yanagida M. Higher order chromosome structure is affected by cold-sensitive mutations in a Schizosaccharomyces pombe gene crm1+ which encodes a 115-kD protein preferentially localized in the nucleus and its periphery. J Cell Biol 1989;108:1195-207.
34. Hutten S, Kehlenbach RH. CRM1-mediated nuclear export: to the pore and beyond. Trends Cell Biol 2007;17:193-201.
35. Ho CY, Wong CH, Li HY. Perturbation of the chromosomal binding of RCC1, Mad2 and survivin causes spindle assembly defects and mitotic catastrophe. J Cell Biochem 2008;105:835-46.
36. Gravina GL, Senapedis W, McCauley D, et al. Nucleo-cytoplasmic transport as a therapeutic target of cancer. J Hematol Oncol 2014;7:85.
37. Landes JR, Moore SA, Bartley BR, et al. The efficacy of selinexor (KPT-330), an XPO1 inhibitor, on non-hematologic cancers: a comprehensive review. J Cancer Res Clin Oncol 2023;149:2139-55.
38. Gravina GL, Mancini A, Sanita P, et al. Erratum to: KPT-330, a potent and selective exportin-1 (XPO-1) inhibitor, shows antitumor effects modulating the expression of cyclin D1 and survivin in prostate cancer models. BMC Cancer 2016;16:8.
39. Jiang Y, Hou J, Zhang X, et al. Circ-XPO1 upregulates XPO1 expression by sponging multiple miRNAs to facilitate osteosarcoma cell progression. Exp Mol Pathol 2020;117:104553.
40. Lin KH, Rutter JC, Xie A, et al. P2RY2-AKT activation is a therapeutically actionable consequence of XPO1 inhibition in acute myeloid leukemia. Nat Cancer 2022;3:837-51.
41. Tai YT, Landesman Y, Acharya C, et al. CRM1 inhibition induces tumor cell cytotoxicity and impairs osteoclastogenesis in multiple myeloma: molecular mechanisms and therapeutic implications. Leukemia 2014;28:155-65.
42. Misund K, Hofste Op Bruinink D, Coward E, et al. Clonal evolution after treatment pressure in multiple myeloma: heterogenous genomic aberrations and transcriptomic convergence. Leukemia 2022;36:1887-97.

43. Liu X, Chong Y, Tu Y, et al. CRM1/XPO1 is associated with clinical outcome in glioma and represents a therapeutic target by perturbing multiple core pathways. J Hematol Oncol 2016;9:108.
44. Nagasaka M, Asad MFB, Al Hallak MN, et al. Impact of XPO1 mutations on survival outcomes in metastatic non-small cell lung cancer (NSCLC). Lung Cancer 2021;160:92-8.
45. Li X, Zou B, Wang S, et al. XPO1-mutant NSCLC without STK11/KEAP1 mutations may predict better survival to immunotherapy. J Transl Med 2021;19:421.
46. Zhu JH, Hong DF, Song YM, et al. Suppression of cellular apoptosis susceptibility (CSE1L) inhibits proliferation and induces apoptosis in colorectal cancer cells. Asian Pac J Cancer Prev 2013;14:1017-21.
47. Liang C, Wang X, Zhang Z, et al. ACOT11 promotes cell proliferation, migration and invasion in lung adenocarcinoma. Transl Lung Cancer Res 2020;9:1885-903.
48. Liu W, Zhou Z, Li Y, et al. CSE1L silencing impairs tumor progression via MET/STAT3/PD-L1 signaling in lung cancer. Am J Cancer Res 2021;11:4380-93.
49. Okamura M, Inose H, Masuda S. RNA Export through the NPC in Eukaryotes. Genes (Basel) 2015;6:124-49.
50. Vaidyanathan S, Thangavelu PU, Duijf PH. Overexpression of Ran GTPase Components Regulating Nuclear Export, but not Mitotic Spindle Assembly, Marks Chromosome Instability and Poor Prognosis in Breast Cancer. Target Oncol 2016;11:677-86.
51. Suzuki T, Koyama Y, Hayakawa S, et al. 1,25-Dihydroxyvitamin D3 suppresses exportin expression in human promyelocytic leukemia HL-60 cells. Biomed Res 2006;27:89-92.
52. Pan LJ, Chen JL, Wu ZX, et al. Exportin-T: A Novel Prognostic Predictor and Potential Therapeutic Target for Neuroblastoma. Technol Cancer Res Treat 2021;20:15330338211039132.
53. Özdaş S, Canatar İ, Özdaş T. Effects of Knockdown of XPO5 by siRNA on the Biological Behavior of Head and Neck Cancer Cells. Laryngoscope 2022;132:569-77.
54. Ding C, Li C, Wang H, et al. A miR-SNP of the XPO5 gene is associated with advanced non-small-cell lung cancer. Onco Targets Ther 2013;6:877-81.
55. Lipowsky G, Bischoff FR, Schwarzmaier P, et al. Exportin 4: a mediator of a novel nuclear export pathway in higher eukaryotes. EMBO J 2000;19:4362-71.
56. Kurisaki A, Kurisaki K, Kowanetz M, et al. The mechanism of nuclear export of Smad3 involves exportin 4 and Ran. Mol Cell Biol 2006;26:1318-32.
57. Teufel A, Staib F, Kanzler S, et al. Genetics of hepatocellular carcinoma. World J Gastroenterol 2007;13:2271-82.
58. Clement PM, Johansson HE, Wolff EC, et al. Differential expression of eIF5A-1 and eIF5A-2 in human cancer cells. FEBS J 2006;273:1102-14.
59. Liu X, Wu S, Yang Y, et al. The prognostic landscape of tumor-infiltrating immune cell and immunomodulators in lung cancer. Biomed Pharmacother 2017;95:55-61.
60. Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39:782-95.
61. Clancy JW, Zhang Y, Sheehan C, et al. An ARF6-Exportin-5 axis delivers pre-miRNA cargo to tumour microvesicles. Nat Cell Biol 2019;21:856-66.
62. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014;20:460-9.
63. Liu N, Liu Z, Zhang W, et al. MicroRNA 433 reduces cell proliferation and invasion in non small cell lung cancer via directly targeting E2F transcription factor 3. Mol Med Rep 2018;18:1155-64.
64. Li J, Chen M, Yu B. miR-433 suppresses tumor progression via Smad2 in non-small cell lung cancer. Pathol Res Pract 2019;215:152591.
65. He Z, Xia Y, Pan C, et al. Up-Regulation of MiR-452 Inhibits Metastasis of Non-Small Cell Lung Cancer by Regulating BMI1. Cell Physiol Biochem 2015;37:387-98.
66. Huang P, Ye B, Yang Y, et al. MicroRNA-181 functions as a tumor suppressor in non-small cell lung cancer (NSCLC) by targeting Bcl-2. Tumour Biol 2015;36:3381-7.
67. Polager S, Ginsberg D. E2F - at the crossroads of life and death. Trends Cell Biol 2008;18:528-35.
68. Denechaud PD, Fajas L, Giralt A. E2F1, a Novel Regulator of Metabolism. Front Endocrinol (Lausanne) 2017;8:311.
69. Wang T, Chen X, Qiao W, et al. Transcription factor E2F1 promotes EMT by regulating ZEB2 in small cell lung cancer. BMC Cancer 2017;17:719.
70. Zhao M, Liu Y, Chang J, et al. ILF2 cooperates with E2F1 to maintain mitochondrial homeostasis and promote small cell lung cancer progression. Cancer Biol Med 2019;16:771-83.

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Supplementary

Table S1 Seventy coexpression genes from cBioPortal significantly associated with exportins mutations

Gene	Correlated gene	Cytoband	Spearman correlation	P value	q value
XPO1	*MSH2*	2p21-p16.3	0.641877	2.24E-61	3.26E-57
	CIP2A	3q13.13	0.641211	3.26E-61	3.26E-57
	POLQ	3q13.33	0.634575	1.30E-59	8.67E-56
	SGO1	3p24.3	0.626094	1.27E-57	6.35E-54
	BUB1	2q13	0.625131	2.12E-57	8.47E-54
	KIF15	3p21.31	0.624534	2.91E-57	9.28E-54
	CKAP2L	2q14.1	0.624323	3.25E-57	9.28E-54
	SASS6	1p21.2	0.615186	3.76E-55	9.38E-52
	TOP2A	17q21.2	0.6122	1.72E-54	3.81E-51
	FANCD2	3p25.3	0.611041	3.08E-54	6.15E-51
CSE1L	*AURKA*	20q13.2	0.700738	1.48E-77	2.97E-73
	EXO1	1q43	0.69397	1.70E-75	1.70E-71
	MCM10	10p13	0.688483	7.23E-74	4.81E-70
	TPX2	20q11.21	0.687557	1.35E-73	6.74E-70
	DEPDC1	1p31.3	0.685141	6.81E-73	2.72E-69
	TTK	6q14.1	0.684709	9.07E-73	3.02E-69
	NEK2	1q32.3	0.684272	1.21E-72	3.46E-69
	AUNIP	1p36.11	0.683399	2.16E-72	5.40E-69
	KIF2C	1p34.1	0.68287	3.07E-72	6.81E-69
	NCAPG	4p15.31	0.681813	6.16E-72	1.23E-68
XPOT	*MARS1*	12q13.3	0.744383	2.24E-92	4.48E-88
	MTHFD2	2p13.1	0.721019	4.34E-84	4.33E-80
	SHMT2	12q13.3	0.706987	1.65E-79	1.10E-75
	RACGAP1	12q13.12	0.68551	5.32E-73	2.66E-69
	NUP107	12q15	0.657224	3.04E-65	1.04E-61
	PARPBP	12q23.2	0.657157	3.16E-65	1.04E-61
	TIMELESS	12q13.3	0.656922	3.64E-65	1.04E-61
	CCT2	12q15	0.64397	6.86E-62	1.71E-58
	DEPDC1	1p31.3	0.636069	5.72E-60	1.27E-56
	DENR	12q24.31	0.634317	1.50E-59	2.92E-56
XPO4	*MPHOSPH8*	13q12.11	0.689765	3.03E-74	6.06E-70
	AKAP11	13q14.11	0.645737	2.50E-62	2.50E-58
	ZC3H13	13q14.13	0.618045	8.64E-56	5.76E-52
	PDS5B	13q13.1	0.615207	3.72E-55	1.86E-51
	ZMYM2	13q12.11	0.611824	2.08E-54	8.29E-51

Table S1 (*continued*)

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Table S1 (*continued*)

Gene	Correlated gene	Cytoband	Spearman correlation	P value	q value
	RNF6	13q12.13	0.598986	1.18E-51	3.92E-48
	PAN3	13q12.2	0.588217	1.93E-49	5.42E-46
	UTP14C	13q14.3	0.587966	2.17E-49	5.42E-46
	NUBP2	16p13.3	-0.57778	2.26E-47	5.02E-44
	RBM26	13q31.1	0.573814	1.33E-46	2.65E-43
XPO5	*RPL7L1*	6p21.1	0.714013	9.10E-82	1.82E-77
	ABCF1	6p21.33	0.696581	2.78E-76	2.77E-72
	HSP90AB1	6p21.1	0.693764	1.96E-75	1.31E-71
	PPP2R5D	6p21.1	0.692099	6.17E-75	3.08E-71
	CDC5L	6p21.1	0.67489	5.49E-70	2.20E-66
	NUP153	6p22.3	0.673024	1.81E-69	6.01E-66
	E2F3	6p22.3	0.663314	7.66E-67	2.19E-63
	UHRF1BP1	6p21.31	0.661677	2.08E-66	5.19E-63
	SRPK1	6p21.31	0.65548	8.59E-65	1.91E-61
	BYSL	6p21.1	0.639986	6.49E-61	1.30E-57
XPO6	*GTF3C1*	16p12.1	0.633405	2.47E-59	4.83E-55
	ATXN2L	16p11.2	0.632172	4.84E-59	4.83E-55
	TBC1D10B	16p11.2	0.582673	2.48E-48	1.65E-44
	USP31	16p12.2	0.542899	5.74E-41	2.87E-37
	SETD1A	16p11.2	0.530376	7.59E-39	3.03E-35
	DNMT1	19p13.2	0.52713	2.61E-38	8.68E-35
	RNF40	16p11.2	0.52402	8.39E-38	2.39E-34
	ZNF646	16p11.2	0.497943	9.55E-34	2.39E-30
	KCTD5	16p13.3	0.497237	1.22E-33	2.70E-30
	ZNF598	16p13.3	0.492875	5.35E-33	1.07E-29
XPO7	*WRN*	8p12	0.766202	5.63E-101	1.12E-96
	CCAR2	8p21.3	0.751744	3.54E-95	3.54E-91
	TNKS	8p23.1	0.744803	1.56E-92	1.04E-88
	ENTPD4	8p21.3	0.715342	3.34E-82	1.67E-78
	CNOT7	8p22	0.67032	9.96E-69	3.98E-65
	CCDC25	8p21.1	0.658894	1.12E-65	3.72E-62
	MCPH1	8p23.1	0.656895	3.70E-65	1.06E-61
	PCM1	8p22	0.644464	5.18E-62	1.29E-58
	INTS9	8p21.1	0.643534	8.78E-62	1.95E-58
	MTMR9	8p23.1	0.641088	3.50E-61	6.98E-58

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Table S2 The enrichment analysis of differently expressed exportins and the 69 most frequently altered neighbor genes in LUAD and LUSC from the cBioPortal and DAVID databases

Category	Term	Count	P value	Fold enrichment
GOTERM_BP_DIRECT	GO: 0007067; mitotic nuclear division	9	3.03E-06	9.828824142
GOTERM_BP_DIRECT	GO: 0051301; cell division	10	4.47E-06	7.738248848
GOTERM_BP_DIRECT	GO; 0006281; DNA repair	8	2.21E-05	9.220041181
GOTERM_BP_DIRECT	GO: 0007062; sister chromatid cohesion	5	5.33E-04	13.14751018
GOTERM_BP_DIRECT	GO: 0006974; cellular response to DNA damage stimulus	6	9.42E-04	7.812655087
GOTERM_BP_DIRECT	GO: 0008283; cell proliferation	7	0.0020821	5.179975322
GOTERM_BP_DIRECT	GO: 0051321; meiotic cell cycle	3	0.0067577	23.89753321
GOTERM_BP_DIRECT	GO: 1901796; regulation of signal transduction by p53 class mediator	4	0.0103605	8.73673257
GOTERM_BP_DIRECT	GO: 0000086; G2/M transition of mitotic cell cycle	4	0.0135485	7.907699553
GOTERM_BP_DIRECT	GO: 0006260; DNA replication	4	0.0187911	6.989386056
GOTERM_CC_DIRECT	GO: 0005654; nucleoplasm	34	7.47E-12	3.477550287
GOTERM_CC_DIRECT	GO: 0005813; centrosome	11	1.85E-06	7.352699531
GOTERM_CC_DIRECT	GO: 0000776; kinetochore	6	8.79E-06	21.09259259
GOTERM_CC_DIRECT	GO: 0005730; nucleolus	13	3.15E-05	4.319428238
GOTERM_CC_DIRECT	GO: 0000922; spindle pole	6	3.72E-05	15.67431193
GOTERM_MF_DIRECT	GO: 0005515; protein binding	59	1.53E-10	1.717767889
GOTERM_MF_DIRECT	GO: 0005524; ATP binding	18	3.54E-05	3.079537853
GOTERM_MF_DIRECT	GO: 0044822; poly(A) RNA binding	13	0.0011181	2.945124406
GOTERM_MF_DIRECT	GO: 0003677; DNA binding	16	0.0014439	2.44466167
GOTERM_MF_DIRECT	GO: 0016887; ATPase activity	5	0.0054088	6.988325882
KEGG_PATHWAY	hsa04114; oocyte meiosis	4	0.0044007	11.26781327
KEGG_PATHWAY	hsa04110; cell cycle	3	0.0541553	7.564882698
KEGG_PATHWAY	hsa00670; one carbon pool by folate	2	0.0593964	31.26818182
KEGG_PATHWAY	hsa03430; mismatch repair	2	0.0680107	27.18972332

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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