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FLCN - A Promising Novel Prognostic Biomarker for Lung Adenocarcinoma (LUAD) Patients

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Abstract: *Rationale:* The tumor suppressor *FLCN* gene mutations are the primary cause of the rare autosomal recessive genetic disorder known as Birt-Hogg-Dubé (BHD) syndrome. Early diagnosis of BHD is difficult since *FLCN* mutation-caused tumors can form in the skin, lungs, kidney, and other organs and are benign. These tumors generate a range of phenotypes. *Methods:* The UALCAN database was utilized to ascertain *FLCN* expression and methylation in LUAD. Additionally, using KM plotter, GEPIA2.0, and cBioPortal, respectively, the survival, validity, and mutation analysis of *FLCN* was ascertained in LUAD. Using STRING and DAVID tools, the pathway and gene enrichment were identified in the presence of *FLCN*. The muTarget database was used to identify the mutant genes. *Results:* The goal of the current study is to examine *FLCN* expression in LUAD tissues. In these patients with LUAD, the study compared the expression of *FLCN* to other clinic-pathological characteristics. When comparing LUAD patients' clinical parameters to those of normal control samples, *FLCN* expression was higher. Additionally, it was discovered that a higher expression of *FLCN* in LUAD patients was linked to a shorter overall and disease-free survival. Results of gene ontology and pathway analysis demonstrated that genes linked with *FLCN* are significantly co-expressed with *FLCN* and are involved in a wide range of distinct molecular functions, biological processes, and pathways. *FLCN* expression was also correlated with promoter methylation levels, genetic alterations, other mutant genes. This crucial information demonstrated the important function that *FLCN* plays in the initiation and expansion of LUAD. *Conclusion:* This research emphasizes how crucial genetic analysis is to the diagnosis and the therapeutic treatment of LUAD.

Keywords: Lung adenocarcinoma; Diagnosis; Treatment; Biomarker

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1. Introduction

Lung cancer is the most commonly diagnosed malignant tumor and the main cause of cancer-related death. It is the second most common cause of new cancer cases in both genders in the United States and the second greatest cause of cancer deaths in females worldwide [1,2]. Lung cancer accounts for more than 27% of all cancer-related

deaths globally, with non-small cell lung cancer (NSCLC) accounting for 80% of cases [2,3]. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), often referred to as non-small cell lung cancer (NSCLC), are the most prevalent subtypes of lung cancer [4,5]. Among NSCLC histological types, lung adenocarcinoma (LUAD) is the most prevalent one. The overall lung adenocarcinoma survival rate is still low despite considerable advancements in lung adenocarcinoma therapeutic approaches, such as surgical treatment, target therapy, and early cancer identification [6]. Currently, cytology screening and imaging examination are sensitive cancer screening methods, however they are ineffective for early identification of lung adenocarcinoma [7,8]. Recently, the clinical results of molecularly targeted therapies for LUAD patients are encouraging [9,10], while drug resistance still blocks curing LUAD patients [11,12]. Moreover, over 50% of patients are still not able to gain a limited benefit from targeted therapy [13,14]. Thus, reliable clinical outcome prediction and early lung cancer identification depend on the establishment of early diagnostic and prognostic biomarkers.

The *FLCN* gene is a tumor suppressor gene also known as the folliculin gene. *FLCN* produces a protein that may aid in controlling cell development and other critical cell processes. Mutated variants of the *FLCN* gene may promote the formation of aberrant cells. Birt-Hogg-Dube syndrome (BHDS) is an inherited disorder associated with a defective *FLCN* gene. Patients with BHDS are more likely to develop kidney cancer, as well as skin and lung cancers. The human *FLCN* gene on chromosome 17p11.2 comprises 14 exons and encodes a highly conserved protein termed folliculin, which is a classic tumor suppressor that regulates cell growth and proliferation [15,16]. An increasing amount of data indicates that the pathogenesis of Birt-Hogg-Dubé (BHD) syndrome (OMIM: 135150), an autosomal recessive condition marked by benign tumors in the skin, lungs, kidneys, and other organs, is mostly caused by mutations in the *FLCN* gene [15]. Age affects the location, development, and course of benign tumors, which can also vary by race or ethnicity [17]. This heterogeneity makes early diagnosis of BHD syndrome difficult and raises the chance of benign tumors becoming malignant [18]. BHD syndrome is linked to the development of cutaneous hamartomas (fibrofolliculomas, FF), numerous lung cysts (LCs), spontaneous pneumothoraces, and renal cell carcinoma (RCC) [19]. The renal malignancies linked with BHD are primarily of the hybrid oncocytic/chromophobe subtype, followed by clear cell renal carcinomas [20]. Approximately 85% of patients with BHD syndrome have multiple FF, which are benign dermatological papules that primarily affect the face and upper torso. These papules typically do not show until beyond the age of 20 [21–23]. Furthermore, in individuals with BHD syndrome, the dermatologic findings could be the only presenting symptoms, thus it's critical to recognize them so that a more thorough systemic evaluation can be carried out.

This study evaluated *FLCN* mutations, expression levels, survival prognosis results, and utilitarian perspectives within the LUAD framework using bioinformatics. This study also looked into the relationship between *FLCN* expression and promoter methylation levels. Numerous databases were used in this investigation, including the UALCAN portal, the Kaplan-Meier tool, the STRING database for protein-protein interactions (PPI), the cBioPortal, the Gene Expression Profiling and Interactive Analysis (GEPIA2.0), the Database for Annotation, Visualization, and Integrated Discovery (DAVID), and the Cancer Genome Atlas (TCGA) informational index. A vast array of functional annotation tools is offered by DAVID to help interpret the biological significance of the lengthy gene list. This study's main contribution was figuring out the *FLCN* expression pattern in LUAD and its potential significance for the onset and management of cancer.

2. Materials and methods

2.1. Expression and methylation analysis by UALCAN

The UALCAN database offers quick access to the cancer multi-omics data gathered from more than 30 distinct cancer types thanks to its user-friendly interface and intuitive features [24]. It performs comprehensive analyses of gene expression, protein abundance, and patient survival across a range of malignant tumor types using a significant amount of data from The Cancer Genome Atlas (TCGA). Researchers may examine and illustrate gene expression patterns linked to various cancer stages, molecular subtypes, and patient socio-demographics using the intuitive UALCAN interface. In the current study, this useful measure was employed to evaluate *FLCN* expression at different phases of a given cancer growth, where this gene exhibits significant dysregulation and is strongly associated with a poor overall survival rate. Using the UCALAN web tool, the *FLCN* promoter methylation level in LUAD was ascertained. In addition, the study assessed *FLCN* promoter methylation data in diverse clinical contexts, accounting for the age, gender, race, and cancer stage of the patient.

2.2. Survival analysis by KM plotter

KM plotter is a widely used tool for overall survival (OS) and disease-free survival (DFS) [25]. This web-based platform examines the impact of particular genes on a patient's propensity to survive various tumor growth types by analyzing vast volumes of clinical data. Researchers can quickly identify prognostic biomarkers and assess the prognostic significance of gene expressions. The main interface of KM Plotter shows Kaplan-Meier survival curves, which offer information on the relationship between patient outcomes and gene expression. Researchers studying the relationship between patient survival and gene expression levels in gastric, ovarian, lung, and breast cancers can benefit from the use of the KM plotter. This study used the KM plotter tool to examine the impact of *FLCN* dysregulation on overall survival (OS) in LUAD patients.

2.3. Expression and survival validation by GEPIA2.0

A well-known web application called GEPIA2.0 is used to predict expression and assess the durability of genetic data [26]. GEPIA2 has broadened the assessment of gene expression from the gene to the transcript levels. It has 198,619 isoforms and 84 cancer subtypes. It also facilitates the analysis of a particular cancer subtype and their interrelationships. Moreover, different cancer subtypes may have different prognoses. Moreover, different cancer subtypes may have different prognoses. Furthermore, as single-cell sequencing has become more widely available, new evaluation standards have emerged. Using the GEPIA 2.0 data set, the relationships between *FLCN* expression and prognosis (OS and RFS) in patients with LUAD cancer were investigated. The relationship between *FLCN* expression and the prognosis (OS and DFS) of LUAD cancer was examined in this study using GEPIA2.0.

2.4. Mutational analysis by cBioPortal

Because of its user-friendly features, the cBioPortal database is mostly used for multidimensional cancer omics data processing [27]. It provides a user-friendly interface for exploring tumor mutations, gene expression, and other genomic data across different cancer types. The portal aims to bridge the gap between complex genomic data and cancer researchers by providing intuitive access to molecular profiles and clinical attributes. Users can interact with the cBioPortal through a simple and flexible interface, intuitive visualization options, and a programmatic web interface. In this study, this data was used to perform mutational analysis of *FLCN* across LUAD cancer.

2.5. STRING AND DAVID analysis

Using the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) [28], a PPI network comprising genes enriched in *FLCN* was extracted in this study. It makes use of an abundance of data to help scientists make sense of the confusing web of interactions between proteins. The study added STRING to the process of making *FLCN* proteins. When compared to other datasets, the STRING data collection is highly regarded for its enhanced quality control, coverage, and high availability of PPI information. Gene expression patterns and literature are just two of the data sources that STRING integrates to provide a single, comprehensive quality score for every communication. It makes use of PPI from computational and experimental methods. The GO and KEGG keywords of *FLCN* and its enriched genes were examined using DAVID [29] with $P < 0.05$ denoting significant findings.

2.6. muTarget

muTarget, an open-access tool, is used for associating mutational status with gene expression alterations across different tumors [30]. Using this tool, different mutant genes responsible for expression alteration in the *FLCN* across LUAD were identified in this study.

3. Results

3.1. Expression analysis of FLCN in LUAD

Employing the UALCAN data set, we inspected the *FLCN* expression in LUAD and normal control samples (**Figure 1**). After further investigation, this study discovered that LUAD malignant growth cells had a low levels of *FLCN* expression than the normal control samples. The strong down-regulation demonstrated the direct correlation between *FLCN* expression and the proliferation of malignant LUAD cells. This discovery raises the possibility that *FLCN*, a therapeutic target or diagnostic marker for LUAD, may play a critical role in preventing the multiplication of malignancy.

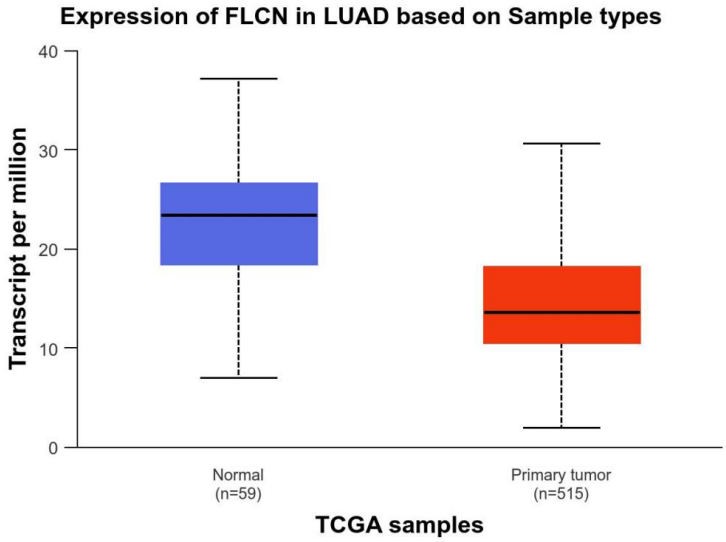

Figure 1. Expression profiling of *FLCN* in LUAD and normal tissue samples.

3.2. Expression analysis of FLCN in LUAD based on different clinical parameters

As a result, this study assessed *FLCN* in LUAD samples using a variety of clinical variables, including the

patient's age, gender, and race in addition to the particular cancer stage (**Figure 2**). When *FLCN* expression was first analyzed in different stages of cancer formation, the study discovered that, in comparison to normal control samples, LUAD samples demonstrated considerably low levels of *FLCN* expression in all stages (**Figure 2A**). Additionally, the study explored *FLCN* expression in LUAD patients and reported that all three racial groups, Asian, African-American, and Caucasian had significantly low levels of *FLCN* expression compared to normal control samples (**Figure 2B**). Furthermore, the study also examined the gender differences in *FLCN* expression in LUAD cancer patients and observed that both male and female patients had significantly low levels of *FLCN* than in normal control samples (**Figure 2C**). Lastly, the study investigated the association between patient age in LUAD and *FLCN* expression and revealed that among LUAD patients, *FLCN* was down-regulated in several age groups (**Figure 2D**). These results highlight *FLCN*'s potential as a helpful biomarker for diagnosis, prognosis and validate its significance in LUAD.

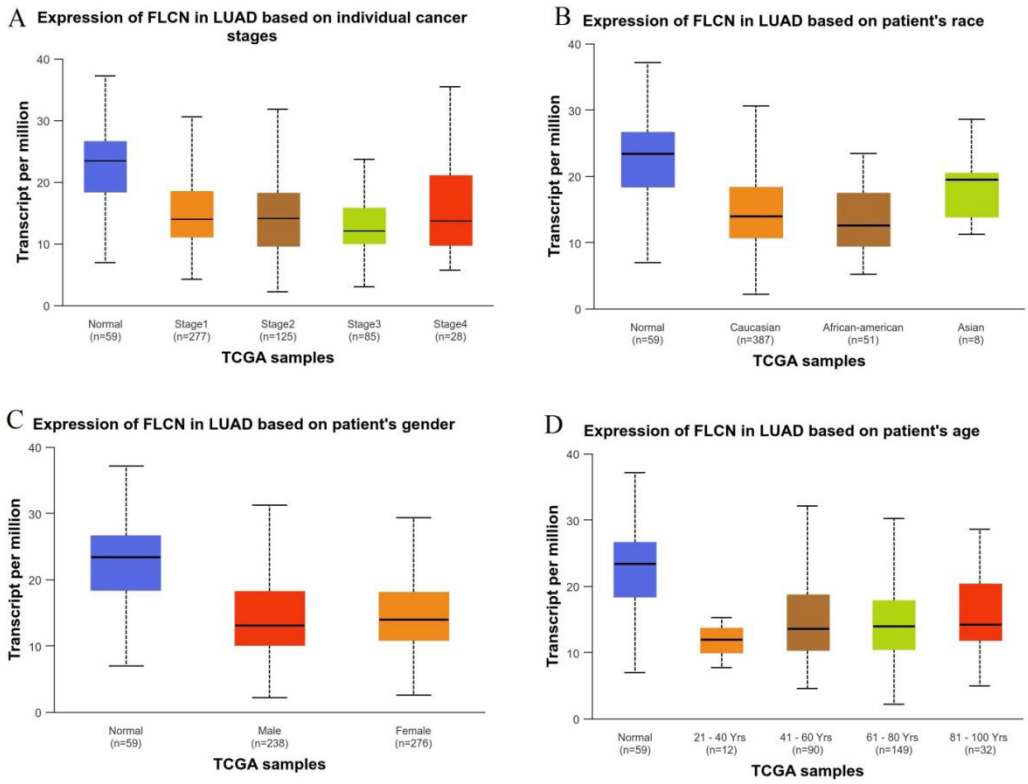

Figure 2. Expression of *FLCN* across different clinical parameters.

3.3. Validation of FLCN expression

The study used GEPIA2 to look at *FLCN* expression in LUAD cells and normal control samples. Compared to normal control samples, LUAD showed noticeably lower *FLCN* expression (**Figure 3A**). Additionally, GEPIA2 data set was used to analyze the relationship between *FLCN* expression and various stages of LUAD development. These results demonstrated a strong correlation between LUAD patient stages and *FLCN* expression. Furthermore, it was shown that the *FLCN* gene had the highest expression in stage IV and the lowest expression in stage III of the LUAD (**Figure 3B**).

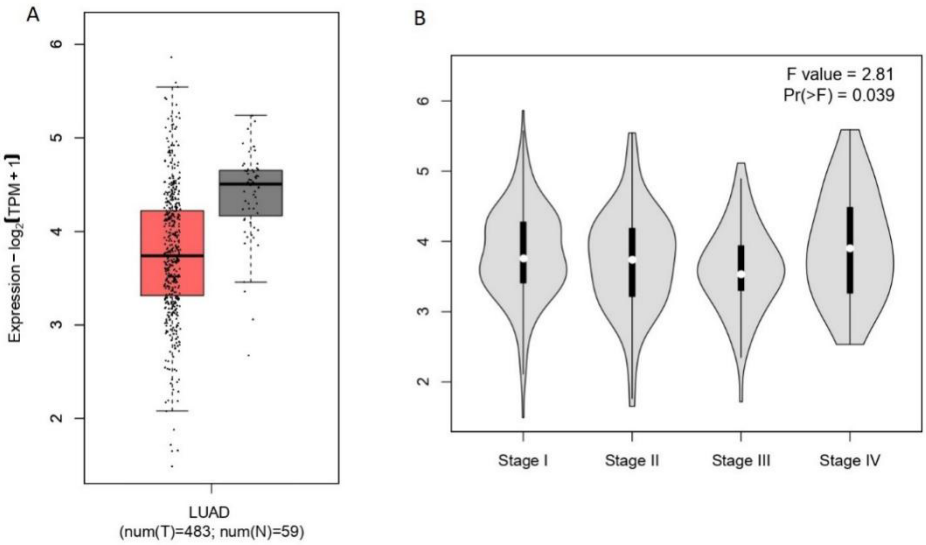

Figure 3. Validation of *FLCN* expression across different stages of LUAD.

3.4. Promoter methylation of FLCN

The study examined the *FLCN* promoter methylation levels in LUAD and normal control samples using the UALCAN online database. The results demonstrated that, in comparison to normal control samples, *FLCN* was hyper-methylated in LUAD samples (**Figure 4**). This result suggests that promoter methylation and *FLCN* expression are positively correlated in LUAD. This correlation demonstrated *FLCN*'s therapeutic potential in the pathophysiology of LUAD, suggesting that this cancer type may target *FLCN* for therapeutic therapies.

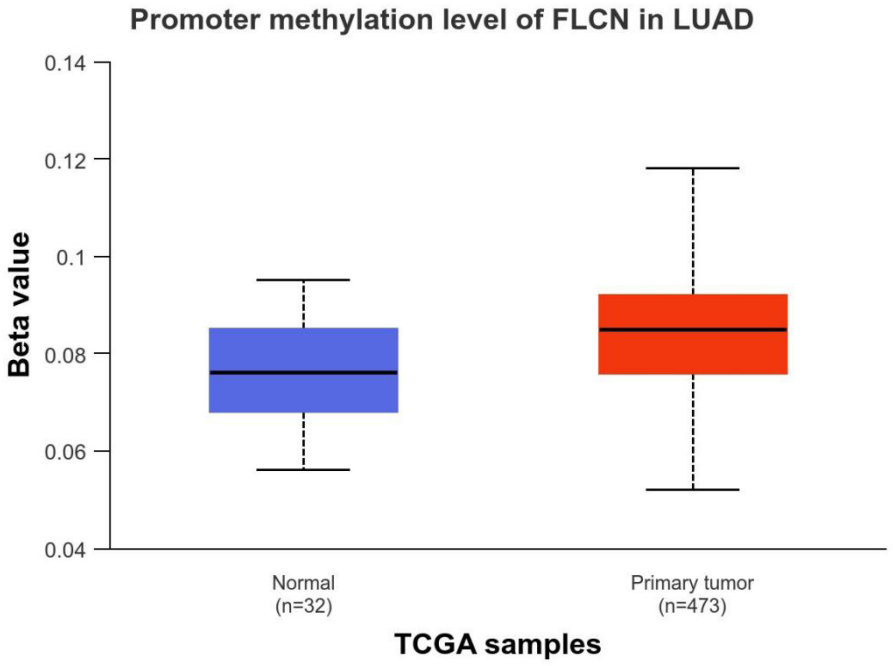

Figure 4. Promoter methylation pattern of *FLCN* in LUAD and normal control samples.

3.5. Promoter methylation of FLCN in LUAD across different clinical parameters

To learn more about the *FLCN* promoter methylation in LUAD, the study looked at several clinical parameters (**Figure 5**). In short, the study looked at *FLCN* promoter methylation and compared LUAD cancer progression phases to normal control data. Crucially, there were variations seen between phases; all four stages displayed hyper-methylation, in contrast to normal control samples (**Figure 5A**). Using the race of the LUAD patients as a criterion, the study examined *FLCN* promoter methylation. This study discovered evidence that all three racial groups (Asian, African-American, and Caucasianhad) had hyper-methylation in the *FLCN* promoter area as compared to normal control samples (**Figure 5B**). Subsequently, the *FLCN* promoter methylation was assessed, and the findings indicated hyper-methylation in both the male and female subjects (**Figure 5C**). Finally, the study examined the relationship between patient age and *FLCN* promoter methylation, finding that all age groups had methylation levels that varied across age groups and were significantly hyper-methylated when compared to normal samples (**Figure 5D**). These comprehensive analyses reveal the unexpected correlation between varying clinical parameters in LUAD and the *FLCN* promoter methylation, leading to a consistent pattern of hyper-methylation in the *FLCN* promoter methylation level in LUAD and highlighting its possible involvement in the genesis of LUAD cancer.

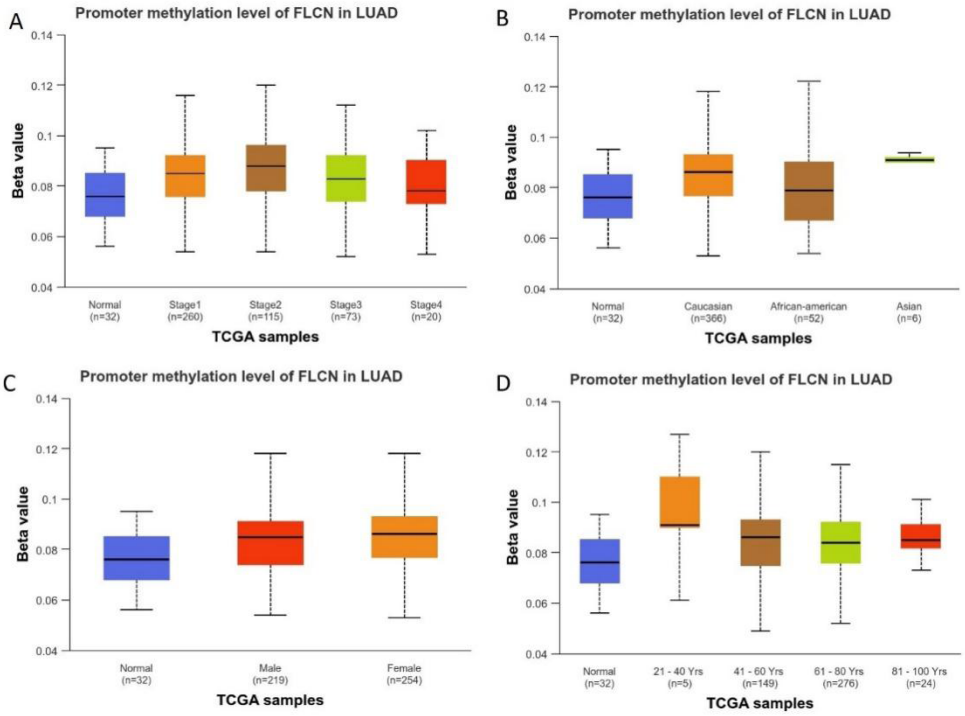

Figure 5. *FLCN* promoter methylation pattern across different clinical parameters.

3.6. FLCN survival analysis

The study used the KM plotter tool to create an assessment for overall survival (OS) and disease-free survival (DFS) in LUAD. These findings indicate that patients with LUAD who expressed low levels of *FLCN* had a better overall survival (OS) than those who expressed high levels of CDON (**Figure 6A**). Furthermore, in a disease-free survival (DFS) trial, LUAD patients with high *FLCN* expression performed worse than those with low *FLCN* expression. The studies emphasize the critical role that *FLCN* plays in determining patient survival

outcomes, highlighting its potential therapeutic utility as a prognostic marker in lung cancer therapy and implying its involvement in the course and progression of LUAD cancer.

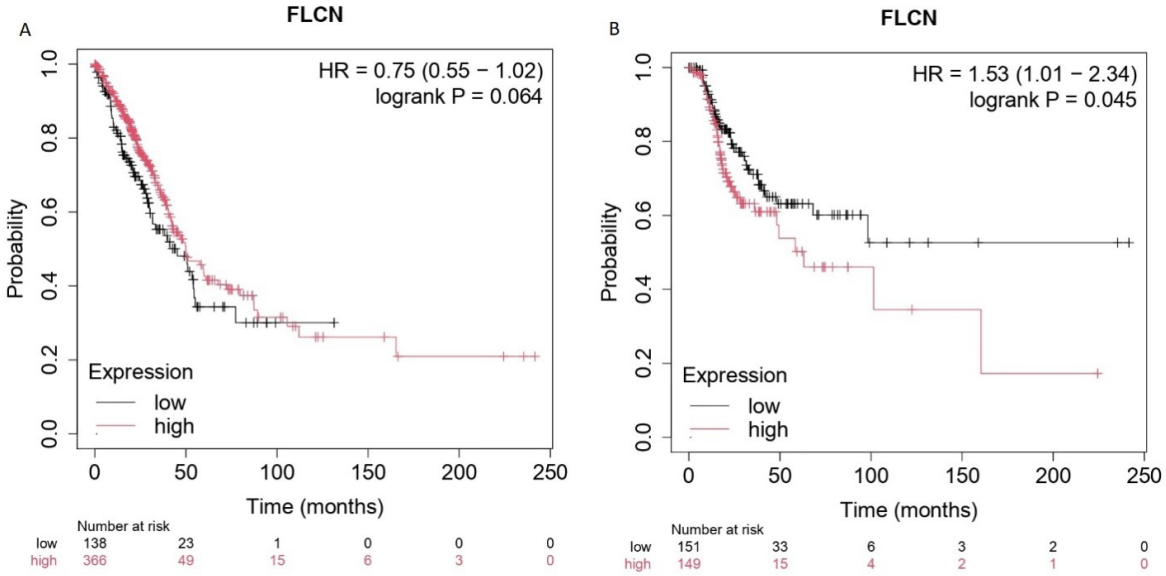

Figure 6. KM survival curve (OS, RFS) of *FLCN* in LUAD patients.

3.7. Survival validation of FLCN

Using the GEPIA2.0 informational tool, this study investigated the prognostic efficacy of *FLCN* expression in LUAD tumor progression. First, the LUAD patients were divided into two groups according to the levels of *FLCN* expression: low and high. In contrast to the high *FLCN* expression group, a low *FLCN* expression in LUAD was associated with great overall survival (OS) (**Figure 7A**). Next, in LUAD, a low *FLCN* expression level was linked with good disease-free survival (DFS) (**Figure 7B**). These results demonstrate the critical role that the *FLCN* gene plays in the initiation and expansion of LUAD cancer.

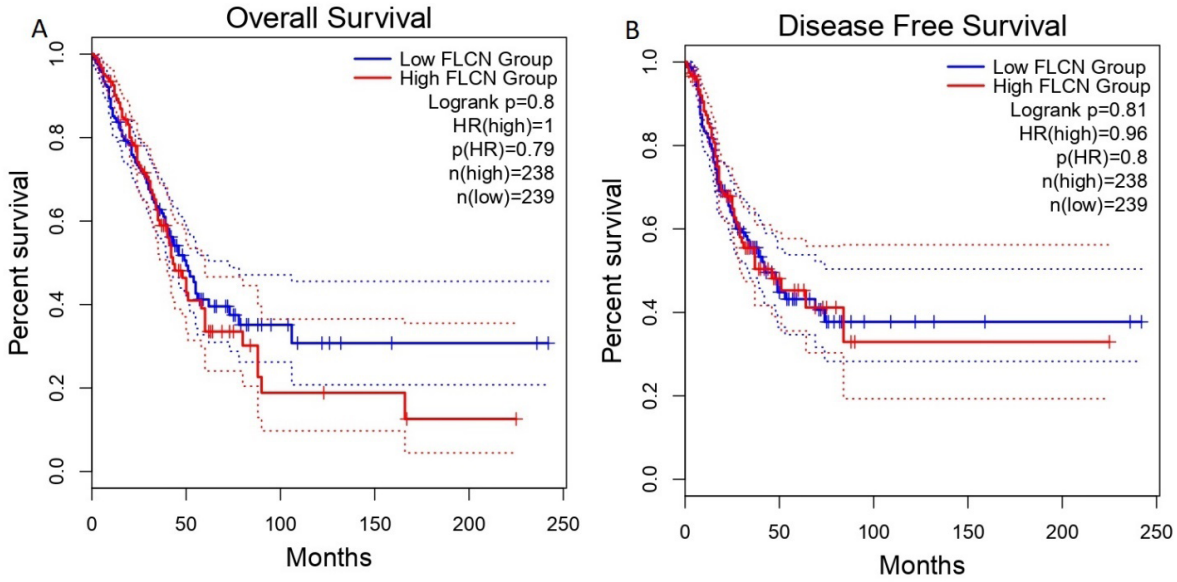

Figure 7. Survival curve (OS, RFS) of *FLCN* in LUAD patients.

3.8. Mutational analysis of FLCN

FLCN genetic changes in LUAD patients were observed using cBioPortal. The result show that genetic alterations in *FLCN* were only seen in 1% of LUAD samples. Among the chromosomal changes analyzed in LUAD were, truncating, in-frame and missense mutations (**Figure 8**), which may be essential to *FLCN* dysregulation in LUAD, even though genetic changes in *FLCN* are uncommon in LUAD.

Figure 8. Oncoplot of *FLCN* in LUAD cancer.

3.9. Protein-protein interaction (PPI) network of FLCN

The structural and functional relationships between the *FLCN* and DEG proteins were examined using the STRING program. The creation of PPI networks demonstrated the diversity of *FLCN* genes by revealing connections between the *FLCN* hub gene and 10 other genes, such as LAMTOR1, LAMTOR4, SLC38A9, RRAGC, RRAGA, LAMTOR2, RRAGB, RRAGD, FNP2, and FNP1. This suggests that *FLCN* is involved in many biological processes, performs a variety of tasks, and interacts powerfully with related genes.

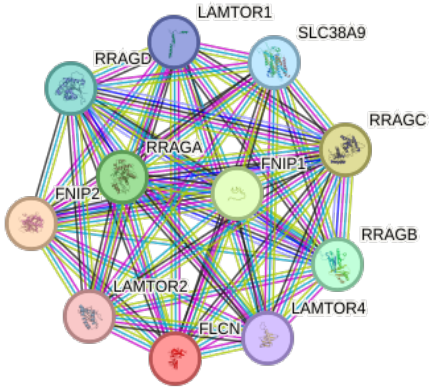

Figure 9. Protein-protein interactions of *FLCN*.

3.10. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The DAVID online tool was used to functionally annotate DEGs. The KEGG pathway-enriched genes and their possible GO (Gene Ontology) classification were studied using terms indicating biological processes, molecular activities, and cellular components related to KEGG pathways. The gene ontology BP analysis revealed that the GEGs are enriched in negative regulation of TORC1 signaling (GO:1904263), positive regulation of TOR signaling (GO:0032008), cellular response to amino acid stimulus (GO:0071230), cellular response to starvation (GO:0009267), protein localization (GO:0008104) (**Table 1** and **Figure 10A**). The gene ontology CC analysis revealed that the GEGs are enriched in FNIP-folliculin RagC/D GAP (GO:1990877), lysosomal membrane (GO:0005765), lysosome (GO:0005764), Gtr1-Gtr2 GTPase complex (GO:1990131), ragulator complex (GO:0071986) (**Table 2** and **Figure 10B**). The gene ontology MF analysis revealed that the GEGs are enriched in GTPase binding (GO:0051020), molecular adaptor activity (GO:0060090), protein-membrane adaptor activity

(GO:0043495), guanyl-nucleotide exchange factor activity (GO:0005085), GTPase activity (GO:0003924) (**Table 3** and **Figure 10C**). The analysis of KEGG enrichment pathways showed that DEGs are involved in mTOR signaling pathway (hsa04150), autophagy – animal (hsa04140), shigellosis (hsa05131) (**Table 4** and **Figure 10D**).

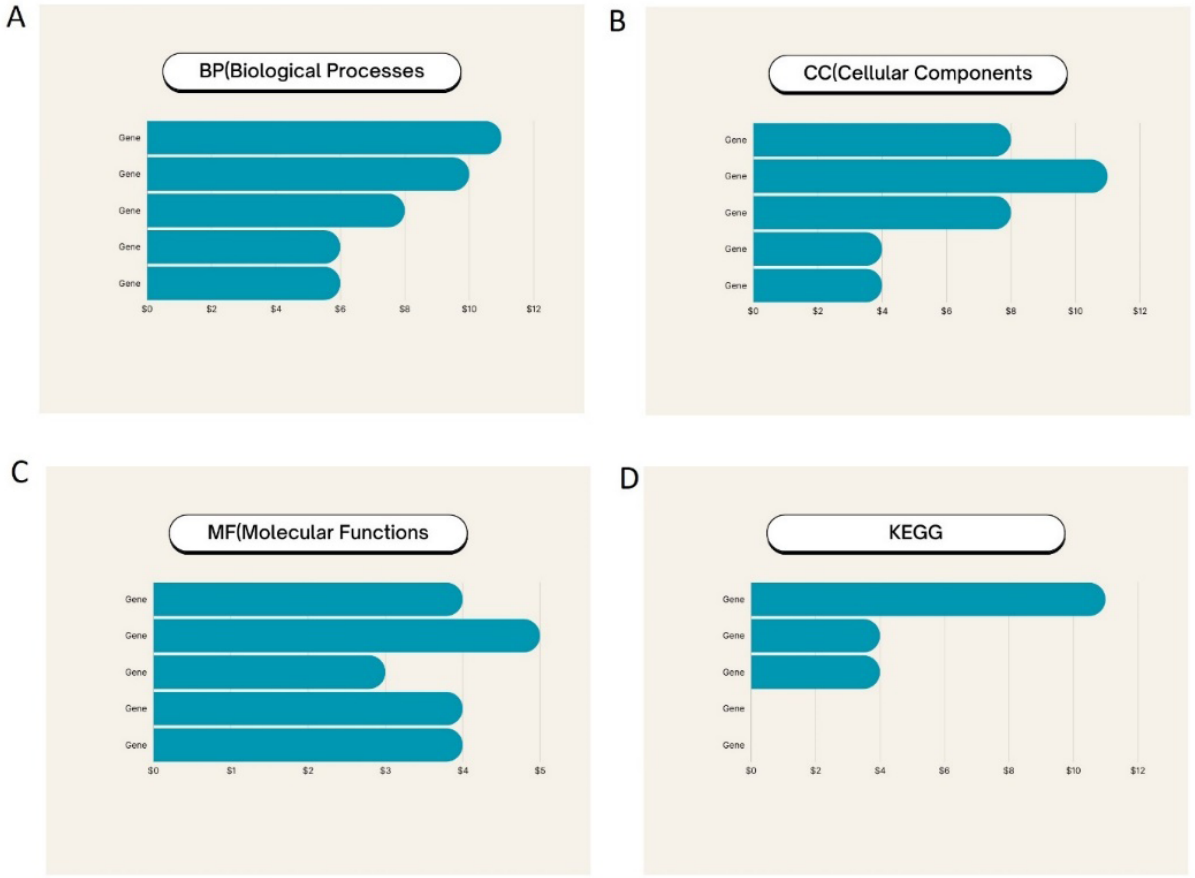

Figure 10. GO and KEGG analysis of *FLCN* by DAVID tool.

Table 1. Gene enrichment analysis (BP)

Gene term	Gene count	Genes	*P*-value
GO:1904263–positive regulation of TORC1 signaling	11	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1, FNIP2	1.4459001395953574E-26
GO:0032008–positive regulation of TOR signaling	10	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1	1.6948069698738623E-24
GO:0071230–cellular response to amino acid stimulus	08	RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9,LAMTOR2, LAMTOR1, LAMTOR4	3.19919449470896E-16
GO:0009267–cellular response to starvation	06	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, FNIP1	1.9672145764390107E-10
GO:0008104–protein localization	06	RRAGA, RRAGC, RRAGB, RRAGD, LAMTOR2, LAMTOR1	6.407977009996166E-9

Table 2. Gene enrichment analysis (CC)

Gene term	Gene count	Genes	*P*-value
GO:1990877–FNIP-folliculin RagC/D GAP	08	*FLCN*, RRAGA, RRAGC, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP2	4.187068176178242E-23
GO:0005765–lysosomal membrane	11	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1, FNIP2	6.402440946010609E-18
GO:0005764–lysosome	08	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR1, LAMTOR4	1.752157273260692E-11
GO:1990131–Gtr1-Gtr2 GTPase complex	04	RRAGA, RRAGC, RRAGB, RRAGD	3.160221627082143E-10
GO:0071986–ragulator complex	04	SLC38A9,LAMTOR2,LAMTOR1, LAMTOR4	1.5793162005874317E-9

Table 3. Gene enrichment analysis (MF)

Gene term	Gene count	Genes	*P*-value
GO:0051020–GTPase binding	04	RRAGC, RRAGB, RRAGD, LAMTOR1	9.791331088746019E-7
GO:0060090–molecular adaptor activity	05	RRAGC, RRAGD, LAMTOR2,LAMTOR1, LAMTOR4	1.7852304320294049E-6
GO:0043495–protein-membrane adaptor activity	03	RRAGA, RRAGC, LAMTOR1	1.343050898930897E-4
GO:0005085–guanyl-nucleotide exchange factor activity	04	SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4	2.0318898449270994E-4
GO:0003924–GTPase activity	04	RRAGA, RRAGC, RRAGB, RRAGD	6.778171602462938E-4

Table 4. Gene enrichment analysis (KEGG)

Gene term	Gene count	Genes	*P*-value
hsa04150: mTOR signaling pathway	11	*FLCN*, RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1, FNIP2	2.5004154144868723E-18
hsa04140: autophagy - animal	04	RRAGA, RRAGC, RRAGB, RRAGD	7.462133456980746E-4
hsa05131: shigellosis	04	RRAGA, RRAGC, RRAGB, RRAGD	0.002287907276650819

3.11. Correlation analysis

The Mann–Whitney U analysis was carried out to identify mutant genes correlated with *FLCN* expression. The study selected the top five mutant genes for LUAD, with $P < 0.05$ and FC >1.4, by using the muTarget database. The top five mutant genes that positively correlated with the expression *FLCN* are PLA2G16, CARS2, SLCO4A1, SCN9A, and BRI3 in LUAD. Collectively, these results suggested that *FLCN* gene expression has a strong correlation with different mutant genes in LUAD (**Figure 11**).

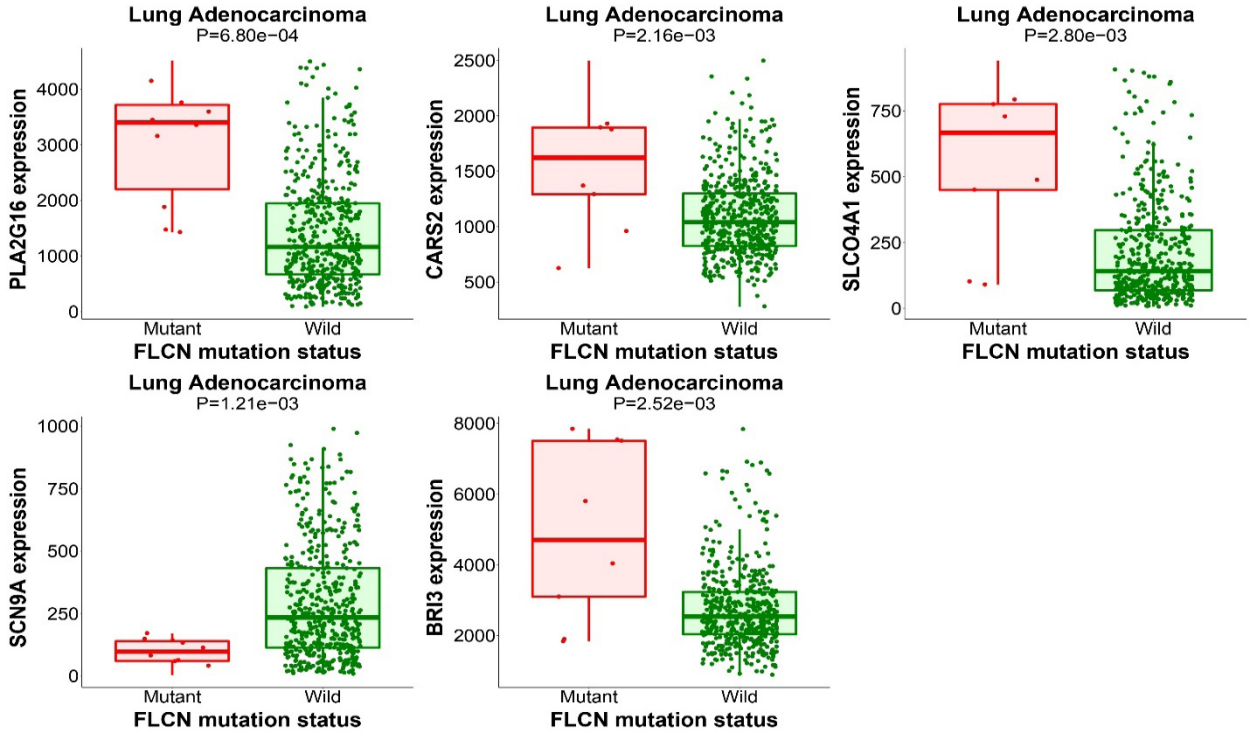

Figure 11. Correlation between *FLCN* expression in LUIAD.

4. Discussion

To conduct an assessment in LUAD, this study examined *FLCN* expression, prognosis, methylation, survival, mutations, and gene enrichment in this research article utilizing a variety of online bioinformatics tools. Furthermore, correlations between *FLCN* expression and crucial mutant genes also assessed. The results illustrated the importance of *FLCN* expression for human health as a putative regulator in the pathogenesis of LUAD and proposed a possible link between *FLCN* expression and the proliferation of LUAD tumors.

Globally, lung adenocarcinoma is the sixth most common cause of cancer-related fatalities. Most LUAD patients who have been diagnosed are in the middle and late stages, with many metastases, and have missed the best time to begin therapy because of the ambiguous early symptoms [31]. The advancement of gene sequencing technologies in recent times has expanded the comprehension of the molecular pathogenesis of lung cancer. This enhances the overall survival of patients with advanced or metastatic diseases and encourages the discovery of new molecular markers and targeted therapies [32]. A growing body of research has shown that hypoxia signaling modulates the development of LUAD, and genes associated with hypoxia may act as predictive indicators for individuals with LUAD. According to reports, hypoxia-stimulated GBE1 expression controls metabolic reprogramming, which aids in the progression of LUAD [33]. The functions of hypoxia-related DNA methylation-driven genes in the development of LUAD are revealed by genome-wide analysis [34]. The poor prognosis of LUAD and medication resistance are caused by hypoxia-induced cell stemness [35].

The human folliculin (*FLCN*) gene encodes at least two major transcript variants on chromosome 17p11.2. Transcript variant 1 encodes the longer isoform that is 3,723 bp in length containing 14 exons of which 11 exons are coding. Transcript variant 2 is a shorter isoform containing 8 exons and uses an alternate splice site in the 3’

coding region to produce a distinctly different carboxy (C)-terminus compared to transcript variant 1. A 3.8 kb *FLCN* transcript was found by northern blot analysis in a wide range of adult tissues, including the brain, heart, placenta, testis, skin, lung, and kidney, as well as in the lung, liver, brain, and kidney of fetuses [16]. In BHD families and in families where spontaneous pneumothorax is the only symptom, more than 150 distinct mutations covering the whole *FLCN* coding area have been found and recorded in the FLCN Leiden Open Variation Database [36]. After discovering *FLCN* mutations in the hereditary kidney cancer form known as BHD syndrome, scientists looked into the possibility that *FLCN* mutations could also be the cause of random renal cancers that shared histological similarities with BHD-associated tumors. On the other hand, there was very little *FLCN* mutation frequency in sporadic kidney cancers [37]. There are now two documented naturally-occurring animal models of BHD, that support *FLCN* role as a tumor suppressor. The Nihon rat model of BHD, a type of Sprague-Dawley rat that spontaneously acquired a germline *FLCN* mutation, develops kidney tumors [38]. The initial indications of *FLCN* possible involvement in mTOR pathway modulation came from studies conducted in vivo in animals lacking in *FLCN*. Mice with *FLCN* inactivation directed towards the kidney distal nephron experienced polycystic kidneys and cystic renal disease before succumbing to renal failure at three weeks of age [39,40]. Studies in *FLCN* deficient *in vitro* and *in vivo* models provide further support for regulation of PPARGC1A by *FLCN*. A genetic analysis of the primary gene responsible for BHD syndrome, the *FLCN* gene, is crucial to the final diagnosis of BHD syndrome. More than 200 pathogenic or probably pathogenic *FLCN* variations are present in the HGMD database. The majority of *FLCN* variations, including nonsense, frameshift, and splice site variants, are truncating variants. Regarding *FLCN* c.1432 + 1G > A, which is also referred to as IVS12 + 1G > A, separate investigations have identified this classical splicing site mutation in individuals with BHD syndrome [23].

The current assessment used the UALCAN database to find *FLCN* expression in LUAD. Studies have indicated that the expression of *FLCN* is elevated in a variety of cancer stages, types of cancer development, age, gender, and racial groups. The primary objective of the flow outcome is to show that LUAD tissues exhibited notably greater levels of *FLCN* expression than normal control samples in relation to the progression of the tumor. The STRING and DAVID tools research also demonstrated the diversity of the *FLCN* gene and how it interacts with other genes to be a crucial part of several biological pathways and activities. Additionally, muTarget online tool was used to assess the correlation between *FLCN* expression and crucial mutant genes. The results indicated that the degree of *FLCN* expression in LUAD was a different unfavorable prognostic factor. Subsequent analyses ought to focus on the prognostic significance of *FLCN* expression at different stages of cancer development.

5. Conclusion

In this study, several bioinformatics-based online databases were used to thoroughly examine *FLCN* in LUAD cancer type. According to this study, there was a significant increase in *FLCN* expression, which was linked to aggressive clinicopathological aspects of LUAD, as well as survival duration and metastasis. When considered collectively, these findings demonstrated that *FLCN* has carcinogenic functions and may be a viable target for treatment in individuals suffering from LUAD.

Disclosure statement

The authors declare no conflict of interest.

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