Am J Cancer Res 2023;13(8):3686-3704
www.ajcr.us /ISSN:2156-6976/ajcr0150234

Original Article

Decoding SEC24 Homolog D, COPII coat complex component accuracy as a signature gene in three human cancers

Weiwei Du[1,2], Yang Sun[3], Wentao Ji[1,2], Tian Luo[1,4], Dandan Zhang[1], Weihong Guo[1], Jianping Liang[1], Yanhua Lv[1], Mengwei Dong[5], Kaixin Li[6]

[1]Department of Respiratory and Critical Care Medicine, Zhongshan City People's Hospital, Zhongshan, Guangdong, China; [2]The Zhuhai Campus of The Zunyi Medical University, Zhuhai, Guangdong, China; [3]Baotou Medical College, Baotou, Inner Mongolia, China; [4]Xinxiang Medical University, Xinxiang, Henan, China; [5]Hebei Provincial Hospital of Traditional Chinese Medicine, Shijiazhuang, Hebei, China; [6]Gansu University of Chinese Medicine, Lanzhou, Gansu, China

Received March 19, 2023; Accepted July 1, 2023; Epub August 15, 2023; Published August 30, 2023

Abstract: Although an increasing body of evidence supports the crucial role of the SEC24 Homolog D, COPII Coat Complex Component (SEC24D) gene in the initiation and progression of cancer, a comprehensive pan-cancer analysis of this gene is still lacking. In this study, we conducted an extensive investigation of SEC24D, aiming to elucidate its potential role and underlying mechanisms across multiple human tumors. Our analysis relied on data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. To validate our findings, we employed RNA sequencing (RNA-seq), targeted bisulfite sequencing (bisulfite-seq) molecular techniques. Our findings revealed elevated mRNA (Messenger RNA) and protein levels of SEC24D in different tumor tissues. However, the up-regulation of SEC24D was significantly correlated with shorter overall survival (OS), metastasis, and various clinical parameters in esophageal cancer (ESCA), lung adenocarcinoma (LUAD), and kidney renal papillary cell carcinoma (KIRP). Expression validation analysis via RNA-seq and targeted bisulfite-seq analyses, further confirmed the higher expression of SEC24D in LUAD cancer cell lines as compared to normal controls. The DNA methylation level of SEC24D was found to be decreased in ESCA, LUAD, and KIRP samples. DNA methylation analysis via bisulfite-seq analysis also validate the lower promoter methylation level of SE24D in LUAD cell lines relative to controls. Moreover, we observed a significant association between the elevated expression of SEC24D and the levels of infiltrating cells, such as B cells, neutrophils, macrophages, CD8+ T cells, and CD4+ T cells. Analysis of SEC24-related genes revealed that "Protein processing in endoplasmic reticulum, SNARE interaction in vesicular transport, Legionellosis, Pathogenic Escherichia coli infection" were mainly involved in the functional mechanism of SEC24D in ESCA, LUAD, and KIRP. Moreover, we also suggested a few valuable drugs (Acetaminophen, Acteoside, Cyclosporine, Polydatin, Estradiol, Estradiol, Quercetin) for treating ESCA, LUAD, and KIRP patients with respect to overexpressed SEC24D. To summarize, this comprehensive pan-cancer study investigated the association between SEC24D expression and clinical parameters in ESCA, LUAD, KIRP. The study provides valuable insights for further exploring the functional and therapeutic aspects of SEC24D and underscores its predictive significance in the carcinogenesis and prognosis of these specific cancer types.

Keywords: SEC24D, cancer, diagnostic, prognostic

Introduction

Cancer initiation and development involve a complex interplay of genetic and molecular alterations that disrupt normal cellular processes [1-3]. Due to its complex nature, cancer kill millions around the globe each year [4, 5]. Despite significant advancements in cancer research and treatment, challenges persist in accurately predicting disease progression, determining optimal treatment strategies, and monitoring treatment responses [6, 7]. The identification of reliable diagnostic and prognostic biomarkers for cancer patients remains an ongoing priority in the field of oncology. These biomarkers can aid in the early detection of cancer, enabling timely intervention and improved patient outcomes [8].

So far, numerous diagnostic and prognostic biomarkers have been identified and investigated in cancer patients [9, 10]. However, it is impor-

tant to acknowledge that many of these biomarkers have certain limitations. Some biomarkers lack sufficient sensitivity and specificity, leading to false-positive or false-negative results [11, 12]. While other biomarkers exhibit variability across different cancer types or individual patients, limiting their universal applicability [13, 14]. Therefore, it is crucial to uncover shared biomarkers that are common across different types of cancer in order to address the challenges posed by tumor heterogeneity.

SEC24 Homolog D, COPII Coat Complex Component (SEC24D) is a member of the SEC24 family, which is involved in the formation of coat protein complex II (COPII) vesicles that mediate protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus [15, 16]. Previous studies have indicated that SEC24D plays a critical role in protein sorting and trafficking within the secretory pathway, influencing cellular functions such as cell growth, proliferation, and differentiation [17, 18]. Despite growing evidence suggesting the critical function of SEC24D in cancer initiation and progression, a comprehensive and systematic pan-cancer analysis of this gene is still lacking. Therefore, the present study aims to investigate the diagnostic, prognostic potential, and detailed mechanisms of SEC24D across multiple human cancers using Bioinformatics and in vitro methodologies.

By decoding the accuracy and significance of SEC24D as a signature gene in esophageal cancer (ESCA), lung adenocarcinoma (LUAD), and kidney renal papillary cell carcinoma (KIRP), we aim to provide valuable insights into its functional role, underlying molecular mechanisms, and potential implications for therapeutic strategies. Such knowledge may contribute to the development of targeted therapies and personalized medicine approaches for patients with these cancers.

Materials and methods

Pan-cancer expression and survival analyses of SEC24D

The TIMER2 database (http://timer.cistrome.org/) serves is a crucial resource for cancer immunology research [19]. This database offers comprehensive and advanced analysis of gene expression and tumor immune infiltrates across diverse cancer types. With its user-friendly interface and robust algorithms, TIMER2 enables researchers to explore the gene expression, tumor microenvironment, assess immune cell abundance, and investigate the prognostic implications of immune infiltration. In the current study, we used TIMER2 platform for Pan-cancer expression analysis of SEC24D in different cancers.

UALCAN (https://ualcan.path.uab.edu/cgi-bin/ualcan-res-prot.pl) is a powerful web resource for cancer researchers, offering valuable insights into gene expression patterns across various tumor types [20]. With its user-friendly interface and comprehensive database, UALCAN enables scientists to explore and analyze RNA sequencing data, visualize differential gene expression, and gain crucial information about protein abundance and patient survival. Its accessibility and accuracy make UALCAN an indispensable tool in cancer research. In the current study, we used UALCAN platform to examine the expression of SEC24D in specific cancer patients, stratified based on clinical variables. This allowed us to gain valuable insights into the role of SEC24D in various cancers and its potential clinical implications.

KM plotter (https://kmplot.com/kmplot.com/analysis) is a robust online tool widely used in cancer research [21]. It provides a comprehensive platform for survival analysis, allowing researchers to assess the prognostic significance of genes in various cancer types. With its vast database and user-friendly interface, KM plotter empowers scientists to explore overall survival and relapse-free survival data, enabling valuable insights into cancer prognosis and potential therapeutic targets. During current research, we used this tool for the pan-cancer survival analysis of the SEC24D.

Expression analysis of SEC24D in metastatic cancer tissues

TNMplot (https://www.tnmplot.com/) is an indispensable tool for expression analysis in metastatic cancer tissues paired with control samples [22]. It provides researchers with a comprehensive platform to investigate the relationship between gene expression patterns and TNM staging in various cancer types. In the current study, this database was used to analyze the expression of SEC24D gene in meta-

static cancer tissue samples paired with normal controls.

Verification of SEC24D expression on additional the cancer genome Atlas and gene expression omnibus datasets

GEPIA (http://gepia.cancer-pku.cn/) and GENT2 (http://gent2.appex.kr/) are widely utilized web-based platforms in cancer research, offering comprehensive gene expression analysis across diverse tumor types [23, 24]. With their user-friendly interface and vast variety of TCGA datasets, these tools enable researchers to validate gene expression patterns and generate interactive plots.

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) is a vital resource for researchers seeking to access and analyze a vast collection of publicly available gene expression data [25]. With its extensive dataset encompassing diverse biological conditions and platforms, GEO provides an invaluable platform for investigating and validating gene expression patterns. In the present study, GEPIA and GENT2 databases were used to validate SEC24D expression on TCGA datasets, while GEO database was utilized to validate SEC24D expression of GEO datasets with the help of GEO2R tool [25].

Protein level expression analysis of SEC24D

The Human Protein Atlas (HPA, (https://www.proteinatlas.org/)) database serves as a valuable resource for researchers in the field of proteomics [26]. This database offers comprehensive and high-quality data on protein expression profiles across various human tissues and cell lines. With its vast collection of immunohistochemistry-based images and transcriptomic data, HPA enables the exploration of protein expression levels across cancer tissue samples paired with controls. For our study, we obtained SEC24D immunohistochemistry (IHC) expression data from various cancer tissues, as well as normal controls, through the HPA database. The IHC analysis employed a polyclonal antibody (HPA041626) specifically targeting SEC24D protein.

Univariate and multivariate survival analysis of SEC24D

In this work, we performed both univariate and multivariate survival analyses of SEC24D gene using GEO datasets through the PrognoScan tool (http://www.prognoscan.org/) [27]. By harnessing the comprehensive gene expression data available in GEO, we assessed the prognostic relevance of SEC24D gene in different clinical variables of the cancer patients.

Promoter methylation analysis of SEC24D

The MEXPRESS database (https://www.mxpresstrans.com/) is a valuable resource for researchers interested in exploring DNA methylation patterns across diverse genomic regions [28]. With its user-friendly interface and comprehensive dataset, MEXPRESS enables researchers to investigate the methylation status of specific genes or genomic regions in various cancer types. In this study, we conduct MEXPRESS to explore promoter methylation level of SEC24D across different can types.

Genetic alteration analysis of SEC24D

The cBioPortal database (https://www.cbioportal.org/) is an indispensable resource for cancer researchers, offering a comprehensive platform for the exploration and analysis of genomic data from various cancer types [29]. With its user-friendly interface and extensive dataset, cBioPortal enables scientists to investigate genetic alterations, identify potential driver mutations, and explore the complex landscape of cancer genomics. In this research, we utilized cBioPortal to analyze SEC24D genetic alterations across different cancers.

SEC24D and immune cell infiltration analysis

In the present study, we used TIMER2 database (http://timer.cistrome.org/) to perform Spearman correlation analysis between SEC24D expression and a variety of immune cells across different cancer patients.

Protein-protein interaction and enrichment of SEC24D

The STRING database (https://string-db.org/cgi/network) is an essential tool for investigating protein-protein interactions and functional networks [30]. With its extensive coverage of known and predicted protein associations, STRING enables researchers to explore the molecular interactions involved in various molecular pathways. This database was used in the current work to construct PPI network of

the SEC24D-associated proteins. DAVID tool is a widely used bioinformatics resource for enrichment analysis of large-scale gene lists [31]. Herein, this tool was used for the enrichment analysis of the SEC24D-associated genes.

Screening of SEC24D-assiciated drugs

The DrugBank database (https://go.drugbank.com/) is a vital resource for researchers in drug discovery and development [32]. This database provides comprehensive information on approved, investigational drugs, and drug-target interactions. We used DrugBank in the current study to screen SEC24D-associated drugs.

In vitro validation of SEC24D expression and methylation status

A total of six cell lines, including two cell lines (HCC15 and HCC44) derived from the primary lung cancer tissues, three cell lines (H-460, A549, and PC-9) derived from the metastatic lung cancer tissue, and one normal cell line (MRC-9) derided from the normal lung tissue, were purchased from the ATCC (American Type Culture Collection). The purchased cell lines were cultured in DMEM (HyClone), supplemented with 10% fetal bovine serum (FBS; TBD), 1% glutamine, and 1% penicillin-streptomycin in 5% CO_2 at 37°C. Total RNA extraction from all these three cells lines was done using TRIzol® reagent method [33], while total DNA was extracted via organic method [34]. Finally, RNA and DNA samples were sent to Beijing Genomics Institute (BGI) company for RNA-seq bisulfite-seq analysis.

After RNA-seq analysis, the gene expression values of the SEC24D were normalized using reads per kilo base million reads (RPKM) and fragments per kilo base million reads (FPKM). While, methylation values were normalized as beta values. The obtained FPKM, and beta values against hub genes in lung cancer and normal control cell line were compared to identify differences in the expression and methylation levels.

Statistics

The gene expression comparisons between cancer and normal sample groups were conducted using t-tests in TIMER2, UALCAN, GEPIA, GENT2, TNMplot, and GEO2R web resources. To evaluate the survival outcomes associated with gene expression levels in cancer patients, Kaplan-Meier analysis and log-rank tests were performed using KM plotter and Prognoscan web resources. Spearman test was employed for correlation analysis. A significance level of $P<0.05$ was chosen as the cutoff criteria to determine statistically significant results. This commonly used threshold allowed us to distinguish between findings that were likely due to chance and those that were considered statistically significant.

Results

Pan-cancer expression and survival analyses of SEC24D

In our study, we conducted a pan-cancer expression and survival analysis of the SEC24D via the UALCAN and KM plotter tools. Our findings revealed that SEC24D was consistently overexpressed in all twenty-two types of cancer samples when compared to their respective normal controls (**Figure 1A**). However, our survival analysis yielded intriguing results, as we observed that the overexpression of SEC24D was specifically associated with the reduced OS of only esophageal carcinoma (ESCA), lung adenocarcinoma (LUAD), and kidney renal papillary cell carcinoma (KIRP) patients (**Figure 1B-D**). This observation suggests that SEC24D may play a significant role in tumorigenesis across ESCA, LUAD and KIRP.

Expression analysis of SEC24D in metastatic ESCA, LUAD, and KIRP cancer tissues

In this part of our study, we focused on analyzing the expression of the SEC24D gene in normal controls, primary and metastatic tissues samples of three specific cancer types including, ESCA, LUAD, and KIRP. Our findings revealed a significant increase in SEC24D expression level in ESCA, LUAD, and KIRP metastatic tissues compared to primary ESCA, LUAD, and KIRP tissues, and normal controls samples (**Figure 2**). This observation suggests that SEC24D may play a crucial role in the metastatic progression of these cancers.

SEC24D expression landscape across different clinical variables

Next, we investigated the expression level of SEC24D across ESCA, LUAD, and KIRP samples

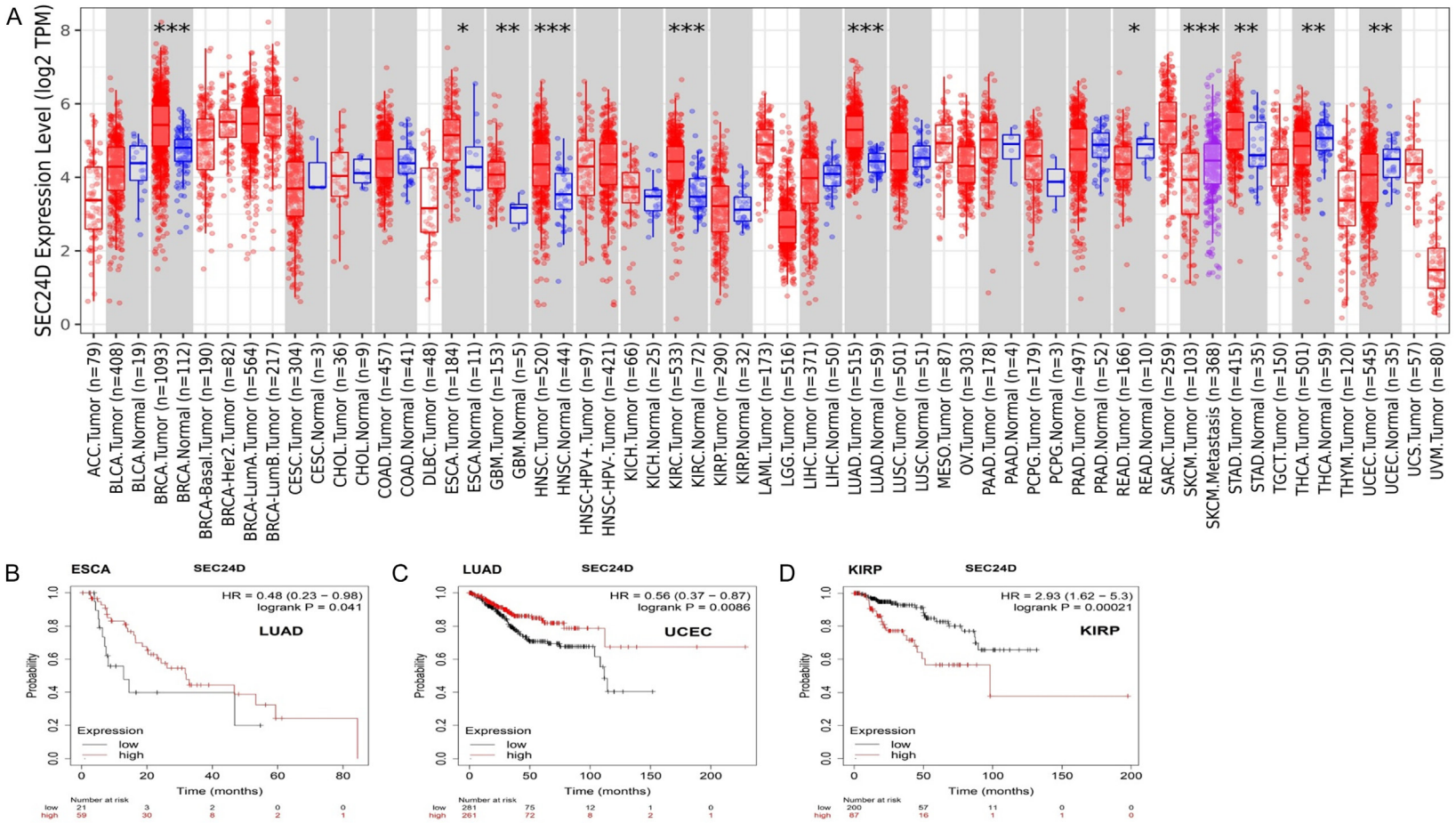

Figure 1. SEC24D expression profiling and survival analysis across different cancers. (A) SEC24D expression profile across various tumor samples and control samples, (B) SEC24D survival analysis across ESCA, (C) SEC24D survival analysis across ESCA, LUAD, and (D) SEC24D survival analysis across KIRP. A *p-value of <0.05 was selected as cutoff criterion.

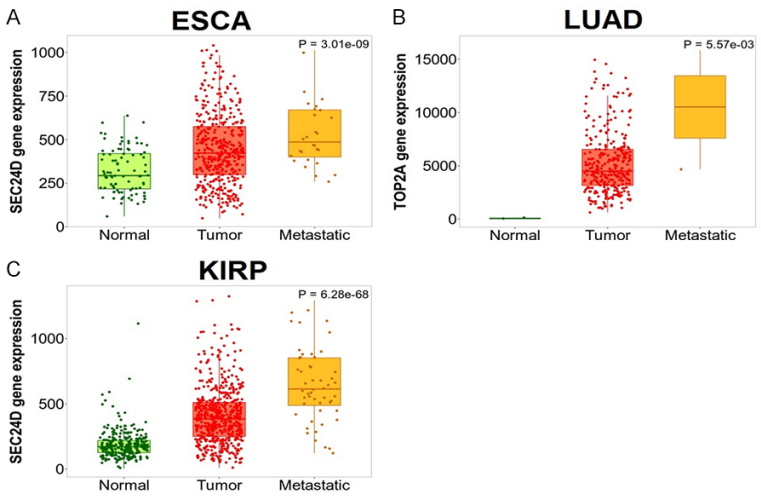

Figure 2. Expression profiling of SEC24D across primary and metastatic ESCA, LUAD, and KIRP samples paired with normal controls. (A) SEC24D expression profiling across primary and metastatic ESCA samples paired with controls, (B) SEC24D expression profiling across primary and metastatic LUAD samples paired with controls, and (C) SEC24D expression profiling across primary and metastatic KIRP samples paired with controls. A *p-value of <0.05 was selected as cutoff criterion.

of various clinical variables and normal control samples. Our findings consistently demonstrated that SEC24D gene expression was significantly up-regulated in ESCA, LUAD, and KIRP samples across different clinical variables (cancer stage, race, gender, and age) when compared to control samples (**Figure 3**). The consistent up-regulation of SEC24D suggests the potential utilization of this gene as a common biomarker in ESCA, LUAD, and KIRP patients of different clinical variables.

Expression validation of SEC24D gene in additional TCGA and GEO datasets

To further validate our findings, we extended expression of analysis of SEC24D by incorporating additional TCGA and GEO datasets of ESCA, LUAD, and KIRP from GEPIA, GENT2, and GEO databases. The inclusion of these independent datasets allowed us to assess SEC24D expression across a larger cohort of patients. Remarkably, our validation analysis consistently supported our initial observations, demonstrating significant up-regulation of SEC24D expression in ESCA, LUAD, and KIRP patients (**Figure 4**). The concordance of these results across multiple datasets strengthens the robustness and reliability of our findings, suggesting that SEC24D up-regulation is a common molecular alteration in these cancer types.

These validation results further underscore the potential significance of SEC24D as a promising biomarker in ESCA, LUAD, and KIRP.

Protein level expression analysis of SEC24D

In order to validate our findings at the protein level, we utilized the HPA database to investigate the expression of SEC24D in ESCA, LUAD, and KIRP samples. Our analysis revealed consistent up-regulation (Staining: high) of SEC24D protein expression in ESCA, LUAD, and KIRP samples relative to controls (Staining: low) (**Figure 5**), which corroborated our previous mRNA expression analysis findings. These results provide further support for the dysregulation of SEC24D in these three cancer types, indicating that the increased mRNA expression of SEC24D translates into elevated protein levels. The alignment of our protein-level validation with the mRNA expression data strengthens the reliability of our findings.

Univariate and multivariate survival analysis of SEC24D

Next, we conducted univariate and multivariate survival analyses via the Prognoscan database to further investigate the prognostic value of SEC24D in ESCA, LUAD, and KIRP. Our results revealed a significant association between higher expression of SEC24D and reduced overall survival (OS) in cancer patients across various clinical variables.

In the univariate survival analysis, we observed that patients with elevated SEC24D expression had significantly shorter OS compared to those with lower expression levels in ESCA, LUAD, and KIRP (**Figure 6**). Furthermore, in the multivariate survival analysis, after adjusting for other clinical variables (cancer stage and gender), the association between high SEC24D expression and poor OS remained significant (**Figure 6**). This suggests that SEC24D expression has a robust prognostic value and is an

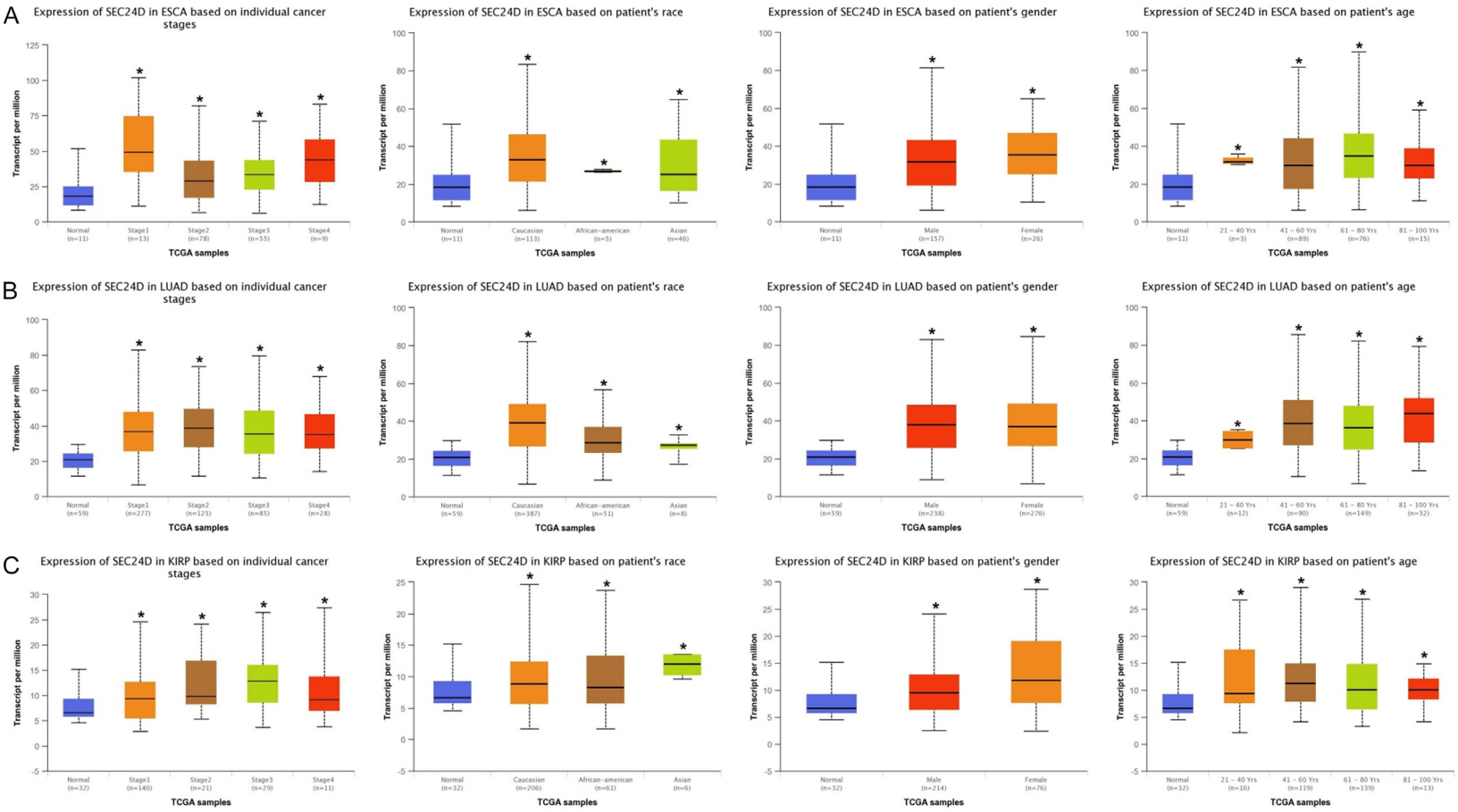

Figure 3. SEC24D expression profiling across ESCA, LUAD, and KIRP patients of different clinical parameters. (A) SEC24D expression profiling across ESCA patients of different clinical parameters, (B) SEC24D expression profiling across LUAD patients of different clinical parameters and (C) SEC24D expression profiling across KIRP patients of different clinical parameters. A *p-value of <0.05 was selected as cutoff criterion.

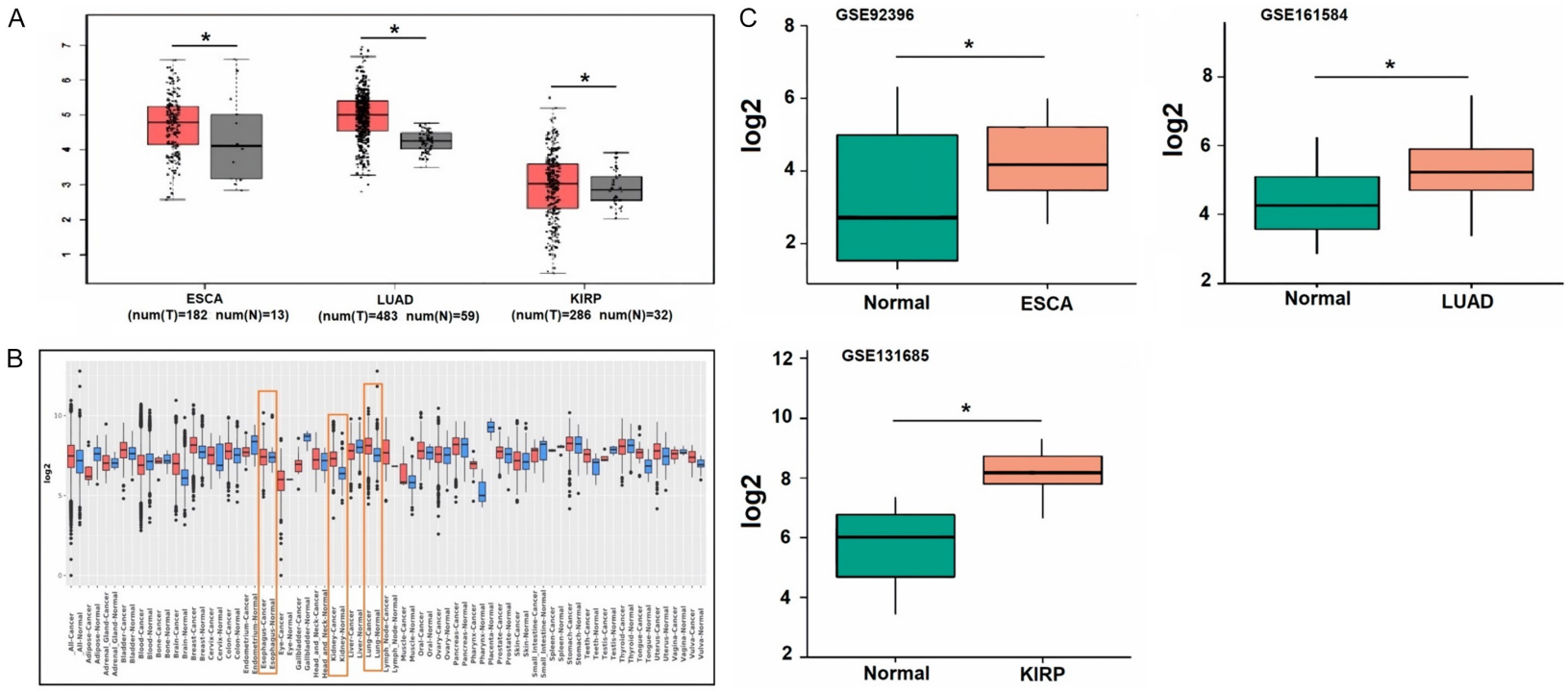

Figure 4. mRNA expression validation of SEC24D using TCGA and GEO ESCA, LUAD, and KIRP datasets via GEPIA, GENT2, GEO database. (A) SEC24D expression validation using TCGA datasets via GEPIA, (B) SEC24D expression validation using TCGA datasets via GENT2, and (C) SEC24D expression validation using GEO datasets (GSE92396, GSE161584, and GSE131685) via GEO database. A *p*-value of <0.05 was selected as a cutoff criterion.

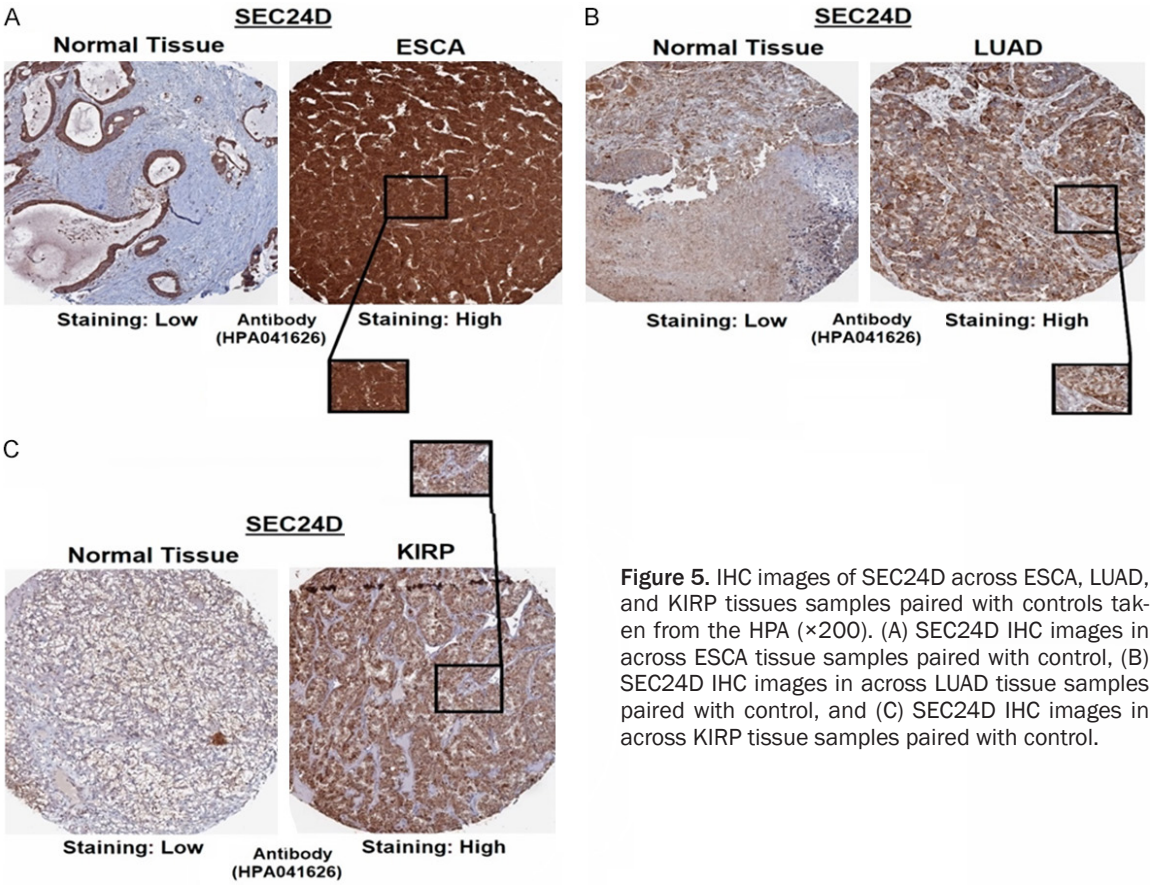

Figure 5. IHC images of SEC24D across ESCA, LUAD, and KIRP tissues samples paired with controls taken from the HPA (×200). (A) SEC24D IHC images in across ESCA tissue samples paired with control, (B) SEC24D IHC images in across LUAD tissue samples paired with control, and (C) SEC24D IHC images in across KIRP tissue samples paired with control.

independent predictor of patient OS, regardless of other clinical factors.

Promoter methylation analysis of SEC24D

We investigated the promoter methylation level of the SEC24D gene using MEXPRESS in ESCA, LUAD, and KIRP patients. Our analysis revealed a consistent pattern of hypomethylation in the promoter region of the SEC24D gene in these cancer types (**Figure 7**). This finding indicates that there is a reduction in methylation level in the promoter regions of the SEC24D gene. The observed hypomethylation suggests a potential mechanism for the up-regulation of SEC24D expression that we previously identified. The identification of hypomethylation in the promoter region of SEC24D in ESCA, LUAD, and KIRP adds another layer of understanding to the molecular characteristics of these cancers.

Genomic alterations in SEC24D

Next, we conducted a mutational analysis of the SEC24D gene using TCGA datasets of ESCA, LUAD, and KIRP via the cBioPortal database. Our analysis revealed that the SEC24D mutations were present in the least number of ESCA (0.5%), LUAD (2.2%), and KIRP (0.4%) cases out of total analyzed cases (**Figure 8**). Moreover, it was also noted that missense mutation was the most common genomic alteration in SEC24D gene across ESCA, LUAD, and KIRP samples. In a nutshell, our findings indicate that genetic alterations in the SEC24D gene are rare in ESCA, LUAD, and KIRP.

SEC24D and immune cell infiltration analysis

We investigated the correlation between SEC24D gene expression and infiltration levels of various immune cell types, including B cells, neutrophils, macrophages, CD4+ T cells, and CD8+ T cells in ESCA, LUAD, and KIRP samples. Our results demonstrated a consistent and positive correlation between SEC24D expression and the infiltration levels of these immune cells in ESCA, LUAD, and KIRP samples (**Figure 9**). These positive correlation suggests a potential involvement of SEC24D in modulating the immune microenvironment within ESCA, LUAD, and KIRP tumors. The up-regulation of SEC24D

A

SEC24D

Variables	Univariate analysis			Multivariate analysis			Dataset
	p value	HR	95% CI	p value	HR	95% CI	
Stage 1	<0.05	2.8	0.5 ~ 12.5	<0.05	2.1	0.2 ~11.5	GSE53624
Stage 2	<0.05	2.9	1.1 ~ 12.2	<0.05	2.4	0.7 ~9.2	
Stage 3	<0.05	2.2	0.9 ~ 11.1	<0.05	11.2	0.2 ~10.9	
Male	<0.05	5.1	1.5 ~ 10.5	<0.05	9.7	0.5 ~7.1	
Female	<0.05	3.4	1.5 ~ 9.7	<0.05	10.3	0.2 ~10.2	

B

SEC24D

Dataset	Variables	Univariate analysis			Multivariate analysis		
		p value	HR	95% CI	p value	HR	95% CI
GSE31210	Stage 1	<0.05	1.1	0.9 ~ 2.7	<0.05	0.5	0.2 ~ 2.3
	Stage 2	<0.05	1.4	1.3 ~ 7.2	<0.05	0.9	0.9 ~ 1.1
	Stage 3	<0.05	1.6	0.7 ~ 8.1	<0.05	2.7	0.5 ~ 1.9
	Male	<0.05	1.1	1.5 ~ 10.5	<0.05	1.8	1.1 ~ 2.5
	Female	<0.05	1.5	0.3 ~ 1.7	<0.05	1.1	0.2 ~ 2.6

C

SEC24D

Dataset	Variables	Univariate analysis			Multivariate analysis		
		p value	HR	95% CI	p value	HR	95% CI
GSE36895	Stage 1	<0.05	2.2	3.3 ~ 7.4	<0.05	1.1	1.4 ~ 3.5
	Stage 2	<0.05	2.1	2.1 ~ 6.3	<0.05	1.7	2.7 ~ 2.5
	Stage 3	<0.05	3.2	3.5 ~ 7.1	<0.05	2.8	3.7 ~ 5.1
	Male	<0.05	1.8	2.9 ~ 6.2	<0.05	8.7	1.4 ~ 3.5
	Female	<0.05	4.2	3.2 ~ 9.4	<0.05	4.3	1.4 ~ 5.5

Figure 6. Univariate and multivariate survival analysis of SEC24D across ESCA, KIRP, and LUAD patients of different clinical variables. (A) Univariate and multivariate survival analysis of SEC24D across ESCA patients of different clinical variables, (B) Univariate and multivariate survival analysis of SEC24D across KIRP patients of different clinical variables, and (C) Univariate and multivariate survival analysis of SEC24D across LUAD patients of different clinical variables. A *p*-value of <0.05 was selected as a cutoff criterion.

may contribute to the recruitment or activation of immune cells, thereby influencing the tumor immune response.

PPI and enrichment of SEC24D

We utilized STRING analysis to explore the functional associations of SEC24D with other genes. Our analysis revealed that SEC24D was associated with 10 other genes, forming a network of interconnected genes (**Figure 10A**). The enrichment of the SEC24D-associated genes in specific KEGG pathways suggests their participation in key molecular pathways. These pathways include "Protein processing in endoplasmic reticulum, SNARE interactions in vesicular transport, legionellosis, and pathogenic Escherichia coli infection" (**Figure 10B**), which are known to be regulated by SEC24D.

Additionally, the GO enrichment of the SEC24D-associated genes provides insights into their potential functions in "COPII vesicle coat, endoplasmic reticulum exist site, vesicle coat, membrane coat, and coated membrane" etc., CC terms (**Figure 10C**), "SNARE binding, zinc ion binding, and transition metal ion binding" etc., MF terms (**Figure 10D**), "COPII-coated vesicle cargo loading, vesicle cargo loading, vesicle targeting, rough ER to cis-Golgi, and COPII vesicle coating", etc., BP terms (**Figure 10E**).

Screening of SEC24D-assiciated drugs

Next, we conducted a screening of SEC24D-associated drugs using the DrugBank database, aiming to identify potential therapeutic options for the treatment of ESCA, LUAD, and KIRP. Analysis results revealed the presence of several drugs, including Acteoside, Cyclosporine, Polydatin, Estradiol, Panobinostat, and Quercetin that demonstrated the capability to decrease the expression of SEC24D (**Table 1**).

Figure 7. SEC24D promoter methylation analysis via the MEXPRESS. (A) across ESCA, (B) across LUAD, and (C) across KIRP. A *p-value of <0.05 was selected as cutoff criterion.

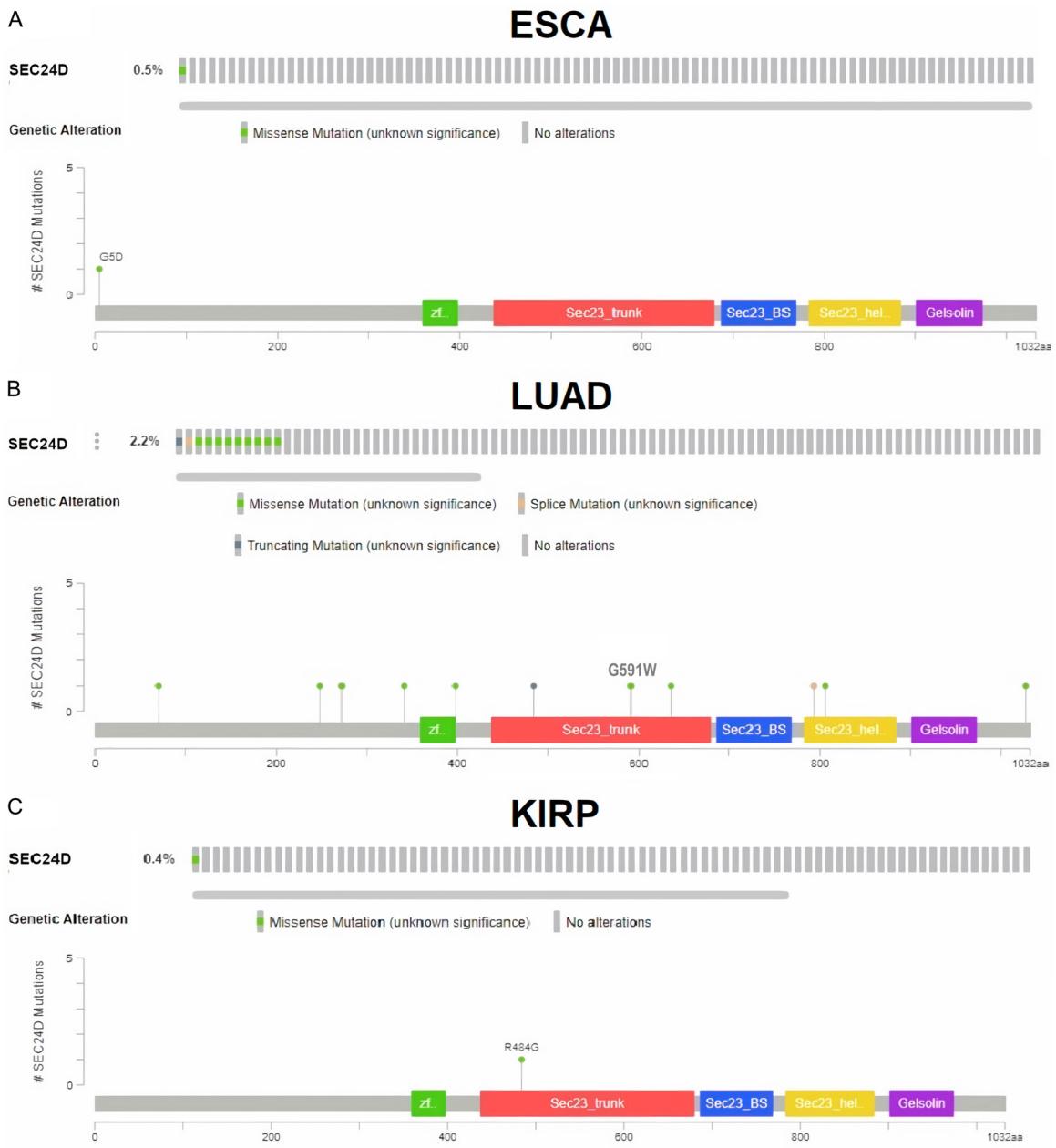

Figure 8. Genetic alterations analysis of SEC24D gene across ESCA, LUAD, and KIRC patients via the cBioPortal platform. (A) Across ESCA samples, (B) Across LUAD samples, and (C) Across KIRP samples.

The identification of such drugs highlights the potential of targeting SEC24D as a therapeutic strategy for ESCA, LUAD, and KIRP.

Experimental in vitro validation of the SEC24D expression and methylation levels

In the current study, by performing RNA-seq and targeted bisulfite-seq analyses of six LUAD cell lines, including two cell lines (HCC15 and HCC44) derived from the primary lung cancer tissues, three cell lines (H-460, A549, and PC-9) derived from the metastatic lung cancer tissue, and one normal cell line (MRC-9) derided from the normal lung tissue, the expression and methylation levels of SEC24D gene were validated. The expression levels SEC24D were validated using FPKM, while methylation level was validated using beta values.

As shown in **Figure 11A**, it was noticed that SEC24D gene expression in both types of LUAD cell lines (derived from primary tumor and metastatic tissues) was notably higher as com-

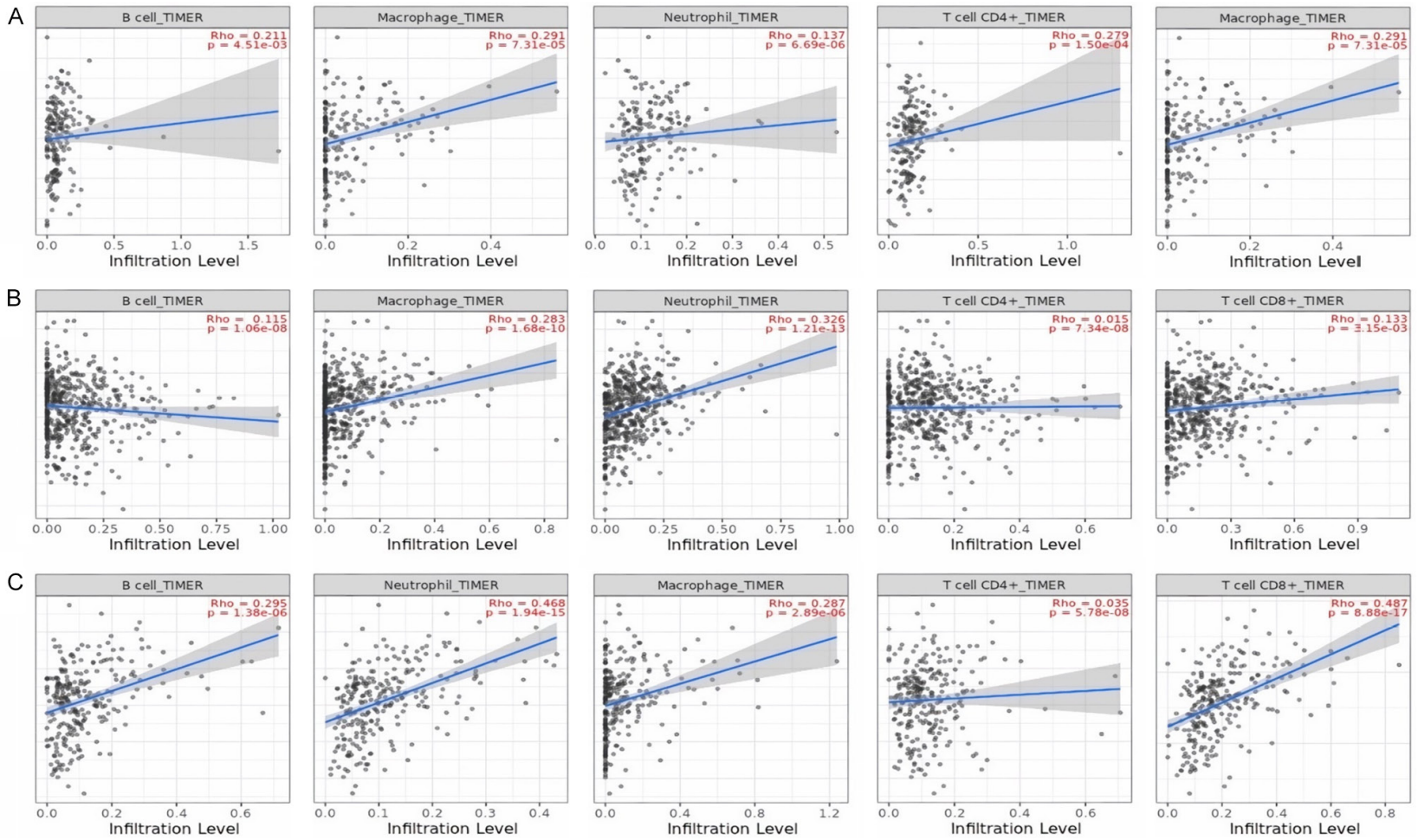

Figure 9. Spearman correlational analysis of SEC24D with different immune cells (B cells, neutrophils, macrophages, CD4+ T cells, and CD8+ T) via the TIMER2 database. (A) Spearman correlational analysis of SEC24D with different immune cells across ESCA samples, (B) Spearman correlational analysis of SEC24D with different immune cells across LUAD samples, and (C) Spearman correlational analysis of SEC24D with different immune cells across KIRP samples. A *p-value of <0.05 was selected as cutoff criterion.

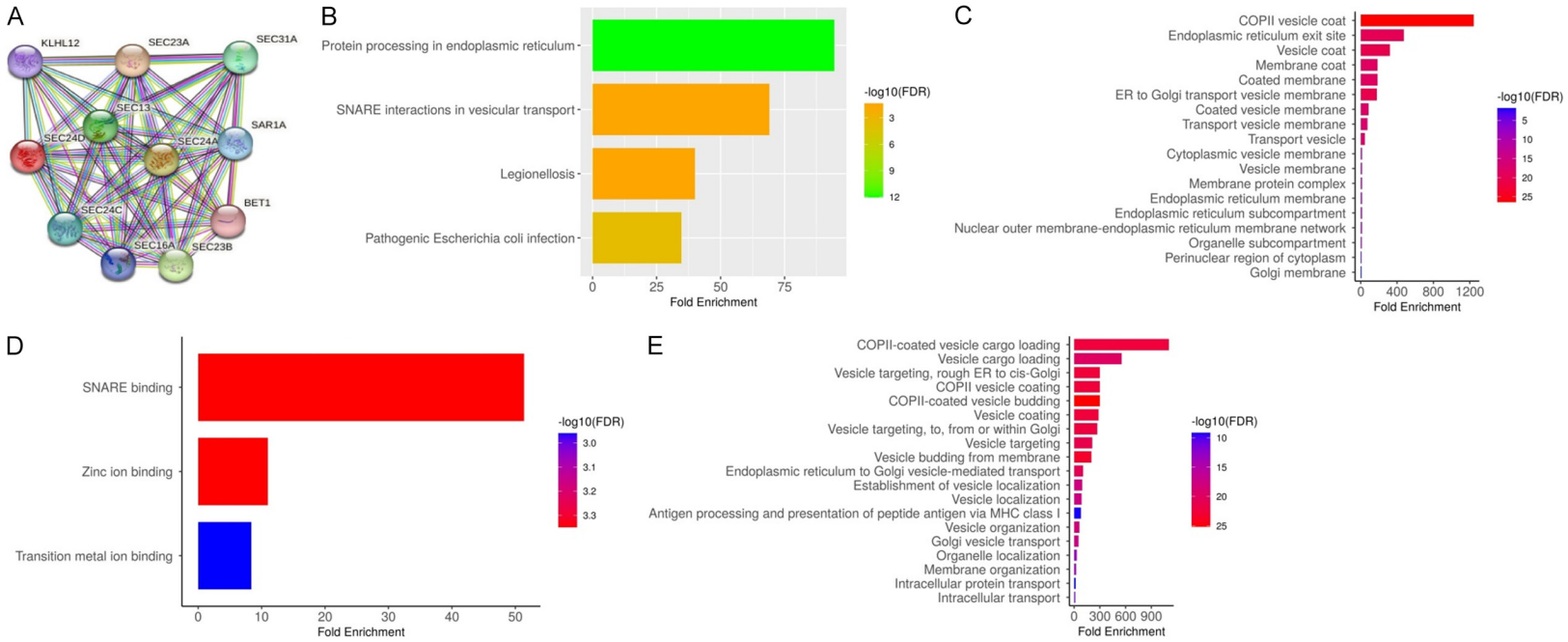

Figure 10. A PPI network development, GO and KEGG enrichment analysis of SEC24D-associated genes. (A) A PPI network, (B) pathway enrichment analysis, (C) CC enrichment analysis results, (D) MF enrichment analysis results, and (E) BP enrichment analysis results. A *p-value of <0.05 was selected as cutoff criterion.

Table 1. DrugBank-based SEC24D-associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	SEC24D	Acetaminophen	Decrease expression of SEC24D mRNA	A20426	Approved
		Acteoside		A20456	
		Cyclosporine		A20661	
		Polydatin		A20456	
		Estradiol		A21424	
		Panobinostat		A21037	
		Quercetin		A20661	

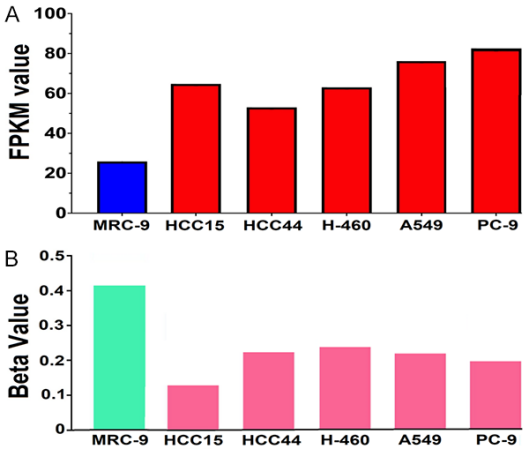

Figure 11. RNA-seq and targeted bisulfite-seq based validation of SEC24D gene expression and promoter methylation levels across two cell lines (HCC15 and HCC44) derived from the primary lung cancer tissues, three cell lines (H-460, A549, and PC-9) derived from the metastatic lung cancer tissue, and one normal cell line (MRC-9). (A) FPKM values based expression plots of SEC24D across analyzed cell lines, and (B) Beta values based methylation plots of SEC24D across analyzed cell lines.

pared to normal cell line. Particularly, the expression of SEC24D was higher in metastatic cell lines as compared to the cell lines derived from the primary tissues (**Figure 11A**). This finding further validates that SEC24D expression may play a role in the metastatic process and could be associated with the acquisition of metastatic properties in cancer cells.

Moreover, we observed lower beta values in LUAD cell lines relative to control cell lines (**Figure 11B**), indicating a hypomethylation pattern of the SEC24D gene. This finding suggests that the regulatory regions of the SEC24D gene may undergo reduced methylation in LUAD, potentially contributing to its dysregulation and aberrant expression.

Discussion

Previously, limited information regarding SEC24D dysregulation in human cancers has been reported in the medical literature. However, studies have identified rare occurrences of genetic mutations in SEC24D and its up-regulation in few specific malignancies. Examples of such malignancies include osteogenesis imperfect, breast cancer, ovarian cancer, and gastric cancer [35-38]. In light of the limited understanding regarding SEC24D gene dysregulation in diverse cancer types, we initiated this study with the aim of conducting a comprehensive investigation into SEC24D gene expression across a range of human cancers through pan-cancer analysis. By exploring SEC24D expression in a pan-cancer context, we sought to broaden our understanding of the potential involvement of SEC24D in different cancers and shed light on its diagnostic and prognostic potentials.

In the present study, we found that SEC24D was widely expressed in a variety of tissues and SEC24D expression is up-regulated in the majority of tumors. Survival analysis revealed that SEC24D overexpression was associated with poor OS of the ESCA, LUAD, and KIRP patients. We further explored the relationship between SEC24D overexpression, metastasis, and clinical parameters. The up-regulated SEC24D expression was found to be associated with different metastasis and diverse clinical parameters across ESCA, LUAD, and KIR.

Promoter methylation of the SEC24D gene has been earlier investigated in various cancers, revealing altered methylation patterns. For instance, a study by Shen et al. reported hypomethylation of SEC24D in colorectal cancer [39]. Similarly, hypermethylation of SEC24D was observed in gastric cancer [40]. To the

best of our knowledge, our study represents the first report demonstrating the association between SEC24D hypomethylation and its downregulation in ESCA, LUAD, and KIRP patients.

Accumulating evidence suggests that genomic mutations play a critical role in tumor progression and influence the response to chemotherapy [41, 42]. For example, in a study conducted by Yang et al., it was reported that mutations in BRCA1 and BRCA2 genes exhibit a significant association with patient survival. This correlation could potentially be attributed to the distinct response these mutations confer towards platinum-based treatment [41]. In a comprehensive study, it was revealed that mutations in specific genes (ESR1, CDH1, RICTOR, and TP53) exhibited a tendency to occur in distinct metastatic sites in breast cancer patients. These findings suggest the potential utility of these mutations as biomarkers or therapeutic targets for patients with metastatic breast cancer [42].

In our study, we found that mutations in SEC24D genes were not prevalent in ESCA, LUAD, and KIRP patients. Previously, mutations in the SEC24D gene have also been the subject of limited investigation in the context of cancer, and available evidence suggests that SEC24D mutations are not commonly observed in various cancer types. A study by Forbes et al. analyzed the genomic landscape of multiple cancers and found a low frequency of SEC24D mutations across diverse tumor types, including breast, lung, colorectal, and ovarian cancers [43]. Similarly, in a pan-cancer analysis conducted by Bailey et al. [44], SEC24D mutations were infrequent across thousands of tumor samples from different cancer types.

Cumulatively, these findings suggest that SEC24D plays a role as an oncogene in the occurrence and advancement of ESCA, LUAD, and KIRP, making it a potential diagnostic and prognostic biomarker with practical applications for patients with these cancers.

Previous studies have identified several biomarkers with limited application in ESCA, LUAD, and KIRP. For instance, in ESCA, the biomarker p53 has been investigated but its clinical utility has been constrained due to its low sensitivity and specificity [45]. In LUAD, the biomarker carcinoembryonic antigen (CEA) has been explored; however, its diagnostic accuracy is limited, particularly in early-stage disease [46]. In KIRP, the biomarker α-methylacyl-CoA racemase (AMACR) has been studied, but its application is restricted due to its relatively low sensitivity and specificity [47]. In this study, we revealed the notable up-regulation of SEC24D expression in ESCA, LUAD, and KIRP patients with different clinical variable. Therefore, SEC24D up-regulation may be used as a new common potential biomarker for ESCA, LUAD, and KIRP patients across heterogeneity barrier.

In our study, we observed a complex infiltration pattern in ESCA, LUAD, and KIRP samples, revealing a positive correlation between SEC24D expression and multiple immune cell types. Specifically, SEC24D expression showed a positive association with the infiltration of B cells, neutrophils, macrophages, CD4+ T cells, and CD8+ T cells in these cancer types. Based on the results, we concluded that SEC24D high expression has a role in altering tumor microenvironment. Emerging evidence also suggests that higher expression of SEC24D plays a role in modulating the tumor microenvironment. SEC24D has been implicated in promoting tumor-associated angiogenesis and immune evasion, contributing to tumor progression [48]. Via the STRING, we identified 10 genes that were enriched with SEC24D. Pathway enrichment analysis revealed that these genes were strongly correlated with different important pathways, including “Protein processing in endoplasmic reticulum, SNARE interactions in vesicular transport, legionellosis, and pathogenic Escherichia coli infection” [49, 50]. The role of these pathways in cancer development and progression is already well acknowledged.

This study encountered a few limitations that should be acknowledged. Firstly, while we successfully validated SEC24D expression in LUAD cell lines, further validation of this gene’s expression in ESCA and KIRP cell lines is necessary. Secondly, the functional investigation of the molecular mechanisms underlying SEC24D expression in the analyzed cancers was not done in this study. Future studies should delve into the molecular mechanisms involved to gain a more comprehensive understanding of SEC24D’s role in tumorigenesis.

Conclusion

In conclusion, this study comprehensively investigated the diagnostic and prognostic implications of SEC24D in ESCA, LUAD, and KIRP through extensive in vivo and in vitro analyses. The findings highlight the clinical importance of SEC24D as a potential molecular biomarker, therapeutic target, and predictive factor for immunotherapy in ESCA, LUAD, and KIRP patients with diverse clinical parameters.

Disclosure of conflict of interest

None.

Address correspondence to: Yanhua Lv, Department of Respiratory and Critical Care Medicine, Zhongshan City People's Hospital, Zhongshan, Guangdong, China. E-mail: lyhzhongshan@163.com.

References

[1] Spilka R, Ernst C, Mehta AK and Haybaeck J. Eukaryotic translation initiation factors in cancer development and progression. Cancer Lett 2013; 340: 9-21.
[2] Bach DH, Zhang W and Sood AK. Chromosomal instability in tumor initiation and development. Cancer Res 2019; 79: 3995-4002.
[3] Smith RCL, Kanellos G, Vlahov N, Alexandrou C, Willis AE, Knight JRP and Sansom OJ. Translation initiation in cancer at a glance. J Cell Sci 2021; 134: jcs248476.
[4] Sylla BS and Wild CP. A million africans a year dying from cancer by 2030: what can cancer research and control offer to the continent? Int J Cancer 2012; 130: 245-250.
[5] Shah U, Shah R, Acharya S and Acharya N. Novel anticancer agents from plant sources. Chin J Nat Med 2013; 11: 16-23.
[6] Elmore LW, Greer SF, Daniels EC, Saxe CC, Melner MH, Krawiec GM, Cance WG and Phelps WC. Blueprint for cancer research: Critical gaps and opportunities. CA Cancer J Clin 2021; 71: 107-139.
[7] Selli C, Dixon JM and Sims AH. Accurate prediction of response to endocrine therapy in breast cancer patients: current and future biomarkers. Breast Cancer Res 2016; 18: 118.
[8] Srivastava A and Creek DJ. Discovery and validation of clinical biomarkers of cancer: a review combining metabolomics and proteomics. Proteomics 2019; 19: 1700448.
[9] Brase JC, Wuttig D, Kuner R and Sültmann H. Serum microRNAs as non-invasive biomarkers for cancer. Mol Cancer 2010; 9: 306.
[10] Yun SJ, Jeong P, Kim WT, Kim TH, Lee YS, Song PH, Choi YH, Kim IY, Moon SK and Kim WJ. Cell-free microRNAs in urine as diagnostic and prognostic biomarkers of bladder cancer. Int J Oncol 2012; 41: 1871-1878.
[11] Jain D, Nambirajan A, Borczuk A, Chen G, Minami Y, Moreira AL, Motoi N, Papotti M, Rekhtman N, Russell PA, Savic Prince S, Yatabe Y and Bubendorf L; IASLC Pathology Committee. Immunocytochemistry for predictive biomarker testing in lung cancer cytology. Cancer Cytopathol 2019; 127: 325-339.
[12] Xu YW, Peng YH, Chen B, Wu ZY, Wu JY, Shen JH, Zheng CP, Wang SH, Guo HP, Li EM and Xu LY. Autoantibodies as potential biomarkers for the early detection of esophageal squamous cell carcinoma. Am J Gastroenterol 2014; 109: 36-45.
[13] McNamara MG, Jacobs T, Lamarca A, Hubner RA, Valle JW and Amir E. Impact of high tumor mutational burden in solid tumors and challenges for biomarker application. Cancer Treat Rev 2020; 89: 102084.
[14] Hu T, Wolfram J and Srivastava S. Extracellular vesicles in cancer detection: hopes and hypes. Trends Cancer 2021; 7: 122-133.
[15] Garbes L, Kim K, Rieß A, Hoyer-Kuhn H, Beleggia F, Bevot A, Kim MJ, Huh YH, Kweon HS, Savarirayan R, Amor D, Kakadia PM, Lindig T, Kagan KO, Becker J, Boyadjiev SA, Wollnik B, Semler O, Bohlander SK, Kim J and Netzer C. Mutations in SEC24D, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta. Am J Hum Genet 2015; 96: 432-439.
[16] Farhan H, Reiterer V, Korkhov VM, Schmid JA, Freissmuth M and Sitte HH. Concentrative export from the endoplasmic reticulum of the γ-aminobutyric acid transporter 1 requires binding to SEC24D. J Biol Chem 2007; 282: 7679-7689.
[17] Sarmah S, Barrallo-Gimeno A, Melville DB, Topczewski J, Solnica-Krezel L and Knapik EW. Sec24D-dependent transport of extracellular matrix proteins is required for zebrafish skeletal morphogenesis. PLoS One 2010; 5: e10367.
[18] Ohisa S, Inohaya K, Takano Y and Kudo A. sec24d encoding a component of COPII is essential for vertebra formation, revealed by the analysis of the medaka mutant, vbi. Dev Biol 2010; 342: 85-95.
[19] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020; 48: W509-W514.
[20] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.

[21] Cai Y, Mei J, Xiao Z, Xu B, Jiang X, Zhang Y and Zhu Y. Identification of five hub genes as monitoring biomarkers for breast cancer metastasis in silico. Hereditas 2019; 156: 20.
[22] Bartha Á and Győrffy B. TNMplot.com: a web tool for the comparison of gene expression in normal, tumor and metastatic tissues. Int J Mol Sci 2021; 22: 2622.
[23] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
[24] Park SJ, Yoon BH, Kim SK and Kim SY. GENT2: an updated gene expression database for normal and tumor tissues. BMC Med Genomics 2019; 12 Suppl 5: 101.
[25] Clough E and Barrett T. The gene expression omnibus database. Methods Mol Biol 2016; 1418: 93-110.
[26] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J and Pontén F. Tissue-based map of the human proteome. Science 2015; 347: 1260419.
[27] Mizuno H, Kitada K, Nakai K and Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics 2009; 2: 18.
[28] Koch A, De Meyer T, Jeschke J and Van Criekinge W. MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. BMC Genomics 2015; 16: 636.
[29] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discove 2012; 2: 401-404.
[30] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ and Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47: D607-D613.
[31] Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007; 8: R183.
[32] Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z and Woolsey J. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res 2006; 34: D668-672.
[33] Rio DC, Ares M Jr, Hannon GJ and Nilsen TW. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc 2010; 2010: pdb.prot5439.
[34] Ghatak S, Muthukumaran RB and Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013; 24: 224-231.
[35] Jiang X, Liu L, Zhang Q, Jiang Y, Huang J, Zhou H and Zeng L. Laparoscopic versus open hepatectomy for hepatocellular carcinoma: long-term outcomes. J BUON 2016; 21: 135-141.
[36] Liu Z, Zhou J, Wang Z and Zhou Z. Analysis of SEC24D gene in breast cancer based on UALCAN database. Open Life Sci 2019; 14: 707-711.
[37] Gui T, Yao C, Jia B and Shen K. Identification and analysis of genes associated with epithelial ovarian cancer by integrated bioinformatics methods. PLoS One 2021; 16: e0253136.
[38] Lu XQ, Zhang JQ, Zhang SX, Qiao J, Qiu MT, Liu XR, Chen XX, Gao C and Zhang HH. Identification of novel hub genes associated with gastric cancer using integrated bioinformatics analysis. BMC Cancer 2021; 21: 697.
[39] Shen L, Toyota M, Kondo Y, Obata T, Daniel S, Pierce S, Imai K, Kantarjian HM, Issa JP and Garcia-Manero G. Aberrant DNA methylation of p57KIP2 identifies a cell-cycle regulatory pathway with prognostic impact in adult acute lymphocytic leukemia. Blood 2003; 101: 4131-4136.
[40] Lamare FA, Khongsti S, Marthong L, Ghosh S, Chenkual S, Dkhar H, Maitra A and Ghosh S. Genome-wide DNA methylation profiling of stomach cancer in the ethnic population of Mizoram, North East India. Genomics 2022; 114: 110478.
[41] Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK and Zhang W. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA 2011; 306: 1557-1565.
[42] Cha S, Lee E and Won HH. Comprehensive characterization of distinct genetic alterations in metastatic breast cancer across various metastatic sites. NPJ Breast Cancer 2021; 7: 93.
[43] Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L, Stefancsik R, Harsha B, Kok CY, Jia M, Jubb H, Sondka Z, Thompson S, De T and Campbell PJ. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017; 45: D777-D783.

[44] Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, Wendl MC, Kim J, Reardon B, Ng PK, Jeong KJ, Cao S, Wang Z, Gao J, Gao Q, Wang F, Liu EM, Mularoni L, Rubio-Perez C, Nagarajan N, Cortés-Ciriano I, Zhou DC, Liang WW, Hess JM, Yellapantula VD, Tamborero D, Gonzalez-Perez A, Suphavilai C, Ko JY, Khurana E, Park PJ, Van Allen EM, Liang H; MC3 Working Group; Cancer Genome Atlas Research Network, Lawrence MS, Godzik A, Lopez-Bigas N, Stuart J, Wheeler D, Getz G, Chen K, Lazar AJ, Mills GB, Karchin R and Ding L. Comprehensive characterization of cancer driver genes and mutations. Cell 2018; 173: 371-385, e18.

[45] Shimada H. p53 molecular approach to diagnosis and treatment of esophageal squamous cell carcinoma. Ann Gastroenterol Surg 2018; 2: 266-273.

[46] Molina R, Marrades RM, Augé JM, Escudero JM, Viñolas N, Reguart N, Ramirez J, Filella X, Molins L and Agustí A. Assessment of a combined panel of six serum tumor markers for lung cancer. Am J Respir Crit Care Med 2016; 193: 427-437.

[47] Hameed O and Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. Semin Diagn Pathol 2005; 22: 88-104.

[48] Wang M, Liu X, Chen T, Cheng X, Xiao H, Meng X and Jiang Y. Inhibition and potential treatment of colorectal cancer by natural compounds via various signaling pathways. Front Oncol 2022; 12: 956793.

[49] Hay JC, Chao DS, Kuo CS and Scheller RH. Protein interactions regulating vesicle transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Cell 1997; 89: 149-158.

[50] Yang JL, Li D and Zhan XY. Concept about the virulence factor of legionella. Microorganisms 2022; 11; 74.