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Original Article

Neurofibromin 2 modulates Mammalian Ste2-like kinases1/2 and large tumor suppressor gene1 expression in A549 lung cancer cell line

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Abstract: Aim: To explore the impact of up- or down-regulation of Neurofibromin 2 (NF2) on the expression of downstream Hippo pathway genes, large tumor suppressor gene1 (LATS1), and phosphorylation of Mammalian Ste2-like kinases1/2 (MST1/2), in lung cancer cells. Methods: A549 lung cancer cells were used. The NF2 was down-regulated by si-RNA interference and upregulated by lentiviral vector mediated overexpression. The LATS1 and MST1/2 expressions were evaluated by real-time PCR and western blot. Results: Down-regulation of NF2 decreased LATS1 and MST1/2 level ($P<0.05$). Overexpression of NF2 increased LATS1 ($P<0.05$) and Mammalian Ste2-like kinases1 (MST1) ($P<0.05$), suggesting LATS1 and MST1 are modulated by NF2 in a lung cancer cell line. Conclusions: NF2 mediates the downstream LATS1 and MST1/2 expressions in a lung cancer cell line.

Keywords: Lung cancer, siRNA interference, overexpression, NF2, hippo pathway

Introduction

Lung cancer causes high morbidity and mortality and is the most common cause of cancer death worldwide. It is estimated that there are 1.6 million new lung cancer cases and about 1.25 million lung cancer deaths each year [1]. In spite of advancement in cancer treatment in recent years, the five-year survival rate of lung cancer remains low. Recurrence of lung cancer is influenced by multiple factors, including chemistry, genetics, and toxin exposure. Like most tumor cells, mutations occur in lung cancer cells resulting in uncontrolled cell proliferation and metastasis [2]. Moreover, the inactivation of contact inhibition in cancer cells enables them to escape apoptosis, which results in proliferation [3, 4]. Studies show that the highly conserved Hippo (Ste20-like kinase Hpo) pathway plays important roles in mediating organ size and tumor formation [5, 6].

Hippo pathway, also known as the Salvador/Warts/Hippo (SWH) pathway, was initially discovered in Drosophila [7]. Hippo pathway plays important roles in mediating cell size, maintaining stable cell number, and controlling cell contact inhibition. Dysregulation of the Hippo pathway results in tumor formation [7]. Hippo pathway is composed of Hpo (hippo), Sav (Salvador), Wts (Warts), Yki (Yorkie), and Mats (Mob as tumor suppressor) in Drosophila. Wts is a NDR (nuclear Dbf2-related) family kinase [8], and its defects can result in spontaneous proliferation of epithelial cells. Sav encodes a scaffolding protein with a WW structural domain. Hpo is the most important component of Hippo pathway. Hippo can bind to Sav and form

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a Hpo/Sav complex, which phosphorylates Wts and activates Mats [9, 10]. The main effector Yki in Hippo pathway was discovered by Huang in 2005 [11]. Yki is the only oncogene in the Hippo pathway, and it can be inactivated by Wts phosphorylation. When the Hippo pathway is inactivated, un-phosphorylated Yki will translocate into the nucleus to initiate gene expression, and promote cell survival and proliferation [12].

The Hippo pathway is highly conserved in evolution. In mammals the Hippo pathway is composed of MST1/2 (the mammalian Hippo orthologs), WW45 (45 KDa WW Domain Protein; Sav in Drosophila), LATS1/2 (Large tumor suppressor gene1/2), YAP (Yes-associated protein), and Mob1 (MOB kinase activator 1A). Transcriptional co-activator YAP and its paralog tafazzin (TAZ) (transcriptional coactivator with PDZ binding motif) act downstream of the Hippo pathway [13]. YAP/TAZ interact with other transcription factors including Smad, Runx1/2, p73, ErbB4, Pax3, and T-box transcription factor 5 (TBX5) to mediate the transcription of a diverse array of genes [14, 15]. LATS1/2 encodes serine and threonine kinase and is the core component of the Hippo pathway [16]. LATS1 is located on chromosome 6q24.25, with a 3393 bp ORF (open reading frame) encoding 1130 amino acid. Recent studies have found that LATS1 plays a role in formation of the spindle body during mitosis [17, 18]. Hence, LATS1 can down-regulate organ size by cell contact inhibition. In addition, LATS1 mediates the cyclin and cyclin b level, causes cell cycle arrest in G2/M, and restrains cell proliferation and growth. LATS2 belongs to the Terra tumor-suppressor family and has a similar structure and function as LATS1 [19, 20]. LATS2 can enhance p53 phosphorylation and promote cell apoptosis [21, 22]. Moreover, LATS1/2 promotes cell apoptosis by increasing the expression of pro-apoptosis proteins or down-regulating anti-apoptosis proteins [23, 24]. Visser et al found that LATS1/2 was down-regulated in leukocythemia, breast, and lung cancers [6].

The signaling cascade from Hpo (MST1/2) to Yki (YAP) is well-studied. However, the upstream regulators of Hippo pathway are still unclear. Merlin and Expanded, a related FERM domain protein, at the upstream of Hippo pathway were discovered by Hamaratoglu in 2009 [26]. Merlin functions with Expanded, and promotes the phosphorylation of Wts, which in turn activates the Hippo pathway [27, 28]. TAZ is the homologous gene in mammal to merlin in Drosophila [29, 30]. NF2 is a tumor suppressor in multiple human cancers [29, 31]. Additionally, studies have shown that inhibition of Hippo pathway promotes the proliferation, migration and metastasis of lung cancer cells [32, 33].

NF2 can inhibit tumor formation by the Hippo pathway, although the mechanism is still unclear. Studies have reported a relationship between NF2 and the susceptibility of lung cancer; however, the conclusions remain contradictory [34-36]. In this study, we investigated the impacts of up-regulation and down-regulation of NF2 on the downstream Hippo pathway genes, LATS1, MST1, and MST2, in a lung cancer cell line.

Materials and methods

Materials

A549 lung cancer cell lines and 293T cell lines were purchased from Shanghai Cell Bank at Sep 9, 2015, Chinese Academy of Sciences, where they were characterized by mycoplasma detection, DNA-fingerprinting, isozyme detection, and cell vitality detection. The information about the cell lines is shown on its website: (http://www.cellbank.org.cn/detail_1.asp?id=283). The cell lines were tested by Cell STR identification before use. Transfection PolyFecter was purchased from WeiShang LiDe Ldt. (Beijing, China). Antibodies, reagents and instruments are listed in Supplementary Table 1. The preparation of major reagents are shown in Supplementary Table 1. The study was approved by the ethics committee of Capital Medical University Electric Power Teaching Hospital.

Methods

NF2-siRNA interference: For siRNA interference experiment, three siRNAs were designed and synthesized. After transiently transfecting the cells, western blot identification was performed and the siRNA with the strongest interference effect was selected for the subsequent experimental testing. A549 cells were seeded onto 6-well plates and cultured until reach 50% confluence. A549 cells were divided into a

siRNA negative control group and a siRNA-NF2 experimental group.

A549 cells were transfected using Lipofectamine RNAiMax Reagent according to the manufacturer's instructions.

RNA isolation: The total RNA of cells was extracted at 48-h post-transfection. Cells were rinsed twice with ice-cold PBS and homogenized in 1 mL Trizol lysate buffer. 0.2 mL trichloromethane was added, vortexed for 10 s, and incubated at room temperature for 3 min. The samples were centrifuged at 12,000 rpm for 20 min at 4°C. The upper aqueous phase was transferred to a new tube and the volume of the aqueous phase was measured. The same volume of isopropanol was added and incubated at -80°C for 1 h. The samples were centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was removed. The RNA pellet was washed with 1 mL 75% ethyl alcohol and centrifuged at 5,000 rpm for 3 min at 4°C. The supernatant was removed and the RNA samples were air dried for 10 min. RNA was dissolved in 40 μL RNase-free water.

Real-time PCR: RNA was reverse-transcribed into cDNA using HiFiScript kits (KangWei century, CW2582). 20 μL reaction system included 5 μL RNA, 1 μL Oligodt [50 pM], and 4 μL dNTP [2.5 mM]. Samples were heated at 65°C for 5 min and cooled on ice. 4 μL Reverse buffer (2×), 0.5 μL Reverse enzyme (200 U/μL), and 5.5 μL RNA-free water were added. The thermal cycles were 42°C for 60 min, 75°C for 15 min, and 4°C forever. Real-time PCR was carried out with KAPA SYBR FAST qPCR Kit Master Mix (2×) (KAPA Biosystems, KK4601). The primers are listed in Supplementary Table 2. The 20 μL reaction system included 10 μL PCR Master Mix (2X), 0.4 μL mRNA forward primers (10 μM), 0.4 μL mRNA reverse primers (10 μM), 2 μL cDNA, 0.4 μL Dye (50X), and 6.8 μL ddH_2O. The PCR cycles were 94°C for 2 min, 94°C for 5 s, 60°C for 15 s, 72°C for 31 s, and 40 cycles. $2^{-\Delta\Delta Ct}$ method was applied to analyze the real-time PCR results. $\Delta\Delta CT=(CT_{target\ gene}-CT_{internal\ reference})$(treatment group)-$(CT_{target\ gene}-CT_{internal\ reference})$(control group).

Western blot (WB): Cells were harvested at 48-h post-transfection. The preparations of WB reagents are listed in Supplementary Table 1. Cells were homogenized in lysis buffer and centrifuged at 10,000 rpm for 10 min at 4°C. 30 μL supernatant was collected and 10 μL 4×SDS loading buffer was added. Samples were incubated at 100°C for 10 min, centrifuged at 12,000 rpm for 1 min, and supernatant was collected. 10 μL sample was loaded onto 10% SDS-PAGE, and run at 80 V for 30 min, and 120 V for 70 min. After electrophoresis, protein samples were transferred onto PVDF membranes. The membranes were blocked with blocking buffer for 1 h, and then incubated with primary antibodies overnight at 4°C. The membranes were washed 3 times with 1×PBST, 10 min each. The membranes were incubated with secondary antibodies for 1 h at room temperature. Membranes were washed 3 times with 1×PBST, 10 min each. Membranes were developed using ECL and exposed to X-ray. Film was scanned by scanner and Gel-Pro analyzer was used to analysis the grayscale for protein quantification. Tubulin was served as an internal reference. WB experiments were repeated 3 times and the results were summarized and analyzed.

The plasmid construction: The plasmids for LX-NF2 were constructed with Vector pWSLV-08 and primer Ef1a-sq (CACTTGATGTAATTCTCCTTGGAAT). The sequence of NF2 is shown in Supplementary Table 2 (Genebank ID: NC_000022.11). LX-NF2 was digested with restriction enzyme NotI and BamHI, and a DNA fragment of 1788 bp was harvested. PWSLV-08 was cut by restriction enzyme NotI and BamHI, and the DNA fragment was inserted into the plasmid. The new expression vector was sequenced and named as pWSLV-08-NF2. The sequence of pWSLV-08-NF2 is shown in Supplementary Table 2, in which the underlines indicate the target gene.

Lentivirus packing: 293T cells were cultured in 10 cm culture dishes for 24 h. Next day, 293T cells were divided into 2 groups, and were transfected with NF2 lentiviral vector (pWSLV-08-NF2) or blank control lentiviral vector (pWSLV-08), respectively. The cells were cultured in media containing transfection reagents for 6-8 h, and then the media were replaced with 10 mL DMEM+10% FBS. On the third day, viral supernatants were harvested and filtered through a 0.45 μm filter membrane for future use. 293T cells transfected with NF2 lentiviral

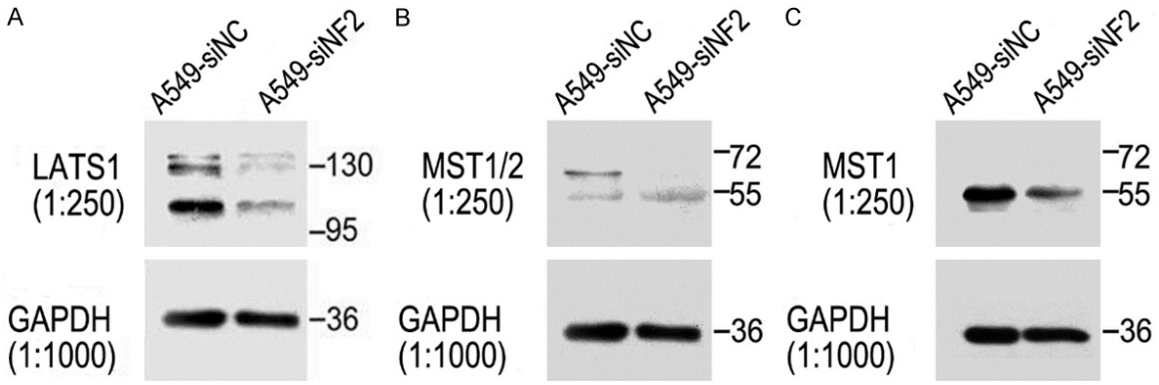

Figure 1. Evaluation of LATS1, MST1/2, and MST1 protein levels using WB. A. LATS1 protein expression (P<0.05, antibody dilution 1:250, molecular weight 127 KD). B. MST1/2 protein expression (P<0.05, antibody dilution 1:250, molecular weight 56 KD). C. MST1 protein expression (P<0.05, antibody dilution 1:250, molecular weight 56 KD). GAPDH was used as an internal reference with a dilution of 1:1000.

vector (pWSLV-08-NF2) are shown in Supplementary Figure 1A.

NF2 overexpression: A549 cells were cultured in 6 cm dishes and divided into 2 groups, NF2 overexpression group or blank control group. The A549 cells were infected with virus harvested from 293T pWSLV-08-NF2 or 293T pWSLV-08, respectively. Successfully infected A549 cells were selected by flow cytometry (FCM). The outcomes before cell sorting (Supplementary Figure 1B) and after cell sorting (Supplementary Figure 1C) indicate the successful screening for NF2 over-expression A549 cells.

Statistical analysis

SPSS 19.0 software was used for the data analysis. All data were presented as mean values ± SEM. One way analysis of variance (ANOVA) or t-test was used for comparisons. Statistical significance was set at P<0.05.

Results

NF2 siRNA interference down-regulates NF2 mRNA and protein expression

The efficiency of si-RNA interference was assessed with real-time PCR. We found that NF2 mRNA expression significantly decreased in NF2-homo-1952 and NF2-homo-774 groups, compared with siNC group (P<0.05). The NF2-homo-774 group showed the best efficiency with 91% decreased in NF2 mRNA expression, compared with siNC group (P=0.002) (Supplementary Figure 2A). Therefore, NF2-homo-774 siRNA was selected for the following studies. We measured the NF2 protein expression in siNC and NF2-homo-774 siRNA groups. We found that the NF2 protein significantly decreased in NF2-homo-774 siRNA group, compared with siNC group (P<0.05; Supplementary Figure 2B, Supplementary Table 3), indicating the successful down-regulation of NF2 using siRNA interference.

Down-regulation of NF2 decreases LATS1, phosphorylation MAST1/2, and MST1

We measured the protein levels of LATS1, phosphorylated MST1/2, and MST1, which are downstream factors of NF2. We found that LATS1, phosphorylated MST1/2 (two bands represented MST1 and MST2, respectively), and MST1 were significantly decreased in the NF2-homo-774 group, compared to the siNC group (P<0.05, **Figure 1** and Supplementary Table 3).

NF2-overexpression

293T cells were transfected with NF2-lentiviral vectors. The NF2 mRNA expressions in 293T cells transfected with pWSLV-08-NF2 (NF2-NF2) and 293T cells transfected with pWSLV-08 (NC-NF2) were measured using real-time PCR. The primers are shown in Supplementary Table 2. We found that NF2-lentiviral vector transfection significantly increased NF2 mRNA expression (53.25 fold increase), compared to the control 293T cells (P=0.015) (Supplementary Figure 3A), demonstrating the achievement of successful NF2 overexpression.

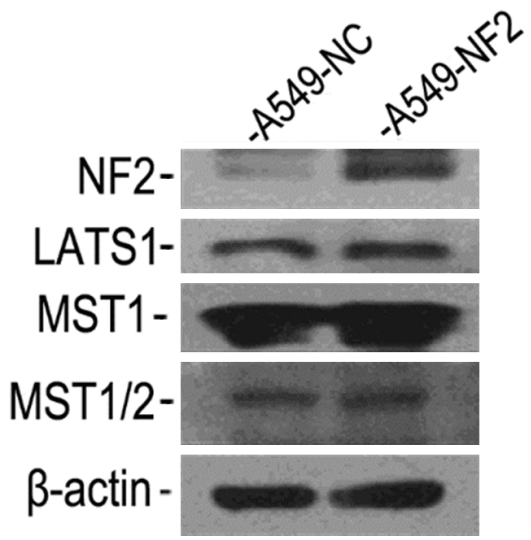

Figure 2. Protein expressions of NF2 and downstream LATS1, MST1, and MST1/2 using WB, P<0.05.

Next, A549 cells were infected with viral supernatants, and NF2 overexpression A549 cells lines were selected with FCM. NF2 mRNA expression was measured with real-time PCR. We found that NF2 mRNA expression significantly increased (4.84 fold increase) in A549 cells infected with NF2 overexpression lentivirus (pwslv-08-NF2), in comparison to the A549 control cells (pwslv-08) (P=0.035) (Supplementary Figure 3B).

We measured the protein levels of LATS1, phosphorylation MST1/2, and MST1. We found that that LATS1 and MST1 were significantly elevated in A549-pwslv-08-NF2 cells, while MST1/2 level was significantly decreased (P< 0.05, **Figure 2** and Supplementary Table 4).

Discussion

Studies have shown that the Hippo pathway is mediated by the interaction of its components [37]. MST1/2 and LATS1/2 are two important components in the Hippo pathway [38-41]. LATS1 mediates the cell cycle and plays an important role in suppressing tumor formation by interacting with other genes in the Hippo pathway [42].

Recent studies have discovered that NF2 is a major regulator of the Hippo pathway. NF2 serves as a scaffold at the plasma membrane to facilitate Hippo signaling. The NF2 tumor suppressor, also known as Merlin (Mer), positively regulates the Hippo pathway in Drosophila [43].

The core of the Hippo pathway is a kinase cascade consisting of STE20-like protein kinase 1 (also known as MST2 and MST1), the large tumor suppressors (LATS1 and LATS2), as well as numerous downstream enzymes, which ultimately regulate cell proliferation, apoptosis, and other functions [44].

Yu et al. [45] demonstrated that NF2 (merlin) can directly activate LATS1/2, or indirectly activate LATS1/2 through MST1/2 activation. Given the vast molecular diversity among cancer types, the relationships between Hippo pathway NF2 and its downstream pathways in different cancers remain poorly understood. Moreover, no study has reported the impact of the upregulation or down-regulation of NF2 on MST1/2 and LATS1/2 expressions in lung cancer.

In this study, we have demonstrated that MST1/2 and LATS1/2 can be modulated by down-regulation or upregulation of NF2 in lung cancer cells using siRNA interference or NF2-overexpression lentiviral vector, respectively. Our results suggest that NF2 has an impact on its downstream genes in lung cancer cells. MST1/2 and LATS1/2 are tumor suppressor genes in the Hippo pathway, and their mRNA or protein expressions can be used as biomarkers in diagnosis of lung cancer.

In the present study, lentiviral vector pWSLV-08 was used to construct plasmid for NF2 overexpression, which is a widely used technique. Successfully transfected A549 cells were screened and separated by FCM, and used in the following real-time PCR and WB experiments.

Based on our results, NF2 may be a direct upstream gene of MST1 and LATS1; however, we cannot rule out the possibility that there may be other genes involved in the NF2, MST1/2 and LATS1/2 pathway. Here we have proposed two mechanisms: first, overexpression of NF2 activates MST1, and then MST1 activates LATS1, resulting in the elevated LATS1. Second, overexpression of NF2 directly activates MST1, MAST1/2 and LATS1 in different ways, respectively.

Our study has limitations. First, we tested the impact of NF2 on MST1/2 and LATS1 expressions only in an *in vitro* lung cancer cell model. In our future studies, we will investigate the upregulation and down-regulation of NF2 in *in vivo* animal models. Second, YAP and TEAD are the effectors in the Hippo pathway [46, 47]. However, we did not measure their expressions in this study. In our future studies we will also evaluate YAP and TEAD expressions in relationship to NF2.

Conclusions

The downstream genes in the Hippo pathway may be mediated by NF2 in lung cancer cells. Therefore, modulating the NF2 level could be a new therapeutic target for suppressing lung cancer.

Disclosure of conflict of interest

None.

Abbreviations

Hpo, hippo; LATS1, Large tumor suppressor gene1; LATS1/2, Large tumor suppressor gene1/2; Mats, Mob as tumor suppressor; Mob1, MOB kinase activator 1A; MST1, Mammalian Ste2-like kinases1; MST1/2, Mammalian Ste2-like kinases1/2; NDR, nuclear Dbf2-related; NF2, Neurofibromin 2; ORF, open reading frame; Sav, Salvador; SWH, Salvador/Warts/Hippo; TAZ, tafazzin; TBX5, T-box transcription factor 5; Wts, Warts; YAP, Yes-associated protein; Yki, Yorkie.

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Supplementary Table 1. Reagents, antibodies, and instruments

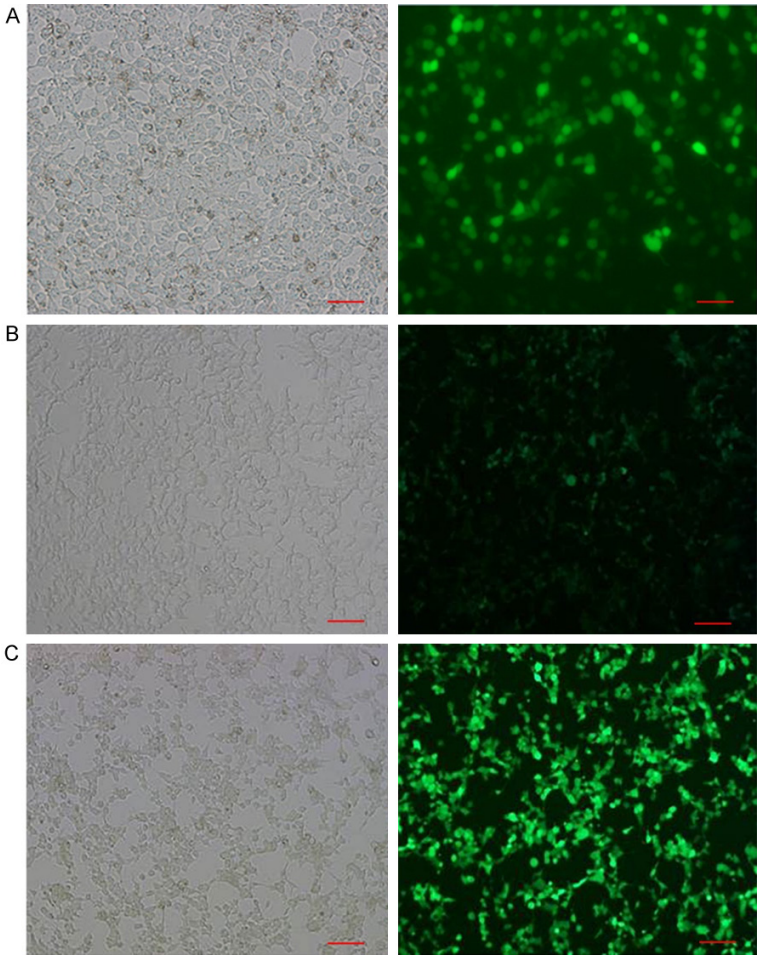
Reagent				
Name			Model/provider	
PBS*			-	
Pancreatin*			-	
DMEM-F12 basic culture medium: Gibco			-	
fetal calf serum Double-antibody: Gibco			-	
Lipofectamine RNAiMax Reagent			-	
Opti-MEM: Gibco			-	
Acrylamide			SuZhou china YaKe company	
N,N'-Methylenebis (acrylamide)			Sigma-Aldrich ShangHai	
Tris			Sigma-Aldrich ShangHai	
Sodium chloride			Sigma-Aldrich ShangHai	
Pierce ECL Western Blotting Substrate			Thermo Fisher Science	
PVDF membrane			INVITROGEN TRADING	
Running Buffer*			-	
Transfer Buffer*			-	
4×SDS Protein Loading Buffer*			-	
Blocking Buffer*			-	
Wash Buffer (PBST)*			-	
M-PER Mammalian Protein Extraction Reagent			Thermo	
Marker of transfection			Fermantas SM0671	
Antibody				
Sheep Anti-rabbit IgG (H+L)			-	
sheep Anti-mouse IgG (H+L)			-	
HRP-conjugated goat anti-Rabbit IgG			1:10000	JIR 111-035-003
Name	Model	Provider	Dilution	AW
NF2 antibody	P35240	ABGENT	1:1000	69 kDa
LAST1 antibody	O95835	ABGENT	1:500	95-135 kDa
MST1/2 antibody	Q13188	ABGENT	1:500	48-85 kDa
MST1 antibody	Q13043	ABGENT	1:500	55 kDa
Instruments				
Name			Provider/model	
Biohazard safety equipment			Thermo	
incubator-co2			Thermo	
fluorescence microscope			Nikon	
cryogenic refrigerator			haier	
Milli-Q ultrapure water producer			Millipore	
-80°C freezer			Thermo	
Tissue Culture Dish			Costar	
Electrophoresis apparatus			QianWei ShangHai company	
Trans-Blot transfer machine			Bio-Rad Laboratories	
Decoloration table			HaiMen City Qilin Medical instrument Factory	
PowerPac™HC electrophoresis apparatus			Bio-Rad	
electrophoresis tank VE-180 and VE-186			Tanon ShangHai	
KODAK X-Omat BT Film			Kodak	
X developing liquid and X fixative			GuanLong shanghai	
Tablet clip X			ShanTou YuJia Medical Instrument	

Note: *, Indicating that there is preparation of the reagent in appendix one; AW, atomic weight; PBS, phosphate buffer saline.

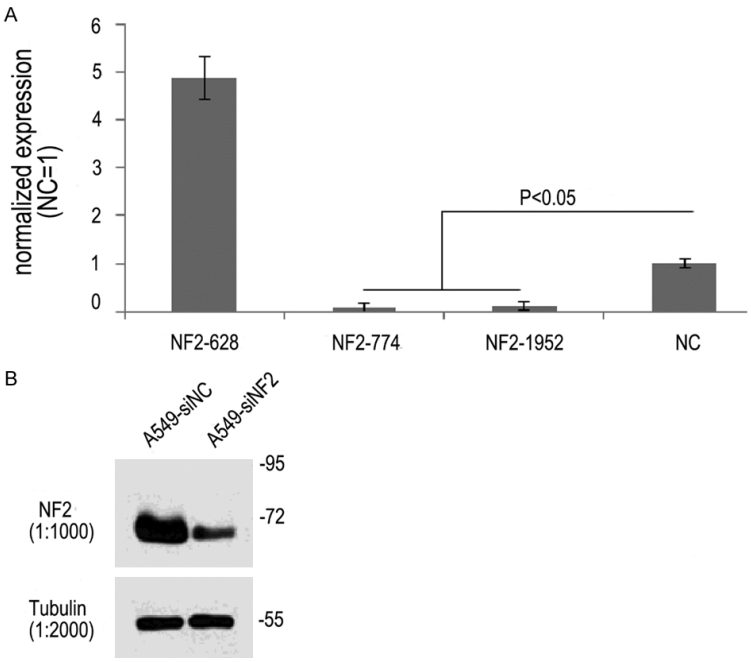
Supplementary Table 2. Primers for Q-PCR

NF2	NF2-F	ACCGTTGCCTCCTGACATAC
	NF2-R	TCGGAGTTCTCATTGTGCAG
GAPDH	GAPDH-F	CCCACTAACATCAAATGGGG
	GAPDH-R	CCTTCCACAATGCCAAAGTT

Note: F, Forward; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; NF2, neurofibromin 2; Q-PCR, Real-time quantitative Polymerase Chain Reaction; R, Reverse.



Supplementary Figure 1. Transfection and sorting of NF2 lentiviral vector. A. 293T cells transfected with NF2 lentiviral vector. B. The A549 cells were infected with NF2-overexpression lentivirus before flow cytometry sorting. C. The A549 cells were infected with NF2 overexpression lentivirus after flow cytometry sorting. Scale bars: 100 μm.

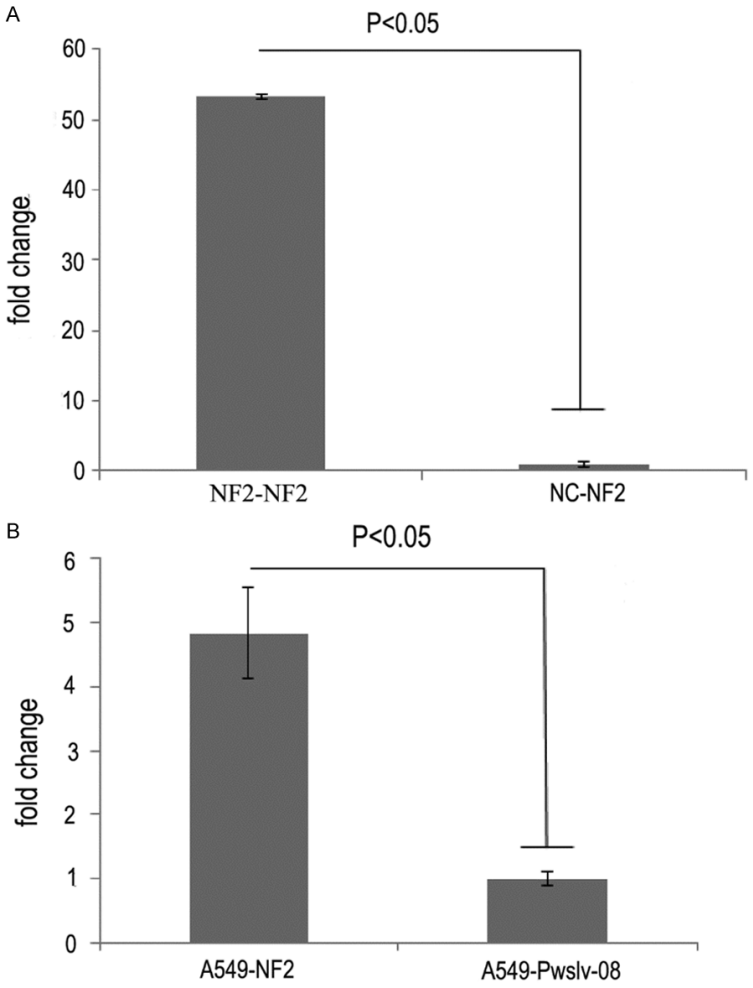


Supplementary Figure 2. siRNA interference with NF2 expression. A. Evaluation of NF2 siRNA efficiency. B. Measurement of NF2 protein expression using WB (NF2 primary antibody 1:1000, molecular weight 69 KD. Tubulin, 1:2000, as an internal reference).

Supplemental Table 3. WB data analysis of siRNA interference experiments

Sample	A549-siNC (mean ± SD)	A549-siNF2 (mean ± SD)	*P* value
NF2	651.18±18.19	121.33±4.17	
Tubulin	292.03±5.09	282.17±6.57	
NF2/Tubulin	2.23±0.10	0.43±0.03	*
LATS1	64032.26±102.44	12157.35±26.55	
GAPDH	75485.36±52.62	68624.69±23.36	
LATS1/GAPDH	0.85±0.01	0.17±0.01	*
MST1/2	44726.12±196.78	512.14±26.7	
GAPDH	71952.34±62.66	65464.55±25.09	
MST1/2/GAPDH	0.62±0.01	0.01±0.00	*
MST1	71670.1±116.51	28969.05±29.73	
GAPDH	83296.64±69.54	78284.54±61.18	
MST1/GAPDH	0.86±0.00	0.37±0.00	*

Note: *, P<0.05, indicating a statistical significance; keeping two decimal places. LATS1/2, Large tumor suppressor gene1/2; MST1/2, Mammalian Ste2-like kinases1/2.



Supplementary Figure 3. mRNA and protein expressions of NF2 and downstream LATS1, MST1, and MST1/2 using real-time PCR. A. NF2 mRNA expression in 293T cells transfected with pWSLV-08-NF2 (NF2-NF2) and in 293T cells transfected with pWSLV-08 (NC-NF2), P<0.05. B. NF2 mRNA expression in A549 cells infected with NF2-overexpression lentivirus (A549-NF2) and in A549 control cells (A549-pWSLV-08), P<0.05.

Supplementary Table 4. WB data analysis of overexpression experiments

Sample	A549-NC (mean ± SD)	A549-NF2 (mean ± SD)	*P* value
NF2	37.16±3.33	155.98±21.75	
β-actin	175.49±7.65	159.92±8.78	
NF2/β-actin	0.21±0.01	0.98±0.19	*
LATS1	109.09±6.45	158.63±8.99	
β-actin	175.13±7.06	161.14±8.07	
NF2/β-actin	0.62±0.06	0.99±0.10	*
MST1/2	81.20±5.64	66.77±3.19	
β-actin	174.46±10.43	161.97±14.26	
NF2/β-actin	0.46±0.01	0.41±0.02	*
MST1	678.01±19.39	784.03±9.48	
β-actin	175.43±13.66	159.60±8.97	
NF2/β-actin	3.89±0.41	4.92±0.22	*

Note: *, P<0.05, indicating statistical significance; keeping two decimal places. NF2, neurofibromin 2; LATS1, Large tumor suppressor gene1; MST1/2, Mammalian Ste2-like kinases1/2; MST1, Mammalian Ste2-like kinases1.