Clinical and prognostic significance of *MYH11* in lung cancer

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Abstract. Myosin heavy chain 11 (MYH11), encoded by the *MYH11* gene, is a protein that participates in muscle contraction through the hydrolysis of adenosine triphosphate. Although previous studies have demonstrated that *MYH11* gene expression levels are downregulated in several types of cancer, its expression levels have rarely been investigated in lung cancer. The present study aimed to explore the clinical significance and prognostic value of *MYH11* expression levels in lung cancer and to further study the underlying molecular mechanisms of the function of this gene. The Oncomine database showed that the *MYH11* expression levels were decreased in lung cancer compared with those noted in the normal lung tissue (P<0.05). Kaplan-Meier plotter results revealed that the decreased *MYH11* expression levels were correlated with poor prognosis in lung cancer patients. Among the lung cancer cases with gene alteration of *MYH11*, mutation was the most common of all alteration types. Coexpedia and Metascape analyses revealed that the target genes were primarily enriched in 'muscle contraction', 'contractile fiber part', 'actin cytoskeleton' and the 'adherens junction'. These results indicated that *MYH11* is a potential novel drug target and prognostic indicator of lung cancer.

Introduction

In 2018, lung cancer was reported as having the highest morbidity and mortality rates of all types of malignancy worldwide, posing a notable risk to human health (1). Although the methods of clinical diagnosis and treatment of lung cancer are improving, the prognosis for patients with lung cancer remains poor. The mortality rate of lung cancer is increasing and the 5-year survival rate is estimated to approximately 18% (2). Therefore, novel targets for drug treatment and prognosis of lung cancer are needed.

Myosin heavy chain 11 (MYH11), which is encoded by the *MYH11* gene, is a smooth muscle myosin belonging to the myosin heavy chain family (3). MYH11 is a contractile protein that slides past actin filaments to induce muscle contraction via adenosine triphosphate hydrolysis (4,5). Previous findings have shown that in aortic tissue, destruction of MYH11 can lead to vascular smooth muscle cell loss, disorganization and hyperplasia, which is one of the mechanisms leading to thoracic aortic aneurysms and dissections (6,7). In previous years, studies have indicated that mutations of the *MYH11* gene are also associated with various types of cancer, including colorectal (8,9), bladder (10), laryngeal (11) and head and neck cancer (12). This may be due to the role of *MYH11* role in cell migration, interaction with cell adhesion proteins and tumor suppression (13,14). However, the association between the expression levels of *MYH11* and lung cancer has rarely been investigated. To the best of our knowledge, only Ma *et al* (15) investigated the key role of *MYH11* in non-small-cell lung cancer. In the present study, the Oncomine, Kaplan-Meier plotter, cBioportal, Coexpedia and Metascape databases were used in order to explore the biological significance of *MYH11* in lung cancer and provide a scientific basis of the underlying molecular mechanism of *MYH11* function in the pathogenesis of lung cancer.

Materials and methods

Oncomine analysis. Oncomine is a large database of cancer microarray experiments and a web-based data-mining platform that is used to screen for differences in gene expression levels between different tumor tissues (16,17). The query terms were set as follows: i) gene: *MYH11*; and ii) analysis type: Lung cancer vs. normal analysis. P<0.05 was considered to indicate a statistically significant difference, fold change was set to 2 and gene rank to 5%. An unpaired t-test was used for the comparison of two groups.

Kaplan-Meier plotter database. Kaplan-Meier plotter online database (kmplot.com) was used to assess the effects of *MYH11* expression levels on survival and prognosis (18). The database was used to query relevant data of lung cancer and the mean

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expression levels of the gene probes. The correlation between *MYH11* expression levels and the survival rate of patients with lung cancer was investigated. P<0.05 was considered to indicate a statistically significant difference. Overall survival, first progression and post progression survival curves were generated using the Kaplan-Meier method and evaluated using the log-rank test.

cBioportal analysis. In order to find mutations and copy number alterations (CNAs) of *MYH11*, genomic data were retrieved from The Cancer Genome Atlas (TCGA) lung cancer datasets using cBioportal (cbioportal.org) (19). The location and frequency of *MYH11* alterations (amplifications, deep deletions and missense mutations) and copy number variance data were assessed.

Coexpedia database. The co-expression genes of *MYH11* in Gene Expression Omnibus (GEO) database were analyzed using Coexpedia (coexpedia.org/) (20). Coexpedia is a database of context-associated co-expression networks constructed from individual series of microarray samples for humans. The total score for each gene was the sum of edge-weights (log-likelihood score) and all connected genes in the network.

Metascape analysis. Metascape (metascape.org) is a powerful web-based tool for gene annotation and gene list enrichment analysis, including biological process (BP), molecular function (MF), cellular component (CC) and a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (21). In the present study, Metascape was used to conduct a pathway and process enrichment analysis of *MYH11* and co-expression genes significantly associated with *MYH11* alterations. In addition, protein-protein interaction (PPI) networks were also analyzed.

Results

Expression levels of MYH11 in different types of cancer. In the Oncomine database, a total of 442 studies were available to compare *MHY11* gene expression levels between different types of cancer and normal tissues. A total of 68 studies were identified as statistically significant in the Oncomine database. In 64 and 4 studies, *MYH11* expression levels were significantly down- and upregulated in cancer tissues compared with normal tissues, respectively. In addition, in 13 studies, decreased *MYH11* gene expression levels were noted in lung cancer compared with normal lung tissues (Fig. 1).

Expression of MYH11 in lung cancer. In the Oncomine database, *MYH11* gene expression levels in all studies investigating lung cancer were obtained. From November 2001 to the present, thirteen studies met the screening threshold criteria, with a total sample size of 1,122 cases (22-30). As shown in Fig. 2 and Table I, the output results indicated that *MYH11* gene expression levels were significantly downregulated in lung cancer tissue compared with normal lung tissue in all 13 studies (median rank=278.0; P=5.38x10^{-8}; Fig. 2).

Expression levels of MYH11 in lung cancer subtypes. In the Oncomine database, *MYH11* gene expression levels results were

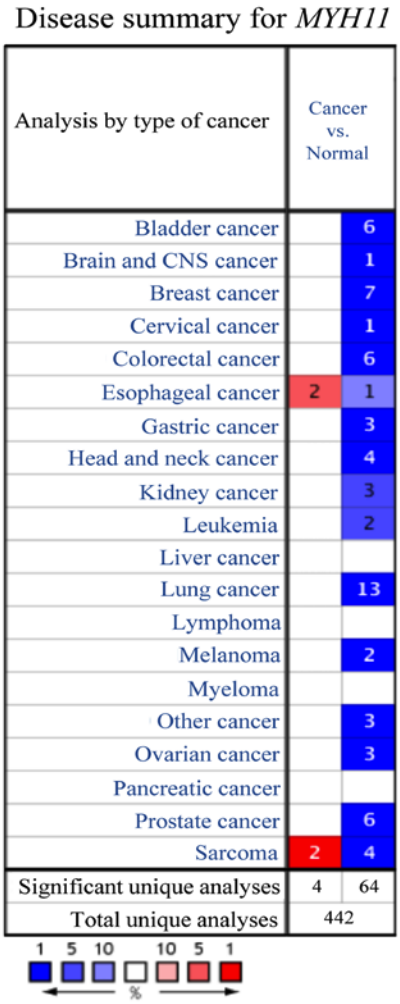

Figure 1. Oncomine analysis of 442 studies investigation *MHY11* gene expression levels in cancer and normal tissues. The query term was, gene: *MYH11*. The 'cancer vs. normal' columns represent the datasets with statistically significant upregulation (red) or downregulation (blue) of *MHY11* in cancer tissues compared with normal tissues. The number in each cell represents the number of analyses that met the threshold and the depth of cell color was determined by the optimal gene rank percentile for the analyses within the cell. Overall, 68 studies were determined as statistically significant in the Oncomine database (64 demonstrated upregulation and 4 demonstrated downregulation of *MHY11* gene expression). *MYH11*, myosin heavy chain 11 gene.

derived from 13 studies investigating lung cancer and the relevant data were compared and analyzed using box-plot analysis (Fig. 3). The aforementioned studies included comparisons between different types of lung cancer (squamous cell lung carcinoma, lung adenocarcinoma, small cell lung carcinoma and large cell lung carcinoma) and normal lung tissue (Table I). The results suggested that the *MYH11* gene expression levels in different lung cancer subtypes were significantly decreased compared with those noted in normal lung tissue (P<0.05; Table I). Compared with normal lung tissue, the specific output results for the lung adenocarcinoma samples in Bhattacharjee *et al* (22), Beer *et al* (23), Landi *et al* (24), Stearman *et al* (25), Hou *et al* (27), Su *et al* (28), Selamat *et al* (29) and Okayama *et al* (30) were 6.39x10^{-9}, 3.17x10^{-12}, 6.23x10^{-25}, 5.14x10^{-8}, 1.7x10^{-15}, 2.72x10-8, 1.62x10^{-17} and 5.2x10^{-10}, respectively. Compared with normal lung tissues, the p-values were as follows for the squamous cell lung carcinoma samples in Bhattacharjee *et al* (5.16x10^{-8})

Table I. Oncomine analysis of 8 datasets analyzing *MYH11* gene expression levels in different lung cancer subtypes. The data were compared with the expression levels of *MYH11* in normal lung tissues.

Author, year	Type of lung cancer	Fold change	P-value	t-test	(Refs.)
Bhattacharjee *et al*, 2001	Lung adenocarcinoma	-20.207	$6.39x10^{-9}$	-8.181	(22)
Bhattacharjee *et al*, 2001	Squamous cell lung carcinoma	-21.362	$5.16x10^{-8}$	-7.066	(22)
Bhattacharjee *et al*, 2001	Small cell lung carcinoma	-12.541	$1.09x10^{-5}$	-5.795	(22)
Bhattacharjee *et al*, 2001	Lung carcinoid tumor	-25.905	$3.97x10^{-9}$	-8.204	(22)
Beer *et al*, 2002	Lung adenocarcinoma	-24.567	$3.17x10^{-12}$	-10.557	(23)
Landi *et al*, 2008	Lung adenocarcinoma	-2.979	$6.23x10^{-25}$	-13.475	(24)
Stearman *et al*, 2005	Lung adenocarcinoma	-16.652	$5.14x10^{-8}$	-7.805	(25)
Talbot *et al*, 2005	Squamous cell lung carcinoma	-2.149	$5.38x10^{-8}$	-6.285	(26)
Hou *et al*, 2010	Large cell lung carcinoma	-5.286	$3.28x10^{-14}$	-12.556	(27)
Hou *et al*, 2010	Lung adenocarcinoma	-3.289	$1.7x10^{-15}$	-9.7	(27)
Su *et al*, 2007	Lung adenocarcinoma	-7.195	$2.72x10^{-8}$	-6.672	(28)
Selamat *et al*, 2012	Lung adenocarcinoma	-2.007	$1.62x10^{-17}$	-10.215	(29)
Okayama *et al*, 2012	Lung adenocarcinoma	-2.717	$5.2x10^{-10}$	-8.48	(30)

MYH11, myosin heavy chain 11 gene.

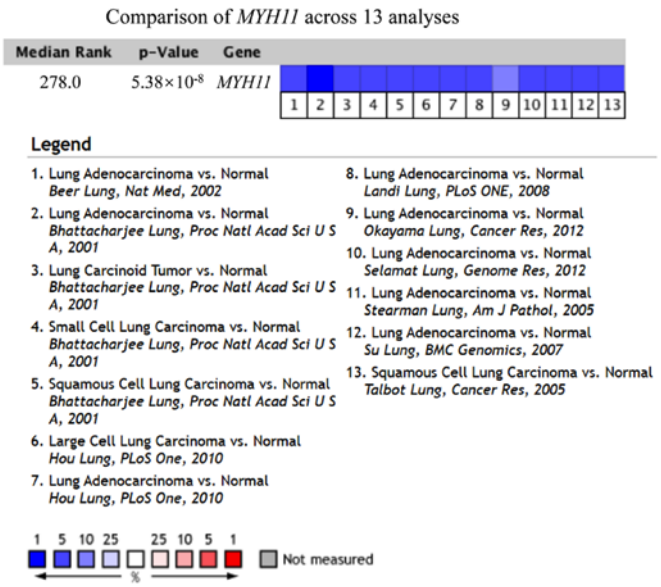

Figure 2. Oncomine analysis of *MYH11* gene expression levels data from 13 lung cancer studies, with a total of 1,122 patients from 2001 (22-30). The rank for a gene was the median rank for that gene across each of the analyses and the P-value for a gene was its P-value for the median-ranked analysis. Red represents increased expression, with deeper colors indicating higher expression and blue represents decreased expression, with deeper colors indicating lower expression. This analysis revealed that *MYH11* gene expression levels were significantly downregulated across lung cancer (median rank=278.0 and P=$5.38x10^{-8}$). *MYH11*, myosin heavy chain 11 gene.

and Talbot *et al* (26) ($5.38x10^{-8}$), the small cell lung carcinoma samples in Bhattacharjee *et al* (22) ($1.09x10^{-5}$), the lung carcinoid tumor samples in Bhattacharjee *et al* (22) ($3.97x10^{-9}$) and the large cell lung carcinoma samples in Hou *et al* (27) ($3.28x10^{-14}$).

Correlation between MYH11 expression levels and prognosis of lung cancer. The correlation between *MYH11* gene expression levels and lung cancer survival rate were analyzed using the Kaplan-Meier plotter analysis tool (Fig. 4). *MYH11* expression levels were positively correlated with overall survival (P=$2.2x10^{-13}$), first progression (P=$8.4x10^{-8}$) and post progression survival (P=0.0043) in patients with lung cancer. These results suggested that *MYH11* exhibited tumor suppressive roles in lung cancer.

Mutations and CNAs of MYH11 in lung cancer. TCGA datasets of all lung cancer samples were selected to investigate mutations and CNAs in the *MYH11* gene. A total of 4,582 cases in 21 studies were included for this analysis (Fig. 5). Among the lung cancer cases with gene alteration of *MYH11*, mutation was the most common alteration type. A total of 30 cases of non-small cell lung cancer were amplified, accounting for

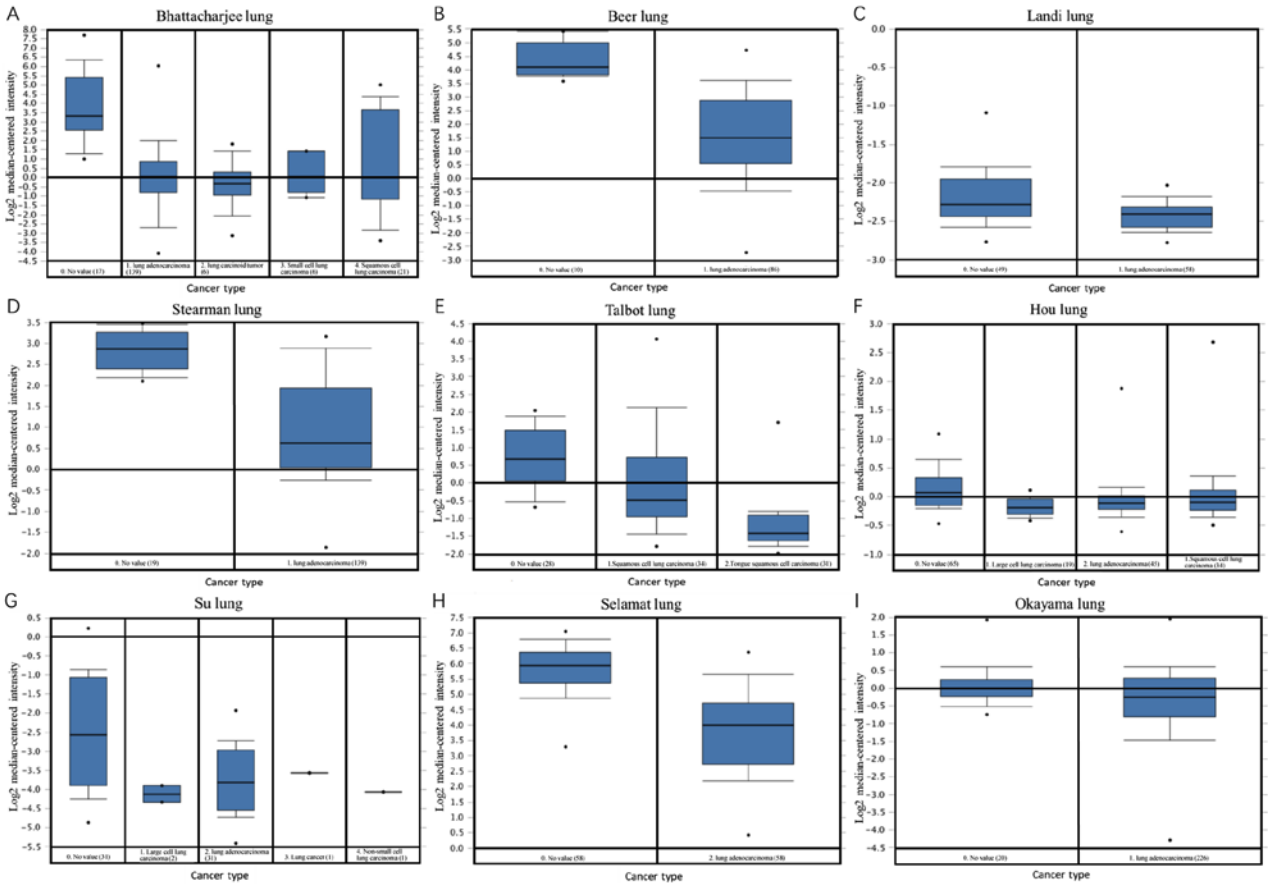

Figure 3. Oncomine analysis. A total of eight datasets, including (A) Bhattacharjee lung, (B) Beer lung, (C) Landi lung, (D) Stearman lung, (E) Talbot lung, (F) Hou lung, (G) Su lung, (H) Selamat lung and (I) Okayama lung datasets were extracted from the Oncomine database for analyzing *MYH11* gene expression levels in different lung cancer subtypes compared with normal lung tissues. The expression profiles were median-centered, log2-transformed and uploaded onto the Oncomine database. This analysis revealed that *MYH11* gene expression levels were significantly downregulated in several lung cancer subtypes. *MYH11*, myosin heavy chain 11 gene.

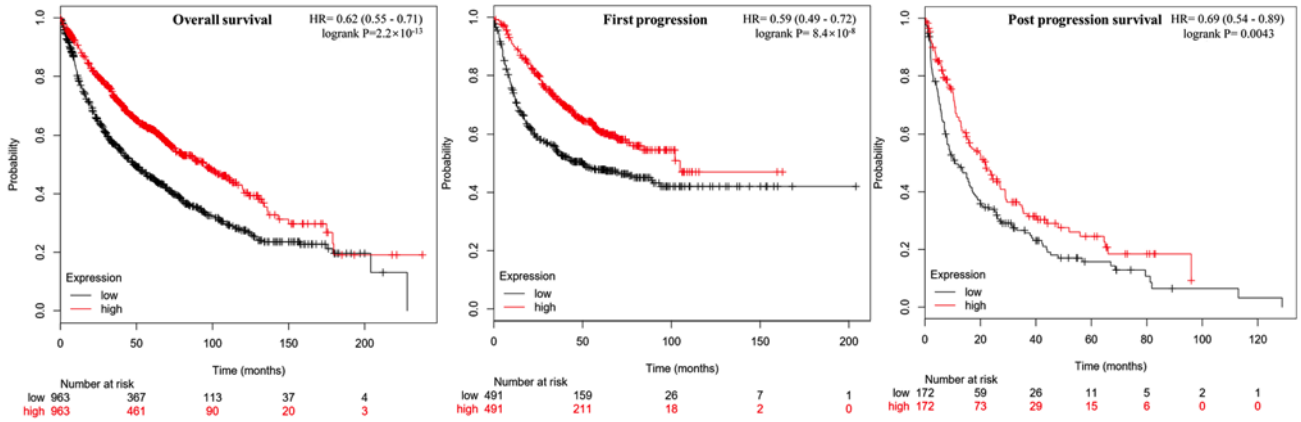

Figure 4. Kaplan-Meier plotter analysis. *MYH11* gene expression levels were positively correlated with overall ($P=2.2x10^{-13}$), first progression ($P=8.4x10^{-8}$) and post progression survival (P=0.0043) in patients with lung cancer. *MYH11*, myosin heavy chain 11 gene; HR, hazard ratio.

7.43%. In lung adenocarcinoma, 99 cases were amplified, 14 cases were deep deletion, and 4 cases were fusion, which accounted for 4.32, 0.61 and 0.17%, respectively. In lung squamous cell carcinoma, 54 cases were amplified, 9 cases were deep deletion, 3 cases were fusion and 2 cases were multiple alterations, which accounted for 3.26, 0.54, 0.18 and 0.12%, respectively. A total of 4 cases of small cell lung cancer were amplified, accounting for 1.74% (Fig. 5 and Table II). There were 169 cases of missense, 22 cases of truncating and 2 cases of in-frame mutations in lung cancer (Fig. 6). The results of the present study demonstrated that the most common type of mutation in lung cancer was missense mutation. A previous study reported that *MYH11* missense mutations in breast cancer are associated with tumor development (31).

Table II. Mutations and copy number alterations of *MYH11* in different types of lung cancer. The alteration frequency of *MYH11* in different lung cancer studies.

	Lung cancer subtype			
Mutation type	Non-small cell, n (%)	Adenocarcinoma, n (%)	Squamous cell carcinoma, n (%)	Small cell, n (%)
Amplification	30 (7.43)	99 (4.32)	54 (3.26)	4 (1.74)
Deep deletion	-	14 (0.61)	9 (0.54)	-
Fusion	-	4 (0.17)	3 (0.18)	-
Multiple alterations	-	-	2 (0.12)	-

MYH11, myosin heavy chain 11 gene.

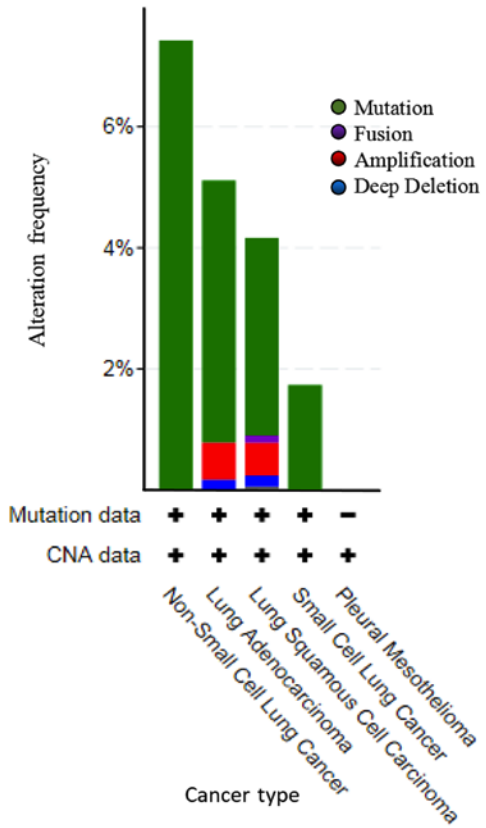

Figure 5. Mutations and CNAs of myosin heavy chain 11 gene alterations in different types of lung cancer. Alteration types included mutations (green), fusions (purple), amplifications (red) and deep deletions (blue). CNA, copy number alteration.

Enrichment analysis of MYH11. The visualization of co-expressed genes was ranked according to the online Coexpedia instructions. The total score for each gene was a sum of edge-weights (log likelihood score) and all connected genes in the network. Then, the functions of *MYH11* and the 30 genes with the highest scores were predicted by analyzing GO and KEGG data using Metascape (Fig. 7A and B). For GO term enrichment analysis, the target genes were mainly enriched in 'muscle contraction', 'contractile fiber part', 'actin cytoskeleton', 'adherens junction', 'actin binding', 'regulation of muscle contraction', 'smooth muscle contraction', 'striated muscle contraction', 'platelet aggregation', 'myosin complex', 'SMAD binding', 'tissue morphogenesis', 'structural constituent of cytoskeleton' and 'positive regulation of transmembrane transport' (Fig. 7A). KEGG analysis revealed that the target genes were mostly enriched in 'vascular smooth muscle contraction', 'cGMP-PKG signaling pathway', 'dilated cardiomyopathy' and 'focal adhesion' (Fig. 7B). Subsequently, the PPI networks and their connections were identified using Metascape. The results demonstrated that the main functions associated with MYH11 protein were 'muscle contraction', 'contractile fiber part', 'actin cytoskeleton', 'adherens junction', 'vascular smooth muscle contraction', 'actin binding', 'regulation of muscle contraction', 'smooth muscle contraction', 'striated muscle contraction', 'platelet aggregation', 'myosin complex', 'SMAD binding', 'tissue morphogenesis', 'structural constituent of cytoskeleton' and 'positive regulation of transmembrane transport' (Fig. 7C).

Discussion

The *MYH11* gene, which encodes a smooth muscle myosin protein, is located at chromosome 16p13.13-13.12 (32). It has been shown that myosin proteins are involved in muscle movement (33). Issouf *et al* (34) reported that *MYH11* is a gene that functions in the molecular mechanisms underlying the contraction of airway smooth muscle in asthma. However, previous studies suggested that *MYH11* was also involved in cell adhesion, migration and tumor suppression (13,14). Previous studies demonstrated that mutations in the *MYH11* gene could promote cancer formation. For example, the *CBFB/MYH11* fusion gene has been implicated in the onset of acute myeloid leukemia (35,36). In addition, *MYH11* gene expression levels have been associated with the prognostic outcome of the bladder urothelial carcinoma, notably in patients with advanced tumors (37). Seitz *et al* (38) demonstrated that *MYH11* gene expression levels were downregulated in breast tumor and metastasis, using microarray and correlation analyses. Disregulated *MYH11* gene expression levels have also been associated with the occurrence of intestinal tumors by affecting the cellular energy balance (39).

In the present study, *MYH11* gene expression levels were significantly decreased in lung cancer tissues compared with that noted in normal lung tissues. These results indicated that the decreased *MYH11* gene expression levels were associated

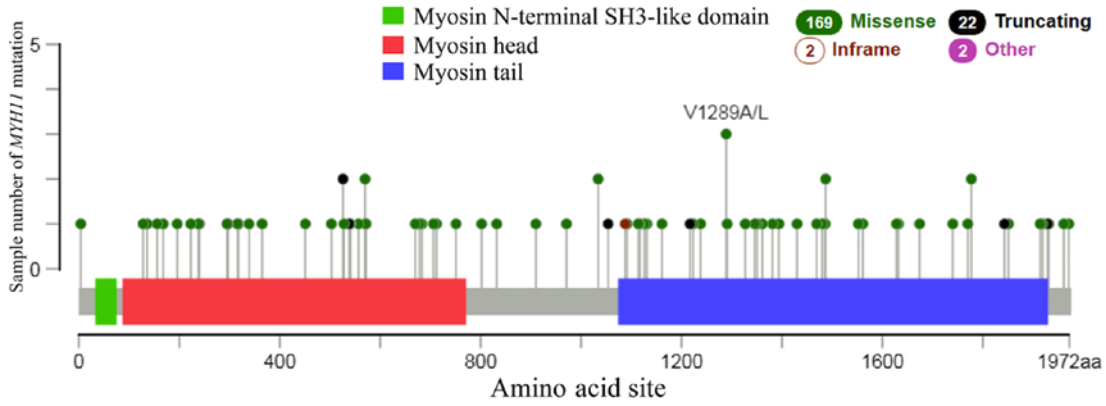

Figure 6. Mutation diagram of myosin heavy chain 11 gene in lung cancer. This graphical view showed the protein domains and the positions of 195 mutations including missense, truncating and in-frame deletions. SH3, SRC Homology 3.

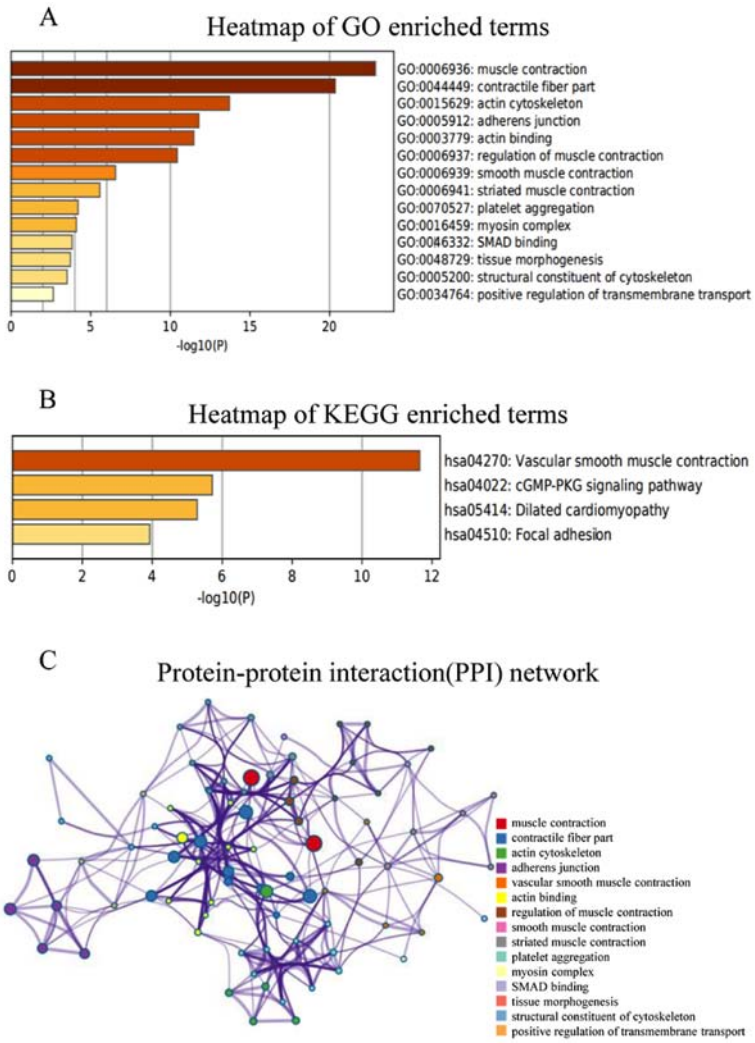

Figure 7. The enrichment analysis of myosin heavy chain 11 gene and co-expression genes using Metascape. (A) Heatmap of GO-enriched terms colored by P-values. (B) Heatmap of KEGG-enriched terms colored by P-values. (C) Protein-protein interaction network. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

with the onset of lung cancer. In addition, decreased *MYH11* expression levels were associated with a less favorable prognosis of patients with lung cancer. These results are in accordance with previous studies from Wang *et al* (9) in colorectal cancer and Ma *et al* (15) in non-small-cell lung cancer, indicating that *MYH11* gene expression levels could be used as a novel biomarker in predicting the prognosis of lung cancer.

Among the lung cancer cases with gene alteration of *MYH11*, mutation was the most common of all alteration

types. Gene-set enrichment analysis showed that the target genes were mainly enriched in 'muscle contraction', 'contractile fiber part', 'actin cytoskeleton' and 'adherens junction'. It has been proposed that myosin-dependent contractile activity in non-muscle cells, similar to that observed in muscle, may also be involved in cell migration (14). Consistent with these findings, the results of the present study suggest that *MYH11* may increase cell migration and adhesion in a mutated manner, in order to increase tumor invasion. In conclusion, the results of the present study demonstrate the potential of *MYH11* as a novel target for the treatment of lung cancer. Despite the use of a large sample size, which increases the accuracy of the results, the present study failed to identify the underlying molecular mechanism of *MYH11* in the development of lung cancer, thus further verification is required.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

MN, XP, HT and XZ designed the present study and drafted the initial manuscript. MX, SL, WS and JW interpreted the data. All authors contributed to analyzing the data, and have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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