Journal of Biosciences and Medicines, 2024, 12, 89-115
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Analysis and Review of Downregulated Actin Cytoskeletal Proteins in Non-Small Cell Lung Cancer

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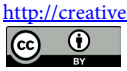
How to cite this paper: Mageed, H.M.A., Sahu, P. and Sundararajan, R. (2024) Analysis and Review of Downregulated Actin Cytoskeletal Proteins in Non-Small Cell Lung Cancer. *Journal of Biosciences and Medicines*, **12**, 89-115.
https://doi.org/10.4236/jbm.2024.124009

Received: February 19, 2024
Accepted: April 9, 2024
Published: April 12, 2024



Abstract

Actin, a highly conserved protein, plays a dominant role in Non-small cell lung cancer (NSCLC). Late diagnosis and the aggressive nature of NSCLC pose a significant threat. Studying the clinic pathological properties of NSCLC proteins is a potential alternative for developing treatment strategies. Towards this, 35 downregulated actin cytoskeletal proteins on NSCLC prognosis and treatment were studied by examining their protein-protein interactions, gene ontology enrichment terms, and signaling pathways. Using PubMed, various proteins in NSCLC were identified. The protein-protein interactions and functional associations of these proteins were examined using the STRING database. The focal adhesion signaling pathway was selected from all available KEGG and Wiki pathways because of its role in regulating gene expression, facilitating cell movement and reproduction, and significantly impacting NSCLC. The protein-protein interaction network of the 35 downregulated actin cytoskeleton proteins revealed that ACTG1, ACTR2, ACTR3, ANXA2, ARPC4, FLNA, TLN1, CALD1, MYL6, MYH9, MYH10, TPM1, TPM3, TPM4, PFN1, IQGAP1, MSN, and ZXY exhibited the highest number of interactions. Whereas HSPB1, CTNNA1, KRT17, KRT7, FLNB, SEPT2, and TUBA1B displayed medium interactions, while UTRN, TUBA1B, and DUSP23 had relatively fewer interactions. It was discovered that focal adhesions are critical in connecting membrane receptors with the actin cytoskeleton. In addition, protein kinases, phosphatases, and adapter proteins were identified as key signaling molecules in this process, greatly influencing cell shape, motility, and gene expression. Our analysis shows that the focal adhesion pathway plays a crucial role in NSCLC and is essential for developing effective treatment strategies and improving patient outcomes.

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Keywords

Non-Small Cell Lung Cancer, NSCLC, Actin, Actin Cytoskeletal Proteins, Focal Adhesion, KEEG Pathway

1. Introduction

Lung cancer, a highly aggressive solid tumor, is the leading cause of cancer-related deaths worldwide, with as high as 1.8 million deaths in 2020 out of the approximately 2.2 million new cases [1] [2]. The five-year survival rate for lung cancer cases ranges from 15% to 22%, depending on the histological subtypes and diagnosis stages [3]. Early prediction of lung cancer could aid in classifying patients according to their risk level to determine the most effective treatment regimens [4]. Several lifestyle and environmental factors are linked to lung cancer, with smoking being the most significant factor (around 85% to 90% of cases) [5]. Exposure to second-hand smoke, radon, metals, and certain toxins also increases the risk [6]. Other risk factors include a history of Hodgkin lymphoma or breast cancer, pulmonary fibrosis, human immunodeficiency virus infection, alcohol consumption, and specific genetic mutations [7] [8] [9] [10].

Although most cases are associated with known risk factors, a small percentage occur in individuals with no identifiable risk factors [11]. Lung cancer is classified into two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), based on the type of cells involved in the cancer. **Table 1** lists the salient differences between these two types of lung cancer [12] [13] [14] [15].

SCLC represents only 15% of lung cancer cases, while NSCLC accounts for about 85% of all lung cancer cases [16]. The primary cause of cellular changes leading to NSCLC is tobacco smoke, which is responsible for around 80% of lung cancer cases in men and 90% in women [17]. Adenocarcinoma and squamous cell carcinoma are the most common subtypes, accounting for 50% and 30% of NSCLC cases, respectively [18]. Squamous cell carcinoma cells display features, such as the presence of intercellular bridges and keratinization, and have a high degree of mutation frequency [19] [20]. NSCLC is also believed to be caused by genetic mutations or changes in lung cells due to exposure to harmful substances such as tobacco smoke, second-hand smoke, radon, asbestos, and air pollution.

Risk factors for NSCLC include age, family history, exposure to second-hand smoke, and exposure to mineral and metal particles or asbestos [21]. Other risk factors include a family history of lung cancer, previous radiation therapy to the chest, and certain lung diseases such as chronic obstructive pulmonary disease (COPD) or pulmonary fibrosis [22].

The diagnosis and selection of effective therapeutic interventions for NSCLC are challenging due to their heterogeneous histological subtypes [23]. NSCLC has a

Table 1. Main differences between SCLC and NSCLC.

Feature	Small Cell Lung Cancer (SCLC)	Non-Small Cell Lung Cancer (NSCLC)
Cell Shape and Size	Small, round cells tightly packed together	Several different types of cells, including squamous cells, adenocarcinoma cells, and large cell carcinoma cells.
Cell Characteristics	Highly aggressive, grows and spreads quickly, early metastasis	Grows and spreads more slowly
Incidence	Accounts for 10% - 15% of all lung cancer cases	Accounts for 85% - 90% of all lung cancer cases
Risk Factors	Strongly associated with smoking, exposure to second-hand smoke, exposure to radon, exposure to asbestos, and a family history of lung cancer	Similar to SCLC, including smoking, exposure to second-hand smoke, exposure to radon, exposure to asbestos, and a family history of lung cancer
Symptoms	Similar to NSCLC, including cough, shortness of breath, chest pain, fatigue, and weight loss. It may also cause neurologic problems, including difficulty with coordination and memory loss	Similar to SCLC, including cough, shortness of breath, chest pain, fatigue, and weight loss
Treatment	Usually treated with chemotherapy and radiation therapy	Treated with surgery, radiation therapy, chemotherapy, targeted therapy, or a combination of these treatments. In some cases, immunotherapy may also be used
location	Tumors are located in the center of the lungs, often near the bronchi	Tumors are in the outer regions of the lungs. They may also be found in the center of the lungs.
Growth rate	Highly aggressive, grows and spreads quickly, early metastasis	Tend to grow and spread more slowly than small cell lung cancer (SCLC) tumors

poor prognosis due to its high proliferative and metastatic potentials [24]. Unfortunately, many cases of NSCLC are diagnosed at advanced stages due to the lack of noticeable symptoms in the early stages [25]. Therefore, new approaches are needed to develop effective therapeutic interventions [26]. Understanding the protein interactions and pathways that drive the progression of lung cancer tumors can lead to the identification of enhanced therapeutic approaches [27] [28].

A better understanding of the molecular pathways and efficient protein networks that drive NSCLC progression has been highlighted [29] [30]. It is beneficial to study the various protein interactions, their functions and structures, as well as metabolism changes at dissimilar stress conditions [31]. Protein expression varies and is mainly regulated at the transcriptional, translational, and

post-translational levels [32]. Cytoskeletal proteins are present in the interstitial cells of the lungs and play a critical role in controlling lung cancer, as they are involved in various stages of immune cell activation and effector function, as well as cellular transformation [33]. Actin is a highly conserved protein involved in various types of cell motility and maintenance of the cytoskeleton [34]. The most notable alteration in lung cancer cells is the downregulation of the majority of proteins involved in the regulation and function of the Actin Cytoskeleton. Additionally, the downregulated proteins play an effective role in killing cancer cells [35]. Preclinical studies have also shown that cell lines of NSCLC are sensitive to focal adhesion kinase inhibition [36].

This study provides a comprehensive analysis of the role of the downregulated actin cytoskeletal proteins in NSCLC, offering insights into their interactions, signaling pathways, and clinical features. The findings of this study have the potential to identify novel biomarkers and drug targets of new therapies and treatment strategies for NSCLC based on targeting these proteins. By examining the interactions among the 35 downregulated actin cytoskeletal proteins [37], the study seeks to identify which proteins are most closely associated with NSCLC.

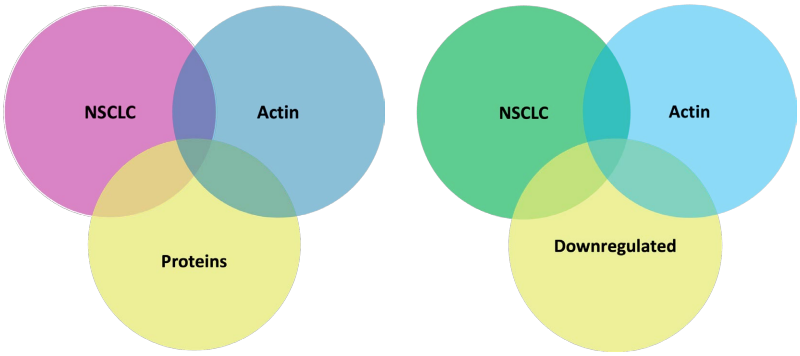
Furthermore, this research investigates the signaling pathways involved in NSCLC development and progression, specifically focusing on the focal adhesion pathway, which plays a crucial role in regulating cell motility, survival, gene expression, proliferation, and differentiation in NSCLC. The paper also analyses the clinical features of NSCLC and how they relate to these actin cytoskeletal proteins, providing insight into the potential for targeting these proteins as a means of treating NSCLC.

2. Methods

Using a literature search on the most common websites, such as Google Scholar and library databases called PubMed [38], downregulated actin cytoskeletal proteins in NSCLC were identified. By using the search functions provided by these databases, we find relevant articles and research on specific topics of our interest *i.e.* NSCLC. In this review, we focus on the combination of keywords *i.e.* "NSCLC" AND "Actin" AND "Proteins" for searching relevant articles. Further, to study the mechanism of action of protein/gene, the keyword combination for searching used was "NSCLC" AND "Actin" AND "Downregulated". The two searching strategies are shown in **Figure 1** as Venn diagrams. The details of the search results are shown in **Table 2**.

Table 2. The search result of the keyword combinations strategy used in the PubMed database.

Set No	Searching For	Database	Useful Hits
S1	"NSCLC" AND "Actin" AND "Proteins"	Engineering Village	120
S2	"NSCLC" AND "Actin" AND "Downregulated"	Engineering Village	21



(a) (b)

Figure 1. The Venn diagram of the two searching strategies.

A similar search was done on Google Scholar with the same keyword combination. Google Scholar, in general, covers a broader selection of articles compared to PubMed, and the top 10 results were selected.

Subsequently, after selecting the 35 downregulated actin cytoskeletal proteins in NSCLC [37], the protein-protein interactions and functional associations of these proteins were analyzed using the STRING database, and key players and pathways involved in the disease could be identified. Moreover, the molecular weight, chromosomes, subcellular locations, and functions of these proteins were studied to understand their roles in the cellular processes of NSCLC. The integration of these characteristics into the analysis involves combining data from various sources, including protein databases (e.g., UniProt, NCBI), bioinformatics tools (e.g., STRING for protein-protein interactions [39], DAVID [40], GO enrichment [41]), and pathway databases (e.g., KEGG [42], Wiki Pathways [43]).

For instance, investigating the biological and functional significance of these proteins involved examining the top ten gene ontology (GO) enrichment terms of biological processes, molecular function, and cellular components. This allowed for a more in-depth understanding of the cellular processes that are affected by the downregulation of these proteins and how they contribute to the development of NSCLC.

Similarly, the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Wiki pathways were considered to explore the underlying mechanism behind the effects of NSCLC. The study focused on the focal adhesion signaling pathway as it plays a crucial role in NSCLC. This pathway has been shown to control gene expression, cell movement, and reproduction, making it a key player in the development and progression of NSCLC [44] [45] [46]. By understanding how downregulated actin cytoskeletal proteins affect this pathway, researchers can pinpoint the molecular mechanisms driving NSCLC and explore new therapeutic avenues.

3. Results

Table 3 lists the 35 downregulated actin cytoskeletal proteins in NSCLC [37], and **Figure 2** shows the string interaction diagram of the proteins that formed a

Table 3. List of 35 downregulated Actin Cytoskeletal proteins.

Protein	Description	Protein	Description	Protein	Description
ACTG1	Actin, cytoplasmic 2	IQGAP1	Ras GTPase-activating-like protein	Sept2	Septin 2
ACTR2	Actin-related protein 2	KRT17	Keratin, type I cytoskeletal 17	TAGLN	Transgelin
ACTR3	Actin-related protein 3	KRT7	Keratin, type II cytoskeletal 7	TAGLN2	Transgelin-2
ANXA2	Annexin A2	MSN	Moesin	TLN1	Talin-1
ARPC4	Actin-related protein 2/3 complex subunit 4	MYH10	Myosin-10 isoform 3	TMSB4X	Thymosin beta-4
CALD1	Caldesmon isoform 2	MYH9	Myosin-9	TPM1	Tropomyosin alpha-1 chain
CTNNA1	Catenin alpha-1	MYL6	Myosin light polypeptide 6	TPM3	Tropomyosin alpha-3 chain
DUSP23	Dual specificity protein phosphatase 23	MYL9	Myosin regulatory light polypeptide 9	TPM4	Tropomyosin alpha-4 chain
FLNA	Filamin-A isoform 2	MYO1C	Myosin-Ic	TUBA1B	Tubulin alpha-1B chain
FLNB4	Filamin-B isoform 4	NDRG1	Protein NDRG1	UTRN	Utrophin
FLNB1	Filamin-B isoform 1	PDLIM7	PDZ and LIM domain protein 7	ZYX	Zyxin
HSPB1	Heat shock protein beta-1	PFN1	Profilin-1	-	-

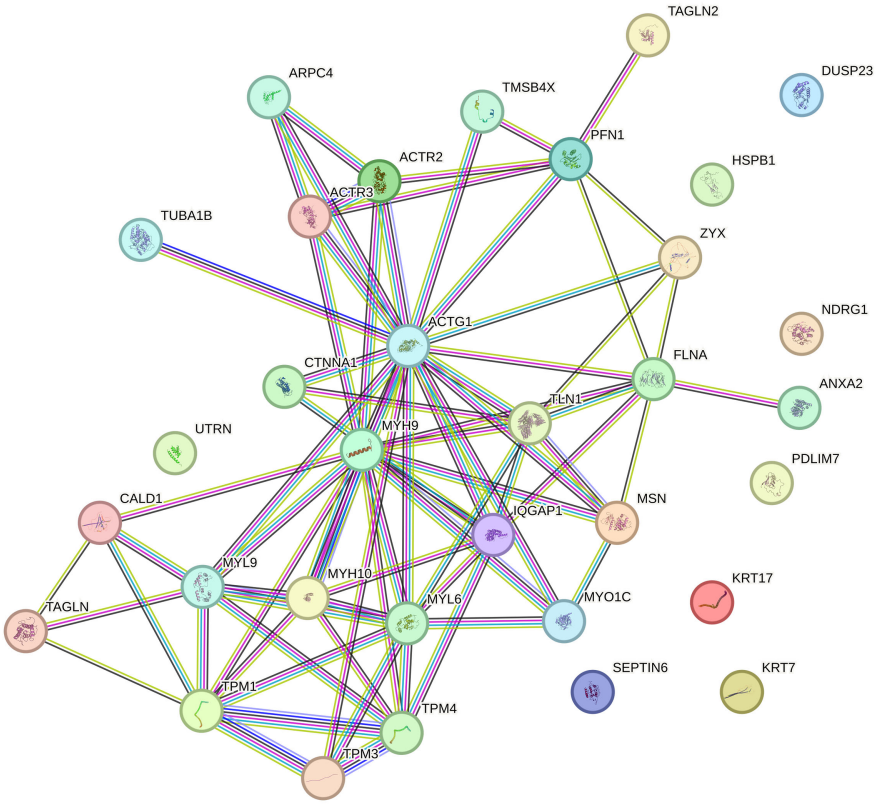


Figure 2. String interaction diagram (high confidence = 0.7) of the significantly downregulated Actin Cytoskeletal proteins.

much-interconnected network [39] [47]. The greater the number of interactions (lines) between proteins, the stronger the interaction between them. Most of the proteins, such as ACTG1, ACTR2, ACTR3, ANXA2, ARPC4, FLNA, TLN1,

CALD1, MYL6, MYL6, MYH9, MYH10, TPM1, TPM3, TPM4, PFN1, IQGAP1, MSN, and ZXY show strong interactions, while HSPB1, CTNNA1, KRT17, KRT7, FLNB, SEPT2, and TUBA1B show medium interactions, and Proteins, UTRN, TUBA1B, and DUSP23 have the fewer interactions.

Table 4 shows the top 10 gene ontology (GO) enrichment terms of biological processes, molecular function, and cellular components for this string diagram, and Figure 2 shows their details.

Table 4. Top 10 GO enrichment terms of biological processes, cellular components, and molecular function for the above string diagram.

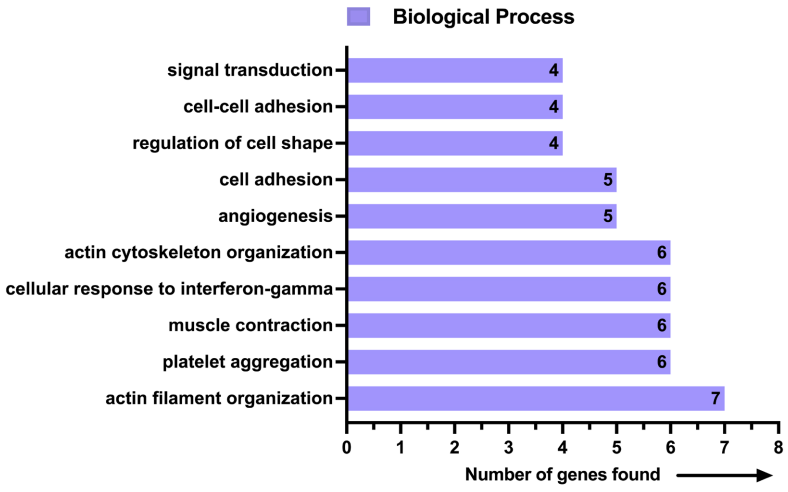
GO Enrichment Term	Description	Number of gene	Genes found
Biological Process	regulation of cell shape	4	MSN, MYH10, TPM1, MYH9
	cell-cell adhesion	4	CTNNA1, TLN1, ANXA2, MYH9
	signal transduction	4	ZYX, FLNB, IQGAP1, NDRG1
	angiogenesis	5	CALD1, FLNA, ACTG1, ANXA2, MYH9
	cell adhesion	5	MYH10, ZYX, CTNNA1, TLN1, MYH9
	platelet aggregation	6	HSPB1, FLNA, MYL9, ACTG1, TLN1, MYH9
	muscle contraction	6	CALD1, TPM3, TPM1, TPM4, UTRN, MYL6
	cellular response to interferon-gamma	6	ACTR3, ZYX, ACTG1, FLNB, MYO1C, ACTR2
	actin cytoskeleton organization	6	FLNA, PDLIM7, PFN1, FLNB, UTRN, ACTR2
	actin filament organization	7	TPM3, TMSB4X, TPM1, ACTR3, TPM4, CTNNA1, MYO1C
Cellular components	myosin II complex	4	MYH10, MYL9, MYH9, MYL6
	brush border	6	FLNA, ACTR3, FLNB, MYO1C, MYH9, MYL6
	adherens junction	8	PDLIM7, MSN, ZYX, CTNNA1, TLN1, ANXA2, MYH9, NDRG1
	stress fiber	10	TPM3, PDLIM7, MYH10, TPM1, ZYX, TPM4, MYL9, FLNB, MYO1C, MYH9
	actin cytoskeleton	15	CALD1, TPM3, FLNA, PDLIM7, TPM1, ARPC4, ACTR3, ZYX, CTNNA1, ACTG1, FLNB, MYO1C, IQGAP1, ACTR2, MYH9
	focal adhesion	15	HSPB1, FLNA, PDLIM7, MSN, ACTR3, ZYX, TPM4, PFN1, CTNNA1, ACTG1, FLNB, TLN1, IQGAP1, ACTR2, MYH9
	extracellular exosome	22	HSPB1, TPM3, FLNA, MSN, MYH10, ARPC4, ACTR3, TPM4, PFN1, TAGLN2, ACTG1, FLNB, TLN1, ANXA2, KRT7, MYO1C, IQGAP1, UTRN, ACTR2, MYH9, MYL6, NDRG1
	cytoskeleton	26	CALD1, HSPB1, TPM3, FLNA, TMSB4X, PDLIM7, MSN, TPM1, ARPC4, ACTR3, TAGLN, TUBA1B, KRT17, ZYX, TPM4, PFN1, TAGLN2, CTNNA1, MYL9, ACTG1, FLNB, TLN1, UTRN, ACTR2, MYH9, NDRG1
	cytosol	29	CALD1, HSPB1, TPM3, FLNA, TMSB4X, PDLIM7, MSN, MYH10, DUSP23, TPM1, ARPC4, ACTR3, KRT17, ZYX, TPM4, PFN1, TAGLN2, CTNNA1, MYL9, ACTG1, FLNB, TLN1, KRT7, MYO1C, IQGAP1, ACTR2, MYH9, MYL6, NDRG1
	cytoplasm	31	CALD1, HSPB1, TPM3, FLNA, TMSB4X, PDLIM7, MSN, MYH10, DUSP23, TPM1, ARPC4, ACTR3, TAGLN, TUBA1B, KRT17, ZYX, TPM4, PFN1, CTNNA1, MYL9, ACTG1, FLNB, TLN1, ANXA2, KRT7, MYO1C, IQGAP1, UTRN, ACTR2, MYH9, NDRG1

Continued

Molecular Function	cytoskeletal protein binding	5	CALD1, MSN, TPM1, ANXA2, ACTR2
	ATP binding	6	MYH10, ACTR3, ACTG1, MYO1C, ACTR2, MYH9
	nucleotide binding	7	MYH10, ACTR3, TUBA1B, ACTG1, MYO1C, ACTR2, MYH9
	structural constituent of cytoskeleton	8	MSN, TPM1, ARPC4, ACTR3, TUBA1B, ACTG1, TLN1, ACTR2
	identical protein binding	8	HSPB1, TPM1, TPM4, CTNNA1, ACTG1, FLNB, ANXA2, MYH9
	RNA binding	9	HSPB1, FLNA, TMSB4X, ZYX, PFN1, CTNNA1, FLNB, ANXA2, MYH9
	cadherin binding	10	CALD1, FLNA, PFN1, TAGLN2, CTNNA1, FLNB, TLN1, IQGAP1, MYH9, NDRG1
	actin filament binding	16	TPM3, FLNA, MYH10, TPM1, ARPC4, ACTR3, TAGLN, TPM4, CTNNA1, FLNB, TLN1, MYO1C, IQGAP1, UTRN, ACTR2, MYH9
	actin binding	19	CALD1, TPM3, FLNA, TMSB4X, PDLIM7, MSN, MYH10, TPM1, ARPC4, ACTR3, TAGLN, TPM4, PFN1, FLNB, TLN1, MYO1C, UTRN, ACTR2, MYH9
	protein binding	33	CALD1, HSPB1, TPM3, FLNA, TMSB4X, PDLIM7, MSN, MYH10, DUSP23, TPM1, ARPC4, ACTR3, TAGLN, TUBA1B, KRT17, ZYX, TPM4, PFN1, TAGLN2, CTNNA1, MYL9, ACTG1, FLNB, TLN1, ANXA2, KRT7, MYO1C, IQGAP1, UTRN, ACTR2, MYH9, MYL6, NDRG1

The GO enrichment analysis of biological processes has identified several noteworthy, downregulated pathways that may shed light on the lung cancer mechanisms as shown in **Figure 3(a)**. Among these pathways, "actin filament organization" with seven genes (TPM3, TMSB4X, TPM1, ACTR3, TPM4, CTNNA1, MYO1C) stands out. This downregulation could disrupt the intricate organization and regulation of actin filaments within cells, potentially influencing cellular morphology and motility, which are pivotal for cancer metastasis [48]. Additionally, "platelet aggregation" (involving HSPB1, FLNA, MYL9, ACTG1, TLN1, MYH9) and "muscle contraction" (involving CALD1, TPM3, TPM1, TPM4, UTRN, MYL6) pathways are downregulated, which could impact tumor growth and cell contractility [49]. The downregulated "cellular response to interferon-gamma" pathway (involving ACTR3, ZYX, ACTG1, FLNB, MYO1C, ACTR2) suggests a potential immune evasion mechanism [50]. Moreover, disruptions in "actin cytoskeleton organization," "angiogenesis," "cell adhesion," and "cell-cell adhesion" pathways, along with altered "regulation of cell shape," may collectively contribute to changes in cell behavior and interactions within the tumor microenvironment. Although "signal transduction" is not significantly enriched, it is worth noting the involvement of genes like ZYX and FLNB [51]. These findings collectively suggest that the downregulation of these biological processes in lung cancer may contribute to disease progression and metastasis.

The GO enrichment analysis of cellular components in lung cancer has unveiled several significant downregulated cellular structures, and potential alterations in subcellular organization associated with the disease, as shown in **Figure 3(b)**. The significance of subcellular localization is assessed based on the known functions of compartments in cell biology and disease pathology [52]. The proteins localized to specific cellular compartments (e.g., mitochondria, nucleus) might be involved in relevant pathways or processes affected in NSCLC [53].



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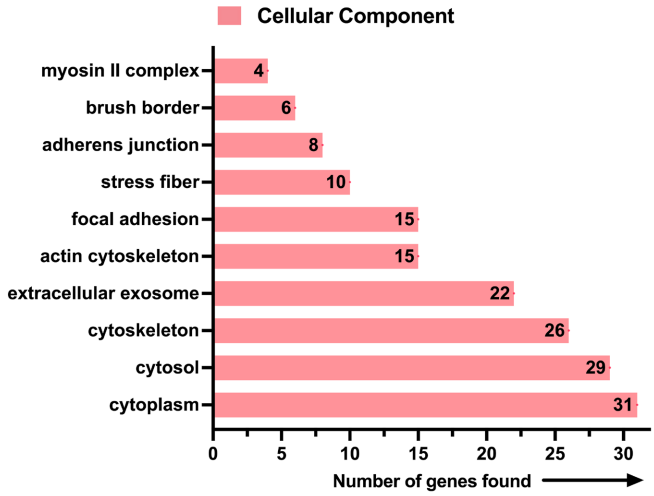

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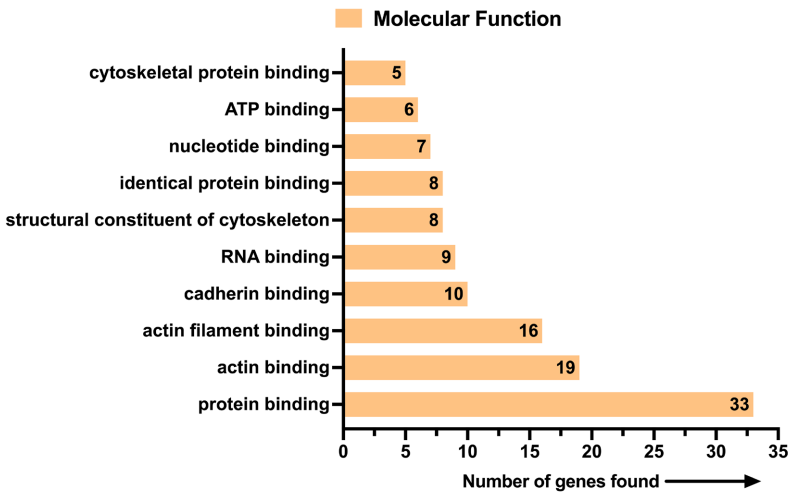

(c)

Figure 3. The top 10 gene ontology (GO) enrichment terms of (a) Biological process (b) Cellular component (c) Molecular function for 35 downregulated proteins.

Notably, "cytoskeleton" and "actin cytoskeleton" are among the most significantly downregulated components, with 26 and 15 genes found, respectively. These structures, comprising genes, such as CALD1, TPM3, FLNA, and others are integral to maintaining cell shape and motility [54]. Furthermore, "stress fiber" and "focal adhesion" structures, with 10 and 15 genes found, respectively, exhibit considerable downregulation [55]. Other components containing genes, such as TPM3, PDLIM7, ZYX, and more are crucial for cell adhesion, migration, and contractility. Interestingly, "extracellular exosome" components with 22 genes are downregulated, suggesting changes in the secretion of cellular components [56]. Additionally, the "cytosol" and "cytoplasm" show downregulation, with 29 and 31 genes, respectively, impacting the intracellular environment and potentially affecting cellular processes. "Adherens junctions" and "brush border" components of cell "membrane" are also affected, altering the cell-cell adhesion and the organization of specialized cell surface structures [57]. These findings collectively suggest a complex reorganization of cellular components in lung cancer, potentially contributing to altered cell behavior and motility within the tumor microenvironment.

The GO enrichment analysis of molecular functions has revealed the molecular alterations as shown in **Figure 3(c)**. The "protein binding" with 33 genes emerged as a central function. While not significantly enriched, its involvement suggests potential disruptions in protein-protein interactions within the cell [58]. Moreover, "actin binding", including CALD1, FLNA, and others (19 genes) is significantly downregulated. This finding indicates potential alterations in the binding of actin filaments, which play crucial roles in cellular motility and structure [59]. "Actin filament binding" is also significantly downregulated, with 16 genes, potentially impacting actin filament dynamics [60]. "Cadherin binding," with 10 genes, suggests changes in cell adhesion, while "RNA binding" (9 genes) and "structural constituent of cytoskeleton" (8 genes) point to potential disruptions in RNA-protein interactions and cytoskeletal integrity, respectively [61]. Additionally, "identical protein binding" and "nucleotide binding" (both with 8 genes) suggest potential changes in protein-protein and nucleotide-protein interactions. Lastly, "ATP binding" (6 genes) and "cytoskeletal protein binding" (5 genes) indicate potential alterations in energy utilization and cytoskeletal protein interactions [62].

Table 5 shows a partial list of various molecular weights and chromosomes of downregulated actin cytoskeletal proteins [63]. The molecular weight of a protein is an important characteristic that influences its physical and chemical properties, such as solubility, stability, and folding [64]. It is also helpful for protein identification, quantification, and purity determination. While there is no specific threshold for significance, proteins with unusually high or low molecular weights compared to the expected range for their family or function might be flagged for further investigation [65].

The chromosome location of a protein-encoding gene is equally essential, as it can provide insights into gene clusters and potential regulatory mechanisms

Table 5. Partial list of various molecular weights and chromosomes of downregulated actin cytoskeletal proteins.

Protein	Molecular weight, Da	Chromosome
ACTG1	41,793	Chr 17-17q25.3
ACTR2	44,761	Chr 2-2p14
ACTR3	47,371	Chr 2-2q14.1
ANXA2	38,604	Chr 15-15q22.2
ARPC4	19,667	Chr 3-3p25.3
CALD1	93,231	Chr7-7q33
MYH9	226,532	Chr22-22q12.3
FLNA	280,739	ChrX-Xq28
MYH10	228,999	Chr17-17p13.1
MYH9	226,532	Chr22-22q12.3
PFN1	15,054	Chr17- 17p13.2

[66]. The significance is often determined by looking for patterns, such as the enrichment of downregulated proteins in specific chromosomal regions, which could suggest a common regulatory mechanism or shared genetic vulnerability in NSCLC [67]. For instance, the genes located on sex chromosomes may exhibit different expression patterns in males and females, while genes on autosomes may be regulated differently depending on their location and proximity to other genes. Furthermore, knowledge of the chromosome location can aid in identifying genetic disorders and diseases associated with specific genes [68].

Table 6 illustrates the intracellular locations and functions of the top five Actin Cytoskeletal proteins. These five proteins were selected due to their dominant contribution to the activities.

Actins are highly conserved proteins that play a crucial role in maintaining the cytoskeleton and various types of cell motility. There are three main groups of actin isoforms in vertebrate animals: Alpha, Beta, and Gamma. Alpha actins are present in muscle tissues and constitute a significant part of the contractile apparatus. Beta and Gamma actins are present in most cell types, where they form the cytoskeleton and mediate internal cell motility. Actin Gamma 1 (ACTG1), which is encoded by this gene, is a cytoplasmic actin found in all cell types. It is involved in various types of cell motility and is expressed in all eukaryotic cells [79].

ACTR2 and ACTR3 are actin-related proteins that are integral components of the actin-related protein 2/3 (ARP2/3) complex subunit 4, a vital player in cell motility and maintaining cellular shape. The ARP2/3 complex plays a critical role in regulating the dynamics of actin filaments. It binds to the sides of actin filaments and is particularly concentrated at the leading edges of mobile cells. ACTR2 and ACTR3 have key functions in actin nucleation and branching, facilitating the formation of intricate actin networks. These networks are essential for driving crucial cellular processes, including cell migration and intracellular transport [80].

Table 6. Partial list of various intracellular locations of top 5 Actin Cytoskeletal proteins.

Protein	Intracellular location	Intracellular function
ACTG1	Cytoplasm/ cytoskeleton	Involved in various types of cell motility and is expressed in all eukaryotic cells [69].
ACTR2	Cytoplasm (but it can also shuttle to the nucleus)	Providing the force for cell motility, mediates actin polymerization upon stimulation by nucleation-promoting factor (NPF), promotes homologous recombination (HR) repair in response to deoxyribonucleic acid (DNA) damage by promoting nuclear actin polymerization, leading to drive motility of double-strand breaks (DSBs) [70] [71] [72] [73]
ACTR3	Both in the Cytoplasm and Nucleus	Regulating actin dynamics at sites of cell-cell adhesion and cell-matrix junctions. It is also involved in the formation of filopodia, which are thin, finger-like protrusions that cells use to sense their surroundings. Filopodia are important for cell migration, guidance, and communication [74].
ANXA2	Extracellular space/ Melanosome	Calcium-regulated membrane-binding protein binds two calcium ions with high affinity, less toxin binds to human cells, and less vacuolization[75] [76].
ARPC4	Cytoplasm/ Nucleus/ Cell projection	providing the force for cell motility, promotes actin polymerization in the nucleus, thereby regulating gene transcription, promotes homologous recombination (HR), and repair in response to DNA damage by promoting nuclear actin polymerization, leading to driving motility of double-strand breaks (DSBs) [77] [78].

A protein-protein string interaction network analysis was conducted to comprehensively investigate the relationship among ACTG1, ACTR2, and ACTR3. This analysis aimed to gain a deeper understanding of the interactions and associations between these proteins within the network. **Figure 4** shows the string interaction diagram of ACTG1, ACTR2, and ACTR3.

By examining the protein-protein string interactions, valuable insights were obtained, shedding light on the intricate relationship. The coordinated actions of these proteins imply that they could be involved in modifying actin dynamics and cytoskeletal rearrangements. These proteins have a number of recognised functional linkages. Moreover, they contribute to essential cellular activities *via* functioning in intracellular trafficking and vesicle transport [81]. Also, the collaboration and interactions among ACTG1, ACTR2, and ACTR3 are essential for controlling cellular migration and motility [82]. ACTG1, ACTR2, and ACTR3 are involved in actomyosin contractility, which is essential for processes like cytokinesis, cell shape changes, and tissue morphogenesis. They participate in the regulation of actin-myosin interactions and the generation of contractile forces. Meanwhile, they participate in signal transduction events, influencing cellular processes such as proliferation, differentiation, and survival. Together, they control the development of lamellipodia, cytoskeletal remodelling, and cellular protrusion, all of which are necessary for cell mobility [83].

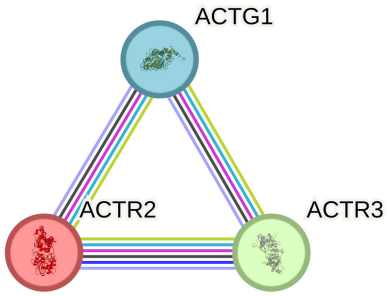

Figure 4. String interaction diagram of ACTG1, ACTR2, and ACTR3.

In addition, they are involved in the force for cell motility and the stimulation of DNA repair by promoting nuclear actin polymerization. Mutations in these genes have been linked to developmental disorders and diseases [84]. Besides, ACTG1, ACTR2, and ACTR3 contribute to cell adhesion and focal adhesion dynamics. They are involved in the formation and turnover of focal adhesions, which are sites of cell-substrate adhesion, and play a crucial role in cell migration, mechano-transduction, and signalling [85]. These functional roles demonstrate how broad and multifaceted ACTG1, ACTR2, and ACTR3 are in cellular processes, including structural support and cell adhesion, as well as contractility, signaling, and interactions with hosts and pathogens.

On the other hand, ANXA2 is a calcium-regulated membrane-binding protein that is responsible for binding two calcium ions with high affinity [86]. This protein has been shown to play a role in diverse cellular processes, including membrane trafficking, exocytosis, and endocytosis. Additionally, it has been discovered that ANXA2 has a major impact on the control of vital cellular processes such as cell adhesion, migration, and invasion in the setting of NSCLC. Studies have shown that ANXA2 is involved in modifying cellular responses and may have an impact on the progression and aggressiveness of NSCLC by decreasing toxin binding to human cells and decreasing vacuolization [87].

The present study utilized KEGG and Wiki pathways to explore the underlying mechanism behind the effects of NSCLC. **Table 7** displays the KEGG and Wiki pathways that were investigated in the study.

The main objective of this study is to analyze the pathways involved in NSCLC and gain insights into the underlying biological processes, with the goal of identifying potential therapeutic targets. To achieve this, the study focused on the focal adhesion pathway, which is known to play a crucial role in the movement and proliferation of NSCLC cells, as well as its impact on gene expression. **Figure 5** depicts the KEGG focal adhesion pathway that was studied in this research.

Figure 5 illustrates that actin string bundles are connected to the integrin family transmembrane receptors via a multi-molecular complex of junctional plaque proteins [88]. Some of the focal adhesion components participate in the structural linkage between membrane receptors and the actin cytoskeleton.

Meanwhile, other components such as protein kinases, phosphatases, and adapter proteins act as signaling molecules, which can greatly impact the reorganization of the actin cytoskeleton, cell shape, and motility, as well as gene expression. It should be emphasized that there is a strong correlation between adhesion and signaling through growth factors. When growth factors bind to their corresponding receptors, it triggers comparable alterations in gene expression and physical structure.

Table 7. List of KEGG and Wiki pathways.

Pathway	Description
KEGG	
hsa04530	Tight junction
hsa05100	Bacterial invasion of epithelial cells
hsa05410	Hypertrophic cardiomyopathy
hsa04520	Adherens junction
hsa04810	Regulation of actin cytoskeleton
hsa05414	Dilated cardiomyopathy
hsa04270	Vascular smooth muscle contraction
hsa04670	Leukocyte transendothelial migration
hsa04260	Cardiac muscle contraction
hsa05132	Salmonella infection
hsa05130	Pathogenic Escherichia coli infection
hsa04510	Focal adhesion
hsa05131	Shigellosis
hsa05205	Proteoglycans in cancer
hsa05135	Yersinia infection
hsa04261	Adrenergic signaling in cardiomyocytes
hsa04921	Oxytocin signaling pathway
Wiki	
WP383	Striated muscle contraction pathway
WP3680	Physico-chemical features and toxicity-associated pathways
WP2272	Pathogenic Escherichia coli infection
WP2572	Primary focal segmental glomerulosclerosis (FSGS)
WP51	Regulation of actin cytoskeleton
WP306	Focal adhesion
WP4217	Ebola virus pathway in host
WP3888	VEGFA-VEGFR2 signaling pathway

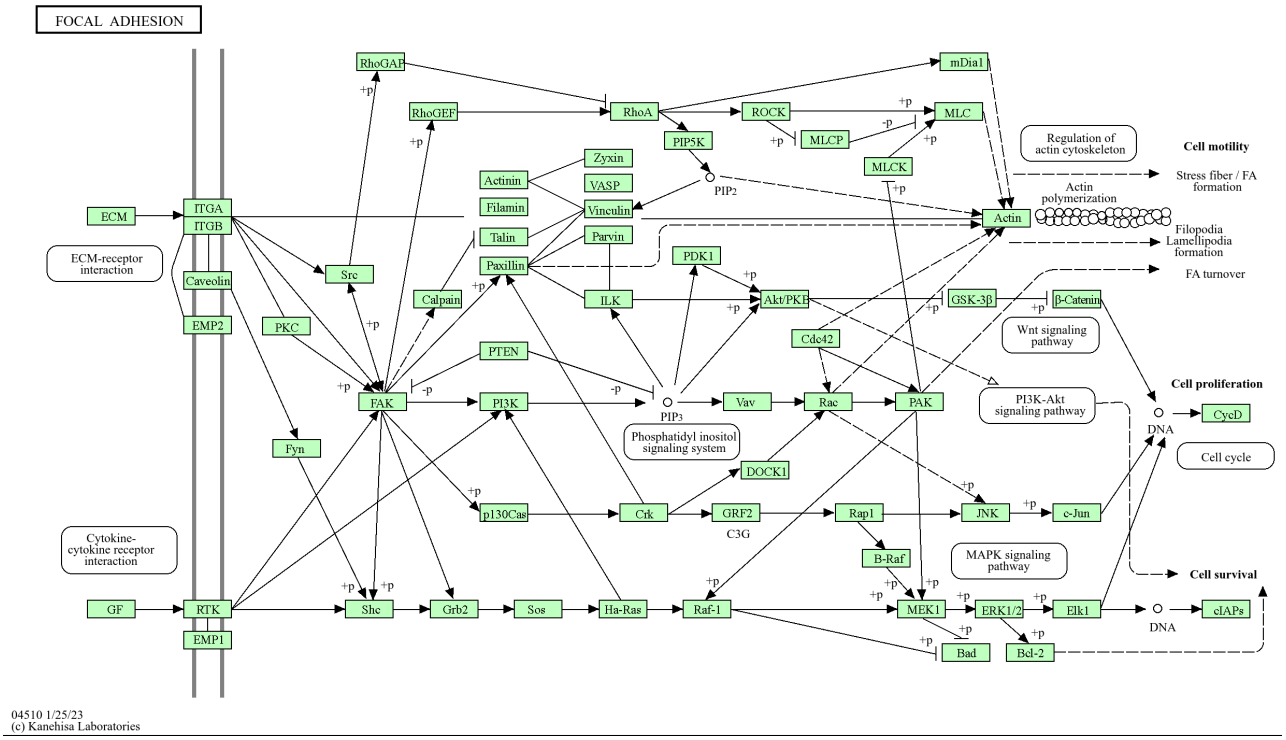

Figure 5. Focal adhesion pathway in NSCLC (highlights in green indicate apoptotic pathways).

The apoptotic pathways are highlighted in green in **Figure 5**. In summary, focal adhesions are crucial in linking membrane receptors with the actin cytoskeleton, while signaling molecules such as protein kinases, phosphatases, and adapter proteins significantly influence cell shape, motility, and gene expression. The correlation between adhesion and growth factor-mediated signaling enables similar morphological changes in gene expression to be initiated by the binding of growth factors to their respective receptors [89]. These insights into the focal adhesion pathway could be useful in identifying potential therapeutic targets for NSCLC treatment.

4. Discussion

The study of the molecular pathology of NSCLC, as discovered by the Cancer Genome Atlas, has identified several significant signaling pathways [90] [91]. Furthermore, Chan *et al.* have studied the molecular pathways in NSCLC and have identified several indicative pathways and specific oncogenic driver alterations that lead to malignant transformations. These researchers stated that kinase protein feeds into multiple downstream pathways [92]. The NSCLC KEGG pathway has also been described to show that molecular mechanisms that are altered in NSCLC include the stimulation of oncogenes in addition to the inactivation of tumor suppressor genes [93]. The focal adhesion pathway presents a new therapeutic target in NSCLC because focal adhesion kinase (FAK) is considered a highly preserved non-receptor tyrosine kinase that plays essential roles in the migration, cellular adhesion, and proliferation of stem cells [94] [95].

FAK is considered one of the main targets for the development of antitumor drugs [96]. Lin *et al.* have suggested that FAK targets are vital in reducing lung cancer mobility and metastasis [97]. Focal adhesion is the close-fitting linking between the cell and the extracellular matrix that allows their communication and signaling [98]. It includes a complex of junctional sign proteins that introduce the actin cytoskeleton to the integrins, and it controls several cellular processes like relocation, proliferation, distinction, apoptosis, and gene expression [99]. According to the focal adhesion sensitivity to mechanical forces, it regulates its construction and role [100]. He L *et al.* found that cell migration declined when focal adhesion genes and platelet-derived growth factor receptors were downregulated in NSCLC [101]. They concluded that focal adhesion could be used as an alternative mechanism in NSCLC.

Moreover, they recommended that combining several proliferation pathways could be effective in reducing tumor cells' growth. According to the NSCLC proteomic profiling, Zhu *et al.* have stated that the NSCLC intrinsic radiosensitivity was principally modified by the signaling pathways of focal adhesion and regulation of the actin cytoskeleton [102]. Cadinu *et al.* have confirmed the kinase proteins' functionality in the NSCLC cell lines' radiation response, and they have defined the NSCLC radiosensitivity-related signaling pathways [37]. On the other hand, Grace K. *et al.* have stated that FAK expression was not associated with survival outcomes in their case study in North America, and it was expressed in the first stage of NSCLC only [103].

Also, MYH9 has been found to improve the cancer cells' stem cell-like biological performance through mTOR signaling pathway activation [104]. Meng Chen *et al.* has concluded that MYH9 expression can be used independently in assessing the prognosis of NSCLC patients [105]. Furthermore, MYH9 expression's downregulation defeats the stem cell-like malignant phenotype of NSCLC. According to Rahul Suresha *et.al*, studying the ACTG1 changes that happen within NSCLC could advance the understanding of tumorigenesis [106]. Additionally, abnormal expression of ACTG1 can be used as a biomarker for the early onset of NSCLC, providing a prospective therapeutic objective and indicating the effectiveness of chemotherapeutic agents currently in use. Atsumi *et al.* have found that focal adhesion-related proteins such as FLNA were strongly co-localized with FAK [107]. This co-localization confirmed that these proteins are the main antigens of the tumorigenic immune cell.

Integrating these analyses provides a holistic view of the role of downregulated actin cytoskeletal proteins in NSCLC. The protein analysis reveals the complex network of interactions among these proteins, GO enrichment analysis categorizes their roles in essential biological processes, and pathway analysis identifies key signaling pathways affected by their downregulation [108] [109]. Together, these analyses offer a comprehensive understanding of how these proteins contribute to NSCLC genesis.

This integrated approach not only explains the interconnectedness of different analyses but also highlights their collective contribution to understanding NSCLC,

paving the way for identifying novel biomarkers and therapeutic targets to improve patient outcomes. Previously, similar studies have shown successful in-vitro treatment strategies for triple-negative breast cancer [110] [111] [112]. Therefore, studying and analysing these interactions and pathways is crucial for developing effective treatment strategies for lung cancer.

5. Conclusions

The protein-protein interactions of the 35 downregulated actin cytoskeleton were studied. Actin proteins, such as ACTG1, ACTR2, ACTR3, ANXA2, and others, had the highest number of interactions, while others showed moderate or lower interactions. Their involvement in various cellular processes, such as cell motility, DNA repair, protein kinase regulation, and calcium-mediated cell processes. ANXA2 has been found to reduce toxin binding and vacuolization in human cells.

The study analyzed the molecular weight, chromosome, subcellular locations, and functions of these proteins to comprehend their involvement in the cellular processes of NSCLC. Additionally, the top ten gene ontology enrichment terms of biological processes, molecular function, and cellular components were examined to understand the biological significance of these proteins. This provided a more detailed understanding of the cellular processes affected by the downregulation of these proteins and how they contribute to the development of NSCLC.

Furthermore, the study examined KEGG and Wiki pathways to investigate the underlying mechanism behind the effects of NSCLC. The results showed that the focal adhesion pathway played a significant role in regulating cell motility, survival, gene expression, proliferation, and differentiation in NSCLC. Focal adhesion was crucial in connecting membrane receptors with the actin cytoskeleton, and protein kinases, phosphatases, and adapter proteins significantly influenced cell shape, motility, and gene expression. Moreover, the correlation between adhesion and growth factor-mediated signaling facilitated the initiation of similar changes in gene expression by binding growth factors to their receptors.

The study suggests that these downregulated proteins play a crucial role in cell motility and maintenance of the cytoskeleton, and their decreased expression can effectively kill NSCLCs. In conclusion, a deeper understanding of gene/ protein interactions and the focal adhesion pathway, as demonstrated in this study, can provide valuable insights for controlling and treating NSCLC by identifying novel biomarkers and drug targets.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

ACTG1:	actin gamma 1
ACTR2:	actin-related protein 2
ACTR3:	actin-related protein 2
ANXA2:	annexin A2
ARP2/3:	actin-related protein 2/3
ATP:	adenosine triphosphate
CALD:1	caldesmon isoform 2
COPD:	chronic obstructive pulmonary disease
CTNNA1:	catenin alpha-1
DNA:	deoxyribonucleic acid
DSBs:	double-strand breaks
DUSP23:	dual specificity protein phosphatase 23
FAK:	focal adhesion kinase
FLNA:	filamin-A isoform 2
FLNA:	filamin A
FLNB4:	filamin-B isoform 4
FLNB1:	filamin-B isoform 1
FSGS:	focal segmental glomerulosclerosis
GO:	gene ontology
HR:	homologous recombination
HSPB1:	heat shock protein beta-1
IQGAP1:	ras GTPase-activating-like protein
KEGG:	kyoto encyclopedia of genes and genomes
KRT17:	keratin, type I cytoskeletal 17
KRT7:	keratin, type II cytoskeletal 7
MSN:	moesin
mTOR:	mammalian target of rapamycin complex 1
MYH10:	myosin-10 isoform 3
MYH9:	myosin-9
MYL6:	myosin light polypeptide 6
MYL9:	myosin regulatory light polypeptide 9
MYO1C:	myosin-Ic
NDRG1:	protein NDRG1
NPF:	nucleation-promoting factor
NSCLC:	non-small cell lung cancer
PDLIM7:	PDZ and LIM domain protein 7
PFN1:	profilin-1
RNA:	ribonucleic acid
SCLC:	small cell lung cancer
Sept2:	septin 2
STRING:	search tool for the retrieval of interacting genes/proteins
TAGLN:	transgelin

TAGLN2:	transgelin-2
TLN1:	talin-1
TMSB4X:	thymosin beta-4
TPM1:	tropomyosin alpha-1 chain
TPM3:	tropomyosin alpha-3 chain
TPM4:	tropomyosin alpha-4 chain
TUBA1:	Btubulin alpha-1B chain
UTRN:	utrophin
ZYX:	zyxin