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Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer

Yang Wang, Qianze Dong, Qingfu Zhang, Zixuan Li, Enhua Wang and Xueshan Qiu[1]

Department of Pathology, First Affiliated Hospital of China Medical University and Department of Pathology, College of Basic Medical Sciences, China Medical University, Shenyang, China

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Yes-associated protein (YAP), the nuclear effector of the Hippo pathway, is a key regulator of organ size and a candidate human oncogene. This study aimed to assess the clinical significance and biological functions of YAP in non-small-cell lung cancer (NSCLC). We investigated the expression of YAP in 92 cases of NSCLC tissue by immunohistochemistry and found that YAP was expressed in 66.3% (61/92) cases and predominantly presented in the nucleus. The expression of YAP in NSCLC was significantly correlated with p-TNM stage ($P = 0.0037$) and lymph node metastasis ($P = 0.0093$). Importantly, YAP expression was associated with short overall survival. Further study in NSCLC cell lines in which YAP was either overexpressed or depleted confirmed that YAP markedly promoted cell proliferation and invasion. These results indicate that YAP plays an important role in NSCLC and might be a useful therapeutic target of NSCLC. (*Cancer Sci* 2010; 101: 1279–1285)

Lung cancer is one of the leading causes of malignancy-related deaths.[1,2] Non–small-cell lung cancer (NSCLC) accounts for up to 80% of all lung cancer cases. Despite progress made in the past decades, the 5-year survival rate for lung cancer remains under 15%.[3] Recent studies suggest that conventional therapies may have reached a therapeutic plateau,[3] so the demanding tasks are to elucidate the mechanisms of tumor biology and to identify new therapeutic targets.

The Hippo signaling pathway, initially identified in *Drosophila*, is a highly conserved potent regulator of cell growth and apoptosis.[4,5] Acting downstream of the Hippo pathway, yes-associated protein (YAP) functions as a transcription co-activator[5,6] which interacts with the PPXY-motif-containing transcription factors, including ErbB4, P73, TEAD, P53BP-2, and Runx2.[7–13] In mammals, the Hippo pathway kinase cascade phosphorylates YAP and induces its cytoplasmic translocation; thus attenuating its interaction with the nuclear binding partners and inhibiting its function.[4–6] YAP is a key regulator of organ size by orchestrating cell proliferation and death. YAP induced the transcription of genes that promote proliferation in murine livers including Ki-67, c-myc, SOX4, and AFP. At the same time, it induced expression of negative regulators of apoptosis, such as the IAP family members BIRC5/survivin and BIRC2/cIAP1, and the BCL2 family gene MCL1 .[4] In addition, YAP is vitally required for development as mice deficient in YAP have an embryonic lethal phenotype.[14]

Recently, YAP has been shown to be a candidate oncogene in the human chromosome 11q22 amplicon.[15–17] YAP expression and nuclear localization was found to be elevated in hepatocellular carcinoma, prostate cancer, colon cancer, ovary cancer, and breast cancer.[18–20] Moreover, some studies have further confirmed the oncogenic function of YAP *in vivo* and *in vitro*. YAP overexpression was able to overcome cell contact inhibition,[20] therefore providing a growth advantage for YAP-overexpressing cancer cells; YAP overexpression in MCF10A cells induced epithelial–mesenchymal transition (EMT), which is often associated with cancer metastasis;[16] transgenic mice with liver-specific YAP overexpression have shown a dramatic increase in liver size and eventually develop tumors.[4,21]

Despite the growing evidences of YAP as a crucial regulator of human cancers, its involvement in NSCLC remains to be clarified. In the present study, we evaluated the expression patterns and clinical significance of YAP in NSCLC. Moreover, we overexpressed and knocked down YAP in NSCLC cell lines to investigate its effects on cancer cell proliferation and invasion.

Materials and Methods

Tissue samples. A total of 98 cases of NSCLC and 20 cases of normal control paraffin-embedded tissue were retrieved from the Pathology Archive of China Medical University from 1995 to 2003. The histological diagnosis and grade of differentiation were evaluated by two independent pathologists, X.S.Q. and Q.F.Z., according to the 2004 World Health Organization classification. Clinicopathological information about the patients was obtained from patient records, and is summarized in Table 1. All of the enrolled patients underwent curative surgical resection without having prior chemotherapy or radiation therapy. The study was approved by the hospital ethical committee.

Cell culture. Human NSCLC cell lines A549 and NCI-H157 (H157) were cultured in RPMI-1640 (Gibco, Invitrogen, NY, USA) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. For transfections, cells were seeded in a six-well plate 24 h before the experiment.

Immunochemistry. Four-μm-thick paraffin-embedded sections on polylysine-coated slides were used for staining. Immunohistochemistry for YAP was performed as follows: slides were deparaffinized in xylene and rehydrated in a graded alcohol series. Antigen retrieval was achieved by microwaving in 10 mmol/L of sodium citrate buffer at pH 6 for 10 min. Peroxidase was blocked with 3% hydrogen peroxide in methanol, and nonspecific protein binding was blocked with 5% goat serum. The primary antibody for YAP (Cell Signaling Technology, Danvers, MA, USA) was diluted (1:100) with phosphate-buffered saline and applied overnight in a humidity chamber at 4°C. Sections were stained in parallel without primary antibody to provide a negative control for each reaction. Antibody binding was then detected by the peroxidase-based EnVision (Dako, Glostrup, Denmark) method. Visualization was performed using DAB chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted.

[1]To whom correspondence should be addressed.
E-mail: qiuxuescmu@hotmail.com

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Table 1. Association of YAP expression in NSCLC with clinical and pathologic factors (χ^2-test)

	Patients (%)	YAP negative (%)	YAP positive (%)	*P*-values
Age (years)				
≤60	46 (50.00)	12	34	0.1226
>60	46 (50.00)	19	27	
Gender				
Female	43 (46.74)	11	32	0.123
Male	49 (53.26)	20	29	
Histology				
Adeno.	54 (58.70)	13	41	0.0199*
SCC	38 (41.30)	18	20	
Differentiation				
Well	31 (33.70)	11	20	0.6672
Moderate	44 (47.83)	13	31	
Poor	17 (18.48)	7	10	
T stage				
T1	30 (32.61)	7	23	0.1435
T2 + T3 + T4	62 (67.39)	24	38	
Nodal status				
N0	54 (58.70)	24	30	0.0093*
N1 + N2	38 (41.30)	7	31	
TNM stage				
I	40 (43.48)	20	20	0.0037*
II–IV	52 (56.52)	11	41	

*$P < 0.05$. Adeno, adenocarcinoma; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; YAP, yes-associated protein.

Two independent, blinded investigators, X.S.Q. and Q.F.Z., examined all tumor slides randomly. Five views were examined per slide, and 100 cells were observed per view at ×400 magnification. We graded the YAP expression according to the distribution, intensity, and percentage of positive cells as described previously.[18] Absence of reactivity was graded as negative. For the cytoplasmic distribution, weak cytoplasmic reactivity was considered as low expression regardless of extent. Strong cytoplasmic reactivity with less than 50% positive cells was graded as low expression, otherwise it was graded as high expression. For the nuclear distribution, nuclear expression in less than 10% of cells was graded as low expression and nuclear expression in more than 10% cells was graded as high expression. Samples with low or high YAP staining were classified as YAP positive.

Plasmid and transfection. The plasmid of pcDNA3.1-hYAP was a kind gift from Dr Subham Basu (Cancer Research UK, London, UK).[22] pcDNA3.1-hYAP or control pcDNA3.1 plasmid was transfected into cells using Attractene Transfection Reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The mRNA level was assessed 48 h later by real-time PCR and protein level was assessed 72 h later by western blotting.

Small-interfering RNA experiment. SMARTpool siRNA for YAP (#M-012200-00) and siCONTROL Non-Targeting siRNA (#D-001210-03-05) were purchased from Dharmacon, (Lafayette, CO, USA). Cells were transfected with siRNA (0.2 nM per six-well plate) using the cationic lipid Dharma*FECT* 1 (Dharmacon, Lafayette, CO, USA) according to manufacturer's instructions. Following transfection, the mRNA level was assessed 48 h later by real-time PCR and protein level was assessed 72 h later by western blotting.

Quantitative real-time PCR. Total cellular RNA was extracted from cells using the RNeasy Mini kit from Qiagen (Hilden, Germany). Reverse transcription of 1 μg of RNA was done using the high capacity cDNA RT kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Quantitative real-time PCR was done using SYBR Green PCR master mix (Applied Biosystems) in a total volume of 20 μL on the 7900HT fast Real-time PCR system (Applied Biosystems) as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 60 s. The sequences of the primer pairs are listed in Table 2. A dissociation procedure was performed to generate a melting curve for confirmation of amplification specificity. β-Actin was used as the reference gene. The relative levels of gene expression were represented as $\Delta Ct = Ct_{gene} - Ct_{reference}$, and the fold change of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ Method. Experiments were repeated in triplicate.

Table 2. Primer sequences

Name	Primer sequences
YAP forward	5′-CGCTCTTCAACGCCGTCA-3′
YAP reverse	5′-AGTACTGGCCTGTCGGGAGT-3′
β-Actin forward[23]	5′-ATAGCACAGCCTGGA-TAGCAACGTAC-3′
β-Actin reverse[23]	5′-CACCTTCTACAA-TGAGCT-GCGTGTG-3′
E-cadherin forward	5′-GGGCTGGACCGAGAGAGTTT-3′
E-cadherin reverse	5′-CCTTGTACGTGGTGGGATTGA-3′
N-cadherin forward	5′-GGGATGTTGAGGTACAGAATCGT-3′
N-cadherin reverse	5′-ATACTGTTGCACTTTTTCTCGATCA-3′
Vimentin forward	5′-TCTCTGGCACGTCTTGACCTT-3′
Vimentin reverse	5′-GCTCCTGGATTTCCTCTTCGT-3′
MMP2 forward	5′-TGTGTTCTTTGCAGGGAATGAAT-3′
MMP2 reverse	5′-TGTCTTCTTGTTTTTGCTCCAGTTA-3′
MMP9 forward	5′-CCTCTGGAGGTTCGACGTGA-3′
MMP9 reverse	5′-TAGGCTTTCTCTCGGTACTGGAA-3′
TGF- β1 forward	5′-AACCCACAACGAAATCTATGACAA-3′
TGF- β1 reverse	5′-AGAGCAACACGGGTTCAGGTA-3′
TGF- β2 forward	5′-CGCTACATCGACAGCAAAGTTGT-3′
TGF- β2 reverse	5′-TCCCAGGTTCCTGTCTTTATGGT-3′

MMP, matrix metalloproteinase; TGF, transforming growth factor; YAP, yes-associated protein.

Western blotting. Total protein from cells were extracted in lysis buffer (Pierce, Rockford, IL, USA) and quantified using the Bradford method. A total of 25 μg of protein was separated using 10% SDS-PAGE and then electophoretically transferred to a PVDF membrane (Millipore, Bedford, MA). The membrane was blocked with 5% dry milk and incubated overnight at 4°C with rabbit polyclonal antibodies against YAP (1:500; Cell Signaling Technology), mouse monoantibody against E-cadherin (1:500; BD Bioscience, San Jose, CA, USA), rabbit polyclonal antibodies against N-Cadherin (1:500; Abcam, Cambridge, MA, USA), rabbit polyclonal antibodies against Vimentin (1:500; Abcam), rabbit polyclonal antibodies against matrix metalloproteinase 2 (MMP2; 1:250; Santa Cruz Biotechnology), and rabbit polyclonal antibodies against MMP9 (1:500; Abcam). After washing, the membrane was incubated with a horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology) at 37°C for 2 h. Protein bands were visualized with the enhanced chemiluminescence (Pierce) and detected using BioImaging Systems (UVP, Upland, CA, USA). The relative protein levels were calculated based on β-actin protein as a loading control.

MTT assay. Twenty-four hours after transfection, cells were plated in 96-well plates in medium containing 10% FBS at about 2000 cells per well, and the quantitation of cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, 20 μL of 5 mg/mL MTT (Sigma, St. Louis, MO, USA) solution was added to each well and incubated for 4 h at 37°C, then the media was removed from each well, and the resultant MTT formazan was solubilized in 150 μL of DMSO. The results were quantitated spectrophotometrically by using a test wavelength of 490 nm.

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Colony formation assay. Forty-eight hours after transfection, cells were planted into 6-cm cell culture dishes (1000 cells per dish) and incubated for 14 days. Cells were then stained with Giemsa and the number of colonies with more than 50 cells was counted.

Cell invasion assay. Cell invasion assay was performed using a 24-well Transwell chamber with a pore size of 8 μm (Corning, NY, USA) and the inserts were coated with Matrigel (BD Bioscience) in serum-free medium. Forty-eight hours after transfection, cells were trypsinized and transferred to the upper Matrigel chamber in 100 μL of serum-free medium containing 3×105 cells and incubated for 16 h. Then the non-invading cells on the upper membrane surface were removed with a cotton tip, and the cells which passed through the filter were fixed with 4% paraformaldehyde and stained with hematoxylin. The number of invaded cells was counted in 10 randomly selected high power fields under microscope. Data presented are representative of three individual wells.

Statistical analysis. The statistical package SPSS 13.0 (SPSS, Chicago, IL, USA) was used for all analyses as described previously.[24] All values are expressed as mean ± SD. The χ^2-test was used to determine the correlation between YAP expression and clinicopathologic factors. The Kaplan–Meier method was used to estimate the probability of patient survival, and differences in the survival of subgroups of patients were compared by using Mantel's log-rank test. A multivariate analysis was performed using the Cox regression model to study the effects of different variables on survival. Other results were analyzed using the Student's *t*-test. All *P*-values were based on the two-sided statistical analysis and a *P*-value of <0.05 was considered to indicate statistical significance.

Results

Expression of YAP in human NSCLCs and correlation with clinical factors. We investigated YAP expression in 92 cases of NSCLC tissue and 20 cases of normal lung tissue by immunohistochemistry. As described above, YAP immunoreactivity was graded as negative and positive. Positive YAP staining was observed in 66.3% (61/92) cases; 32.6% (30/92) was defined as high expression and 33.6% (31/92) as low expression. The YAP protein was mainly accumulated in the nucleus with a lesser cytoplasmic presence (Fig. 1). As for the normal lung tissues, only 15% (3/20) of cases had strong nuclear expression, and the expression was limited to type II pneumocytes (Fig. 1f) which compose the regenerative compartment of the alveolus.

We analyzed the relationship between YAP expression and the clinicopathologic factors. As described in Table 1, YAP expression was significantly correlated with pTNM stage ($P = 0.0037$) and nodal status ($P = 0.0093$). In addition, lung adenocarcinoma had a higher YAP expression rate than squamous cell carcinoma ($P = 0.0199$). No significant difference in the YAP status was observed according to the age, gender, tumor size, and differentiation. We analyzed the relation of YAP expression to the overall survival rate and found that the overall survival was significantly lower in patients with YAP-positive NSCLC than in patients with YAP-negative NSCLC ($P = 0.0427$; Fig. 2) Multivariate survival analysis revealed that

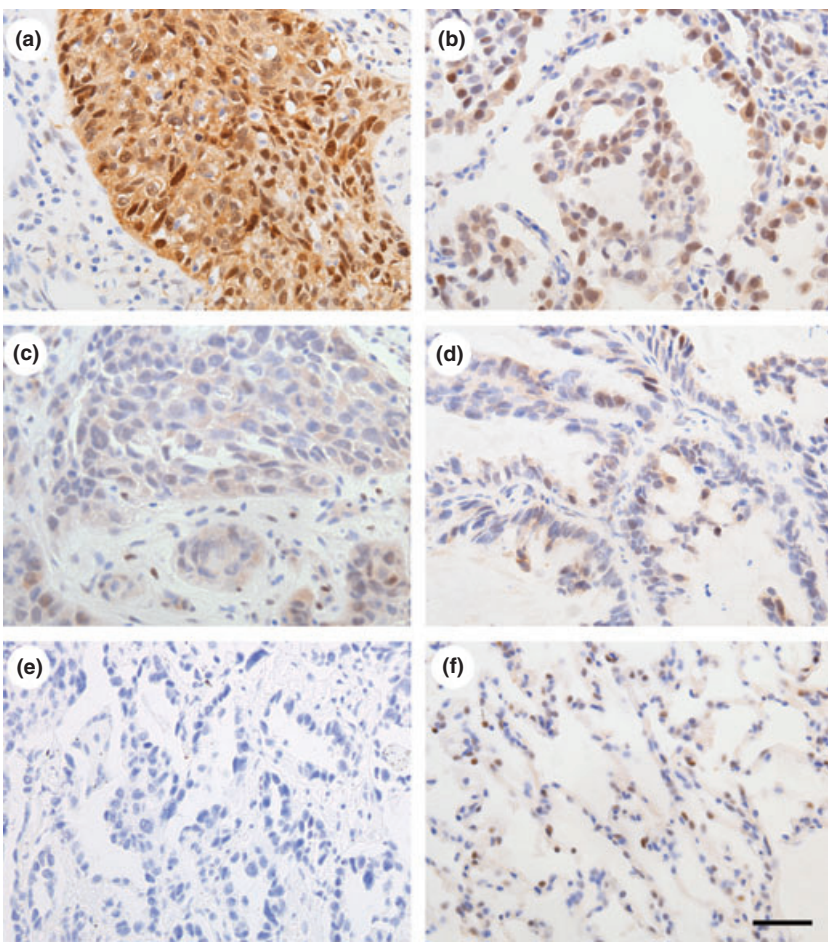

Fig. 1. Expression of yes-associated protein (YAP) in normal lung and non-small-cell lung cancer (NSCLC). (a) High YAP expression in NSCLC with both cytoplasm and nucleus present. (b) High YAP expression mainly in the nucleus of NSCLC. (c) Low YAP expression in NSCLC, staining in both cytoplasm and nucleus of tumor cells. (d) Low YAP nuclear expression in NSCLC. (e) Negative YAP expression in NSCLC. (f) YAP expression in normal lung epithelium with strong nuclear YAP present in the type II pneumocytes. Original magnification, ×400; scale bar, 50 μm.

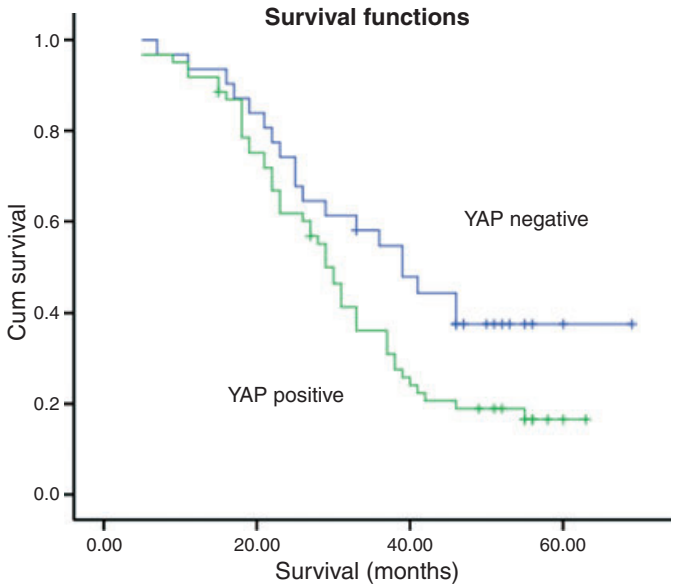

Fig. 2. Survival curves of patients with positive and negative yes-associated protein (YAP) expression.

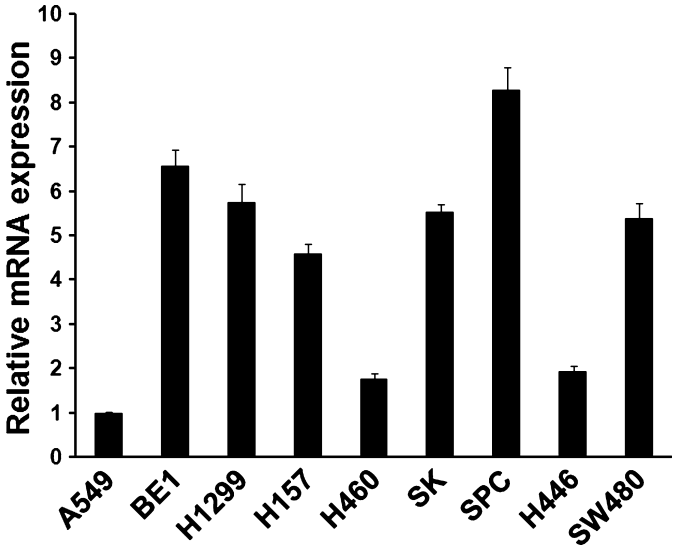

Fig. 3. Relative yes-associated protein (YAP) mRNA expression in cell lines. Seven non-small-cell lung cancer (NSCLC) cell lines, one small-cell lung cancer cell line (H446), and one colon cancer cell line (SW480) were measured by real-time PCR. The fold change of YAP expression was calculated by the $2^{-\Delta\Delta Ct}$ Method. Columns, mean; bars, SD.

the nodal status of the tumor was the significant and independent prognosticator ($P = 0.0025$; Cox regression model) (Table 3).

YAP promotes proliferation of lung cancer cells. To determine whether YAP plays a role in NSCLC cell proliferation, MTT and colony formation assays were performed. Relative expression level of YAP was analyzed by real-time PCR in a panel of lung cancer cell lines. The A549 cell line had a lower YAP level while the H157 cell line had a higher level (Fig. 3).

We overexpressed YAP in A549 cells. Transfection of pcDNA3.1-hYAP plasmid significantly increased YAP mRNA and protein levels compared with control empty vector (Fig. 4). Overexpression of YAP in A549 cells led to a significant increase in the A549 cell growth rate (Fig. 5a) and foci numbers (YAP overexpression *vs* control: 299 ± 21 *vs* 68 ± 8, $P < 0.001$) (Fig. 5b,c).

We also knocked down YAP expression in H157 cells using YAP-specific siRNA. Efficient depletion of YAP expression was confirmed by real-time PCR and western blot analysis (Fig. 4). YAP depletion in H157 cells reduced the proliferation rate (Fig. 5a) and impaired the potential of colony formation (control *vs* YAP siRNA: 194 ± 17 *vs* 102 ± 12, $P < 0.001$) (Fig. 5b,c).

YAP increases the invasion of lung cancer cells. It has been reported that YAP overexpression in MCF10A cells induced EMT and increased cell invasion. Consistently, we observed that up-regulated YAP expression was significantly correlated with lymph node metastasis in NSCLC tissues. To address the impact of YAP on NSCLC cell invasion, we performed Matrigel invasion assay. As shown in Figure 6, overexpression of YAP in A549 cells stimulated cell invasion ($P < 0.05$) when compared with control empty vector. YAP-depleted H157 cells showed a weaker invasion ($P < 0.05$) ability compared with control siRNA-transfected cells. To further explore the mechanisms by which YAP promoted NSCLC cell invasion, we explored the expression of several important EMT-related molecules in YAP-overexpressed A549 cells and YAP-depleted H157 cells. As shown in Figure 7, the mRNA levels of E-cadherin, N-cadherin, Vimentin, MMP2, MMP9, transforming growth factor-β1 (TGF-β1), and TGF-β2 were examined by quantitative real-time

Table 3. Multivariate analysis for predictive factors in patients with NSCLC (Cox regression model)

Factor	*P*-values	RR	95% CI
Tumor status	0.9755	1.009	0.561–1.816
Nodal status	0.0025	2.223	1.324–3.730
YAP	0.4219	1.267	0.711–2.257
Gender	0.8188	1.070	0.600–1.909
Age	0.3483	0.786	0.475–1.301
Histology	0.3246	0.731	0.391–1.364

CI, confidence interval; NSCLC, non-small-cell lung cancer; RR, risk ratio; YAP, yes-associated protein.

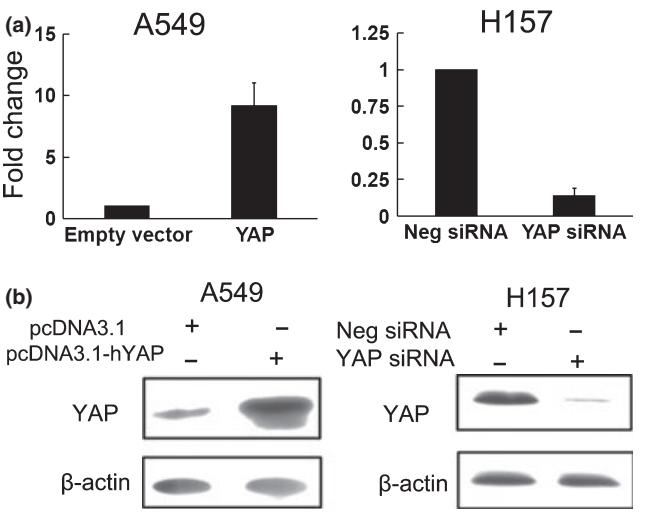

Fig. 4. Yes-associated protein (YAP) expression in lung cancer cells transfected with YAP or YAP specific siRNA. (a) Real-time PCR; and (b) western blot analysis showed the effects of YAP overexpression and YAP silencing. Left, A549 cells transfected with pcDNA3.1-hYAP and empty vector. Right, H157 cells transfected with specific YAP siRNA and negative control siRNA.

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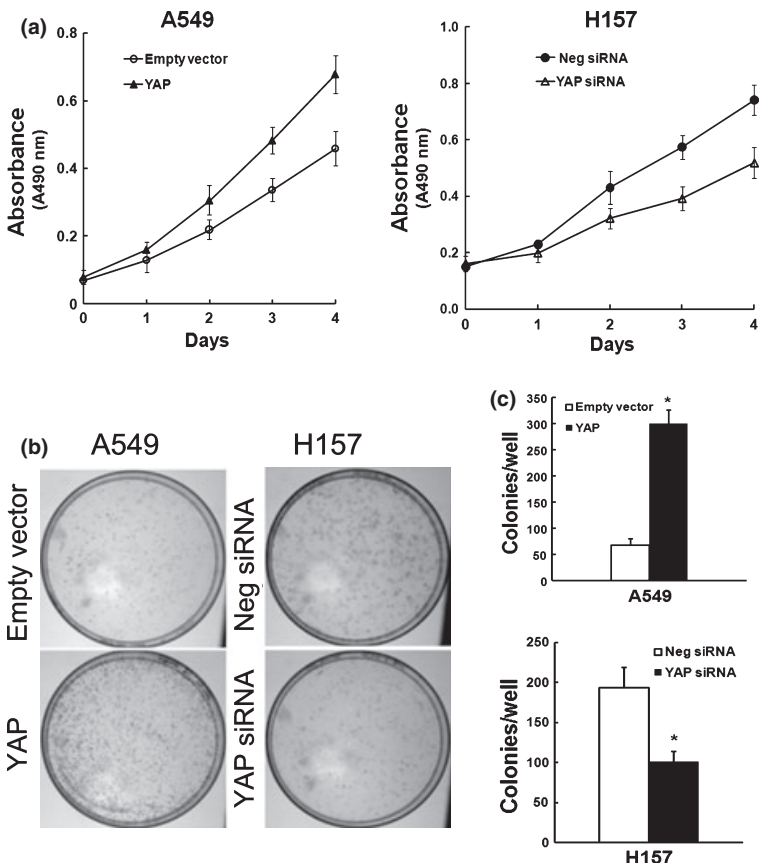

Fig. 5. Effects of yes-associated protein (YAP) on the proliferation of non-small-cell lung cancer (NSCLC) cell lines. (a) MTT assay indicated that overexpression of YAP in A549 cells promoted proliferation and that knockdown of YAP in H157 cells suppressed proliferation. Points, mean results of three independent experiments; bars, SD. (b) Colony formation assay indicated that YAP overexpression significantly promoted cell proliferation in A549 cells (YAP overexpression *vs* control: 299 ± 21 *vs* 68 ± 8, $P < 0.001$) and that YAP down-regulation markedly suppressed proliferation in H157 cells (control *vs* YAP siRNA: 194 ± 17 *vs* 102 ± 12, $P < 0.001$). (c) Columns, mean for three experiments; bars, SD; *$P < 0.05$.

RT-PCR. Meanwhile the changes of protein levels of E-cadherin, N-cadherin, Vimentin, MMP2, and MMP9 were evaluated by western blotting. However, we did not observe remarkable changes to their expression. The only interesting finding was that both the mRNA and protein levels of MMP9 were slightly decreased after YAP silencing in H157 cells. E-cadherin expression in H157 cells was undetectable. YAP depletion did not change E-cadherin status in H157 cells. Thus we did identify E-cadherin changes after the silencing of YAP in H157 cells.

Discussion

Overexpression of YAP has been implicated in tumor progression in various human cancers.[16,18,19,25] However, the expression of YAP and its correlation with clinicopathologic factors have not been defined in lung cancer. In this study, we demonstrated that overexpression of YAP in NSCLC tissues was significantly correlated with lymph node metastasis and pTNM stage. Furthermore, YAP overexpression was associated with poor prognosis of lung cancer patients. In addition, we revealed that YAP overexpression in lung cancer cells promoted cell proliferation and invasion while its depletion impaired cell viability.

In previous studies, YAP expression and nuclear localization was found to be elevated in hepatocellular carcinoma, prostate cancer, colon cancer, and breast cancer.[18–20] A study of 28 cases of lung adenocarcinoma subjected to tissue microarray showed that YAP was expressed in 54% of cases.[18] Clinical study in 177 hepatocellular carcinoma patients showed that YAP could serve as an independent predictor for hepatocellular carcinoma-specific disease-free survival and overall survival.[19] Consistent with these findings, the present report showed that the level of YAP was increased in NSCLC tissues, which was mainly accumulated in the nucleus with a lesser cytoplasmic presence. Moreover YAP expression correlated with pTNM stage ($P = 0.0037$) and lymph node metastasis ($P = 0.0093$). Importantly, positive YAP expression was associated with reduced overall survival in NSCLC patients, but did not serve as an independent predictor for patient survival. Taken together, these results indicate that YAP is an oncogenic protein overexpressed in NSCLC and is associated with lung cancer progression.

As a candidate oncogene, YAP has been shown to be a potent regulator of cell growth. Overexpression of YAP in the liver of transgenic mice can expand liver mass from 5% of bodyweight to about 25% and eventually leads to tumor growth.[4] YAP overexpression stimulates proliferation and increases saturation cell density in monolayer culture of NIH-3T3 cells.[20] To address the impact of YAP on proliferation in NSCLC, we overexpressed and silenced YAP in NSCLC cell lines. We found that overexpression of YAP resulted in a marked increase of cell growth rate, while depletion of YAP greatly suppressed cancer cell proliferation. Moreover, consistent with previous studies,[20] we found that YAP was able to overcome cell contact inhibition. The YAP-overexpressed A549 cells kept proliferating even after reaching confluency and resulted in a higher saturation density than vector control cells.

It is confirmed that YAP overexpression in MCF10A cells triggered EMT,[16] which is often associated with cancer cell invasion and metastasis. In this study, we showed that YAP overexpression significantly promoted cancer cell invasion and YAP siRNA konckdown blocked cell invasion. To further explore the mechanisms by which YAP promoted NSCLC cells, we explored the expression of several important EMT-related molecules. We did not observe remarkable changes to these molecules, but YAP still significantly affected the invasiveness. It could be argued that there are other functional aspects of YAP contributing to the regulation

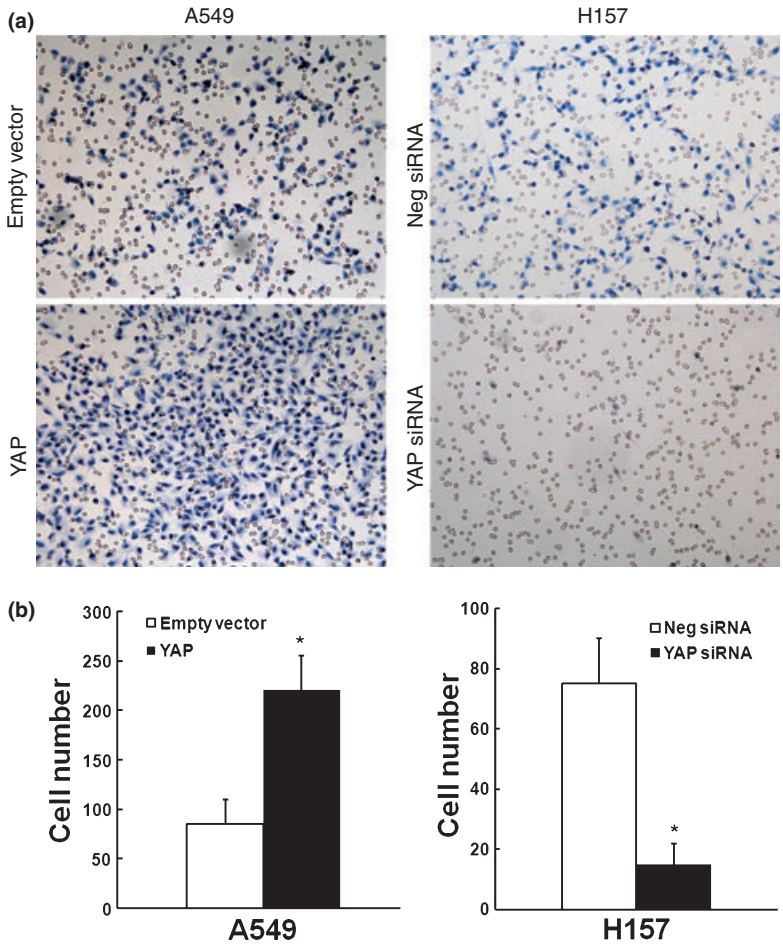

Fig. 6. Yes-associated protein (YAP) promotes cell invasion *in vitro*. (a) Transwell assay indicated that overexpression of YAP in A549 cells significantly promoted cell invasion. Knockdown of YAP in H157 cells greatly suppressed tumor cell invasion. Original magnification, ×200. (b) Graphs show the number of cells invaded through the Transwell after 16 h of incubation. The number of invaded cells was counted in 10 randomly selected high power fields (×400) under microscope. Columns, mean for three experiments; bars, SD; *$P < 0.05$.

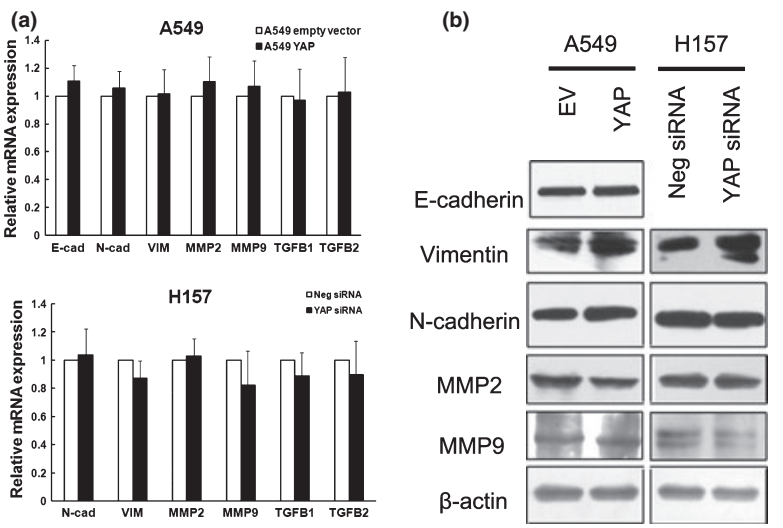

Fig. 7. Effects of yes-associated protein (YAP) on epithelial–mesenchymal transition (EMT)-related molecules. (a) Real-time PCR and (b) western blotting showed the expression changes after overexpressing YAP in A549 cells and silencing YAP in H157 cells. The expression of E-cadherin (E-cad), N-cadherin (N-cad), Vimentin (VIM), matrix metalloproteinase 2 (MMP2), MMP9, transforming growth factor-β1 (TGFB1), and transforming growth factor-β2 (TGFB2) were explored. Columns, mean; bars, SD.

of invasion besides the effect on EMT. Together, these results suggested YAP had an important effect on lung cancer cell growth and invasion, which is in line with our clinical data that YAP correlated with lymph nodal status and stage.

However, YAP was also reported to be a tumor suppressor as its gene locus was deleted in some breast cancers with a correlated loss of YAP protein expression.[26] In addition, some studies have identified YAP as a transcriptional coactivator promoting anti-tumoral effects on the basis of its interaction with p53 family members, p73, and p53-binding protein-2.[27] These results present a rather intriguing question: is YAP1 an anti- or pro-tumorigenic protein? One possible explanation is that pathways are not fixed but can dynamically change depending on the context.[28] Thus, it is possible that YAP function

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changes depending on the upstream input, and the variations of binding partners' repertoire between cells.

In conclusion, this study identifies YAP as an oncoprotein overexpressed in NSCLC which is important for the regulation of malignant cell growth and invasion. Moreover, YAP expression contributes to the poor prognosis of NSCLC, making it a candidate target protein for future cancer therapeutics. Additional work is needed to elucidate the molecular functions of YAP in NSCLC.

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Disclosure Statement

The authors have no conflict of interest.

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