Increased STIP1 and Hsp90 Correlate with Progression and Prognosis of Lung Adenocarcinoma

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Research Article

Keywords: Lung adenocarcinoma,LAC,stress-inducible phosphoprotein 1, STIP1, heat shock protein 90, Hsp90

Posted Date: February 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-226260/v1

Abstract

Background: Stress-inducible phosphoprotein 1 (STIP1) and heat shock protein 90 (Hsp90) have been found to be correlated with malignant tumors. The aim of this investigation was to study the relationship between their expressions and lung adenocarcinoma (LAC).

Methods: The expressions of STIP1and Hsp90 in LAC cells and tissues were tested by immunohistochemistry and western blot; the correlation between their expressions and clinicopathological parameters of LAC was analyzed by survival analysisand multiple regression analysis.

Results: Expressions of STIP1 and Hsp90 were higher in A549 cells and LAC tissues than that in 16 human bronchial epithelial cells (16HBE cells) ($P<0.05$) and adjacent normal lung tissues ($P < 0.05$). The expression of STIP1 and Hsp90 in LAC showed a strong positive correlation ($P < 0.05$) and significantly correlated with lymph node metastasis ($P < 0.05$), advanced clinical stage ($P < 0.05$) and shorter survival ($P < 0.05$) of LAC.

Conclusions: Increased expressions of STIP1 and Hsp90 were closely related to malignant biological behavior of LAC, suggesting that they could be used as potential biomarkers and prognostic indicators for LAC.

Introduction

Lung cancer is one of the most malignant tumors with the fastest growth in morbidity and mortality and the greatest threat to population health and life[1]. In the past 20 years, the incidence of lung cancer has been reported to increase significantly in China [2] and the incidence of lung adenocarcinoma (LAC) has exceeded that of lung squamous cell carcinoma (LSCC) [2]. Clinical studies show that LAC is closely related to specific carcinogenic driver genes and the structural changes (mutation, overexpression or rearrangement) of these specific oncogenes can trigger and promote the development of LAC cells [3–5]. So far, it has been known that lung cancer patients associated with driving genetic mutations have reached 62% of the total number of lung cancers[6, 7]. In any case, it is believed that there are more unknown driving genes associated with the development of LAC that have not been discovered [8].

Although the human genome map has been drawn, the function of the protein encoded by the gene is extremely complicated, so the study of protein function has become the focus of the post-genome era [9]. The STIP1 gene is located in the human chromosome 11q13 coding region and consists of 14 exons with a protein molecular weight of 63 kDa, which is located near many cancer-related genes [10]. It is reported that up-regulated STIP1 promotes the growth, colony formation and migration of liver cancer cells [11] and high STIP1 expression is correlated with advanced T stage of colorectal cancer [12]. Heat shock protein 90 (Hsp90) plays an important role in protein folding, subunit assembly, and regulation of cell growth and survival [13, 14]. It has been found to be highly expressed in some solid tumors, which is closely related to tumor progression and prognosis [15, 16]. The purpose of this study was to quantify the

difference in expressions of STIP1 and Hsp90 in LAC cells and tissues, and to verify their associations with LAC clinical parameters.

Material And Methods

Cell culture

Human LAC cell lineA549 purchased from Life Sciences Cell Bank of Chinese Academy of Sciences. 16HBE cells were purchased from the cell bank of the state key laboratory of environment and genetics, Xi'an Jiaotong University. The 16HBE cells were cultured in RPMI 1640 medium with 10% FBS, 100 g/mL penicillin and 100 g/mL streptomycin. The A549 cells were cultured in culture flasks (culture medium preparation: 10% fetal bovine serum + 1% double antibody + 89% DMEM high glucose medium). The incubator environment was set to 37°C and with a 5% of CO2 content. When the cell density at the bottom of the bottle reached 80%, the cells were passaged. The cells in logarithmic growth were harvested for total protein extraction.

Patients selection

From January 2013 to December 2015, LAC samples (LAC tissue and adjacent normal tissue which is at least 3cm away from the cancer tissue) from a total of 72 patientsundergoing surgery were collected (Affiliated Hospital of Jining Medical University, Jining, Chinaand Gansu Provincial Hospital, Lanzhou, China). Important clinical data for patients were extracted (gender, age, smoking status, cell differentiation, lymph node metastasis and clinical staging) (Table 1). Survival data were defined as the time from the time of surgery until the time of death or loss of follow-up. The study was approved by the Institute's Ethics Committee of Affiliated Hospital of Jining Medical University, Jining, China. The patient's consent and informed consent were obtained before the collection of tissue samples from the patient.

Table 1
Clinicopathological parameters in patients with lung adenocarcinoma (N = 73)

Group	Parameters	Cases	%
Gender			
	Male	43	58.9
	Female	30	41.1
Age			
	< 60	37	50.7
	≥ 60	36	49.3
Smoking status			
	Yes	35	47.9
	No	38	52.1
Degree of differentiation			
	Poor	32	43.8
	Moderate	22	30.1
	Well	19	26.1
Lymphatic metastasis			
	No	31	42.5
	Yes	42	57.5
TNM staging[]			
	III	45	61.6
	III	28	38.4

TNM staging: T = primary tumor; N = regional lymph nodes; M = distant metastasis; [], The 8th edition of the TNM classification for non-small lung cancer.

Western blot

The cultured A549 cells were lysed with RIPA lysate to extract total cellular protein. The BCA method was used to determine the protein concentration of each sample.After the boiling water bath denaturation treatment, an equal amount of total protein was subjected to polyacrylamide gel electrophoresis. After electrophoresis, the total protein was transferred to a PVDF membrane. After the PVDF membrane was blocked with a 10% skim milk powder solution, the corresponding primary antibody was added and incubated at 4°Covernight. The PVDF membrane was rinsed and then incubated with a secondary

antibody at 37°C for 45 min. The PVDF membrane was illuminated by ECL method and was exposed in a dark room with X-ray film.

Tissue microarray (TMA) construction

The tissue sections were observed and labeled under the light microscope. A tissue array instrument was used to locate and drill a hole with a diameter of 1mm on the recipient wax block. A tissue column with a diameter of 1 mm was drilled from the tumor tissue and inserted into the hole of the recipient wax block. The prepared tissue chip wax block was incubated in a 37°C incubator for 1 h. After cooling at room temperature, it was cut into 4 μm thick tissue sections and placed in an oven at 60°C overnight. The sections were subjected to HE staining to see if the samples were accurate, and the qualified sections were kept at -4°C for use.

Immunohistochemistry (IHC)

The expressions of STIP1 (dilution concentration of mouse anti-human STIP1 polyclonal antibody, 1:500; Abcam) and Hsp90 (dilution concentration of mouse anti-human Hsp90 polyclonal antibody, 1:300; Boster) in tissues were detected by immunohistochemical staining. A positive expression of STIP1 in thyroid cancer was used as a positive control for STIP1 and a positive expression of Hsp90 in breast cancer was used as a positive control for Hsp90. PBS buffer instead of primary antibody was used as a negative control. The expressions of STIP1 and Hsp90 were stained as tan and brownish yellow, which were distributed in the cytoplasm of LAC cells. The semi-quantification analysis of IHC were performed by the following criteria: 0 for tumor cells without staining; medium staining was 1 point; more than 75% of tumor cells were stained for 2 points; more than 1 point is considered a positive expression [17].

Statistical analysis

SPSS 20.0 software was used for statistical analysis. The chi-square test and Fisher's exact test were used for comparison of counting data between groups. The one-way ANOVA was used to compare the measurement data. Correlation analysis between the enumeration data was performed by the cruskal-wallis rank sum test.Kaplan-meier method was used to analyze the survival data. Bilateral test was taken and $P < 0.05$ was considered statistically significant.

Results

Expression levels of STIP1 and Hsp90 in A549 cells are higher than that in 16HBE cells

A Western blot method was used to determine the expression levels of STIP1 and Hsp90 in A549 and 16HBE cells, we found that the expressions of STIP1 and Hsp90 in A549 cells were higher than that in 16HBE cells (Fig. 1A). Quantitative analysissuggested that the expression levels of STIP1 (0.88 ± 0.17) and Hsp90(0.79 ± 0.27) in the A549 cells were higher than that in 16HBE (0.46 ± 0.13 for STIP1; 0.52 ± 0.19 for Hsp90, $P < 0.001$) (Fig. 1B).

Expression levels of STIP1 andHsp90 in LAC tissues are higher than that in adjacent normal lung tissues

The expression rate of STIP1 in cancer adjacent normal tissues was 21.9% (16/73) and 57.7% (42/73) in LAC tissues, showing that the expression of STIP1 in LAC was higher than that in non-cancerous lung tissues($P < 0.001$)(Table 2; Fig. 1C; Figs. 2A - D). The expression of Hsp90 also showed a significant increase in LAC tissues (43/73; 68.9%), compared with cancer adjacent normal tissues (24/73; 32.9%) ($P = 0.002$) (Table 2; Fig. 1D; Figs. 2E - H).

Table 2
Comparison of STIP1 and HSP90 expression in LAC and adjacent normal tissues (73)

Items	Groups	N	Comparison of expression rates			
			Negative (%)	Positive (%)	χ^2 value	*P* value
STIP1						
	Normal	73	57(78.1)	16(21.9)	19.337	< 0.001
	LAC	73	31(42.5)	42(57.7)		
Hsp90						
					9.958	0.002
	Normal	73	49(67.1)	24(32.9)		
	LAC	73	30(41.1)	43(68.9)		

LAC, lung adenocarcinoma; HSP90, heat shock protein 90; STIP1, stress-inducible phosphoprotein 1.

Expression of STIP1 is associated with poor differentiation, lymph node metastasis and advancedstage of LAC

Compared with well differentiation (6/19; 31.6%), no-lymph node metastasis (3/28; 9.6%) and early stage (16/45; 33.3%), STIP1 was highly expressed in LAC tissues with poor differentiation (24/32; 75%) ($P = 0.019$), lymph node metastasis (39/42; 92.9%) ($P < 0.001$), and advanced stage (26/28; 92.8%) ($P = 0.002$), suggesting that elevation of STIP1 was associated with malignant biological behavior of LAC (Table 3; Figs. 3A - C).

Table 3
Correlation between STIP1 expression and clinical features of LAC (n = 73)

Items	Groups	N	Expression of STIP1 in lung tissues			
			Negative (%)	Positive (%)	χ^2 value	*P* value
Gender						
	Male	43	19(44.2)	24(55.8)	0.127	0.722
	Female	30	12(40)	18(60)		
Ages						
	< 60	37	16(43.2)	21(56.8)	0.019	0.892
	≥ 60	36	15(41.7)	21(58.3)		
Smoking						
	Yes	35	18(51.4)	17(48.6)	2.211	0.137
	No	38	13(34.2)	25(65.8)		
Differentiation						
	Poor	32	8(25)	24(75)	7.896	0.019
	Moderate	22	10(40.9)	12(59.1)		
	Well	19	13(68.4)	6(31.6)		
Lymph node metastasis						
	No	31	28(90.4)	3(9.6)	50.51	< 0.001
	Yes	42	3(7.1)	39(92.9)		
TNM staing						
	III	45	29(66.7)	16(33.3)	10.037	0.002
	III	28	2(7.2)	26(92.8)		

LAC, lung adenocarcinoma; TNM, stage of lung cancer; STIP1, stress-inducible phosphoprotein 1.

Expression of Hsp90 is associated with lymph node metastasis and advancedstage of LAC

A higher expression of Hsp90 was observed in lymph node metastasis (37/42; 88.1%) (*P*< 0.001)and advanced LAC tissues (24/28; 85.7%) (*P*< 0.001)compared to no-lymph node metastasis (6/31; 19.4%) and early stage of LAC (9/45; 42.2%), suggesting that elevation of Hsp90 was associated with metastasis and progression of LAC (Table 4; Figs. 3D - F).

Table 4
Correlation between HSP90 expression and clinical features of LAC (n = 73)

Items	Groups	N	Expression of HSP90 in lung tissues			
			Negative (%)	Positive (%)	χ^2 value	*P* value
Gender						
	Male	43	17(39.5)	26(60.5)	0.105	0.746
	Female	30	13(43.3)	17(56.7)		
Ages						
	< 60	37	16(43.2)	21(56.8)	0.143	0.705
	≥ 60	36	14(38.9)	22(61.1)		
Smoking						
	Yes	35	15(42.9)	20(57.1)	0.086	0.769
	No	38	15(39.5)	23(60.5)		
Differentiation						
	Poor	32	9(34.4)	23(65.6)	3.016	0.221
	Moderate	22	10(45.5)	12(54.5)		
	Well	19	11(57.9)	8(42.1)		
Lymph node metastasis						
	No	31	25(80.6)	6(19.4)	34.815	< 0.001
	Yes	42	5(11.9)	37(88.1)		
TNM staging						
	III	45	26(57.8)	19(42.2)	13.487	< 0.001
	III	28	4(14.3)	24(85.7)		

LAC, lung adenocarcinoma; TNM, stage of lung cancer; HSP90, heat shock protein 90.

Expressions of STIP1 and Hsp90 in LAC showed a strong positive correlation

STIP1 and Hsp90 had a positive co-expression rate of 38.4% (28/73) and a co-negative rate of 54.8% (40/73). The *Pearson* correlation coefficient was 0.86 ($P < 0.001$) and the *Spearman* correlation coefficient was 0.86 ($P < 0.001$), which indicated that there was a significant positive correlation between

the expressions of STIP1 and Hsp90.The *Kappa* value was 0.859($P < 0.001$), indicating that there was a consistent trend between their expressions.

Expressions of STIP1 and Hsp90 are negatively correlated with the survival of LAC patients

As shown in Table 5, compared with patients with negative STIP1(49.83 ± 1.23 months; 95% CI = 47.43–52.24), LAC patients with STIP1 expression(29.59 ± 1.07 months; 95% CI = 27.49–31.69) had shorter survival(Log Rank, P < 0.001; Breslow, P < 0.001) (Figs. 4A and B). In addition,survival of LAC patients with expression of Hsp90(30.46 ± 1.58 months; 95% CI = 28.03–32.85)was shorter than those without expression of Hsp90 (49.34 ± 1.39 months; 95% CI = 46.74–51.95)(Log Rank, P < 0.001; Breslow, P < 0.001) (Figs. 4C and D).

Table 5
Correlation analysis between the expressions of STIP1 and Hsp90 and the survival of patients with LAC

Items	Groups	Mean	Standard Deviation	95% Confidence Interval		Log Rank		Breslow	
				Lower bound	Upper bound	Chi-square	P-value	Chi-square	P-valve
STIP1	Negative	49.83	1.23	47.43	52.24	65.11	< 0.001	52.35	< 0.001
	Positive	29.59	1.07	27.49	31.69				
Hsp90	Negative	49.34	1.39	46.74	51.95	50.06	< 0.001	43.74	< 0.001
	Positive	30.46	1.58	28.03	32.85				

STIP1, stress-inducible phosphoprotein 1; HSP90, heat shock protein 90.

Multiple regression analysis of clinical parameters and overall survival of patients with LAC

As shown in Table 6, a total of 8 parameters were included in the analysis, including gender, age, smoking or not, degree of tissue differentiation, lymph node metastasis, TNM staging, and expression of STIP1 and Hsp90. Finally, three parameters, TNM staging (P value = 0.004; OR value = 2.991; 95% CI = 1.405–6.366), STIP1 (P value = 0.001; OR value = 9.614; 95% CI = 2.463–37.52) and Hsp90 expression (P value = 0.015; OR value = 3.585; 95% CI = 1.279–10.028), were included in the regression equation. The results suggested that TNM staging, STIP1 and Hsp90 expression were risk factors for shortening the survival of patients with LAC and the regression equation based on COX regression was $H(t)=[h_0(t)]e^{(1.612\ X6 + 2.223\ X7 + 1.277\ X8)}$.

Table 6
Multiple regression analysis of correlation between clinical parameters and overall survival of patients with LAC

Variables (X)	Items (single parameter grouping)	P value	Odds ratio value	95% CI for odds ratio	
				lower	upper
Gender (X1)	Male (X_{1-0}) vs. female (X_{1-1})	0.918	-	-	-
Age (X2)	< 60 (X_{2-0}) vs. ≥60 (X_{2-1})	0.297	-	-	-
Smoking (X3)	Yes (X_{3-0}) vs. no (X_{3-1})	0.264	-	-	-
Differentiation (X4)	Poor (X_{4-0}) vs. moderate (X_{4-1}) vs. well (X_{4-2})	0.127	-	-	-
Lymphatic invasion (X5)	Positive (X_{5-0}) vs. negative (X_{5-1})	0.657	-	-	-
TNM (X6)	I-II (X_{6-0}) vs. III (X_{6-1})	0.004	2.991	1.405	6.366
STIP1 (X7)	Negative (X_{7-0}) vs. positive (X_{7-1})	0.001	9.614	2.463	37.52
Hsp90 (X8)	Negative (X_{8-0}) vs. positive (X_{8-1})	0.015	3.585	1.279	10.028
Risk function:		$H(t)=[h_0(t)]e^{(1.612\ X6 + 2.223\ X7 + 1.277\ X8)}$			

LAC, lung adenocarcinoma; CI, confidence interval; TNM, stage of lung cancer; STIP1, stress-inducible phosphoprotein 1; HSP90, heat shock protein 90.

Discussion

Lung cancer is one of the major diseases that cause cancer-related death. Among them, LAC is the most common pathological type, and it has gradually increased in recent years [1]. Studies have shown that some gene mutations and protein expression abnormalities are closely related to the occurrence and development of LAC, and some molecular targeted drugs have been used in clinical practice [3, 18, 19]. STIP1 is a is an adaptor protein that combines heat shock protein 70 and heat shock protein 90 to modulate biological effects such as transcription, translation, and protein folding, and promotes the growth of tumor cells [12, 20, 21]. According to a recentmeta-analysis, the high expression of STIP1 was related to lymph node metastasis, advanced TNM stage and shorter survival of patients compared with that of tumor tissues with low STIP1 expression, which was expected to be a molecular marker of malignancy or provide new ideas for the development of targeted drugs [22]. However, the malignant tumors included in themeta-analysis have only ovarian cancer, liver cancer, gastric cancer, thyroid cancer, and colon cancer. This also reflects the current lack of research on the expression pattern of STIP1 in

LAC. In our study, we investigated the association and prognostic significance of STIP1 and Hsp90 with LAC clinical features by methods of molecular biology and biostatistics.

We first used the Western blot method to determine the expression patterns of STIP1 and Hsp90 in A549 and 16HBE cells and found that the expressions of STIP1 and Hsp90 in A549 cells were higher than that in 16HBE cells. Some studies have observed that the expression of STIP1 is increased in other tumor cells and tissues, and suggests that STIP1 expression plays a role in the occurrence, invasion and metastasis of malignant tumors [23, 24]. We found that the expressions of STIP1 and Hsp90 in LAC tissues were higher than that in adjacent normal lung tissues. These results from our study suggest that STIP1 and Hsp90 may play an importantrole in the occurrence and development of LAC, and the synergistic increase of the two may be risk factor for LAC. Hsp90 has been found to be highly expressed in the serum and tissues of lung cancer patients and up-regulation of Hsp90 is related to the occurrence, development and outcome of lung cancer [25]. We found that the expression of STIP1 was associated with poor differentiation, lymph node metastasis and advanced stage of LAC and that the expression of Hsp90 was related to lymph node metastasis and advanced stage of LAC. Lymph node metastasis and poor differentiation are the malignant biological characteristics of tumors and are often used to determine the treatment of tumors and to predict the prognosis of tumors [26–28]. Previous studies suggest that STIP1 is elevated in patients with lymph node metastasis and advanced clinical stages, and indicate that STIP1 expression may serve as a potentially valuable biomarker for cancer [11, 12, 20–22, 24]. As a chaperone, Hsp90 can activate signals of some cancer-related proteins, kinases and transcriptional regulatory proteins, which lead to the proliferation of tumor cells and promote the development, migration and metastasis of tumors [25]. However, inhibition of Hsp90 expression has shown inhibition of lung cancer cell proliferation, migration and metastasis, which involves complex signaling pathways including induction of apoptosis, inhibition of vascular endothelial growth factor (VEGF) and EGFR pathways [15, 16, 29, 30]. Combining our findings with previous studies, we hypothesized that the high expression of STIP1 and Hsp90 may promote the progression and invasion of LAC cells, thus speeding the development of LAC.

STIP1 has been shown to act as an adaptor which can guide Hsp90 to Hsp70 in cytoplasm to form a customer protein complex and ultimately regulate its molecular chaperone activity, which play complex roles in RNA splicing, signal transcription, protein folding, signal transduction and cell cycle regulation [10, 31]. Overexpression of STIP1 in cancer cells is associated with regulation of the JAK2-STAT3 signaling pathway. The interaction of STIP1 and HSP90 promotes maturation of the JAK2 protein and forms a scaffold complex that transduces JAK2-STAT3 signaling. However, inhibiting the expression of STIP1 can block the interaction between STIP1-Hsp90 and showed an antitumor effect[23]. From our study, we deduce that STIP1 and Hsp90 have synergistic effects in the occurrence and development of LAC, and the common increase in both may play a role in the progress of LAC. During our follow-up, 64 out of 73 patients were followed up with complete survival data. We found that patients with expressions of STIP1 and Hsp90 had a shorter survival than those without expressions, which means that STIP1 and Hsp90 are prognostic risk factors for LAC. A meta-analysis points out that cancerpatients with high STIP1 expression often have shorter overall survival (hazard ratio = 2.15) compared with those with lower

STIP1 expression [22]. A clinical pathology analysis suggests that STIP1 expression is significantly associated with tumor size, lymph node metastasis, and TNM stage, and patients with higher STIP1 expression have a shorter overall survival [32]. Our analysis suggested that TNM staging, STIP1 and Hsp90 were included in the regression equation, indicating that advanced TNM staging, STIP1 and Hsp90 are risk factors for shortening the survival of patients with LAC.A study of ovarian cancer suggests that patients with STIP1 expression have worse survival (high STIP1 = 76 months; low STIP1 = 112 months) [21]. And, high expression of STIP1 is associated with the poor prognosis of breast cancer patients and HER-2 positive expression [20]. The expression of Hsp90 in lung cancer tissue has been found to beincreased and thesurvival is shorter inpatients with high expression than in those with low expression [33]. And, the high expression of Hsp90 in lung cancer promotes the metastasis and proliferation of lung cancer [34–36]. However, inhibition of Hsp90 expression has been found to block proliferation, migration and metastasis of lung cancer cells [36].

Conclusions

Elevated expressions of STIP1 and Hsp90 in LAC cells and tissues closely related to lymph node metastasis, advanced clinical stage and shorter survival of LAC, suggesting that they could be used as potential biomarkers and prognostic indicators for LAC.

Abbreviations

2D-LC-MS/MS, two-dimensional liquid chromatography/tandem mass spectrometry; 16HBE, 16 human bronchial epithelial cells; ALK, anaplasticlymphoma kinase; EGFR, epidermal growth factor receptor; Hsp90, heat shock protein 90; iTRAQ, isobaric tags for relative and absolute quantification; IHC, immunohistochemistry; LAC, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; MET, mesenchymal to epithelial transition factor; RET, rearranged during transfection; ROS1, proto-oncogene tyrosine-protein kinase ROS; STIP1, stress-inducible phosphoprotein 1; TMA, tissue microarray; VEGF, vascular endothelial growth factor.

Declarations

Ethics approval and consent to participate

The study was approved by the Institution Ethics Committee of the First Affiliated Hospital, Xi’an Medical University, Xi’an, China. All patients in this study signed an informed consent form before being included in the study. We specifically declare that all research strategies and methods involved in this research are implemented in accordance with the Declaration of Helsinki.

Consent to publish

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None.

Funding

None.

Authors' contributions

BX R, YW Z, JY W, SC Y, MQ G, and SH J participated in the design and coordination of the study, carried out the critical appraisal of studies, statistical analysis of studies and wrote the manuscript. All authors have read and approved the manuscript, and ensure that this is the case.

Acknowledgements

None.

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Figures

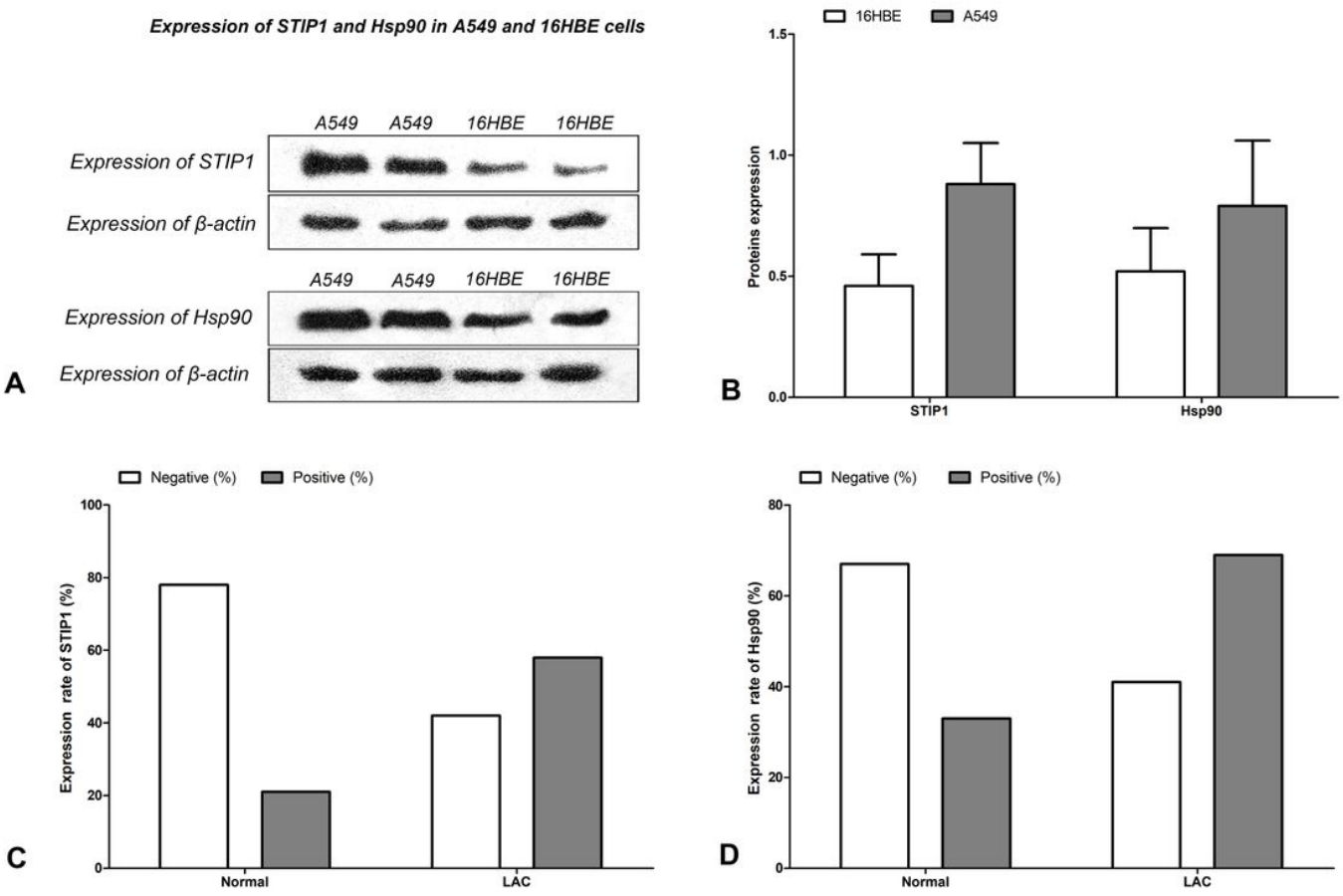

Figure 1

Protein expressions of STIP1 and Hsp90 in A549 and 16HBE cells. (A) Western blot showed that the expressions of STIP1 and Hsp90 in A549 cells were higher than that in 16HBE cells. (B) Quantitative analysis suggested that the expression levels of STIP1and Hsp90 in the A549 cells was higher than that in 16HBE cells. (C) Expression of STIP1 in LAC tissues was higher than that in adjacent normal lung tissues. (D) Expression of STIP1 in LAC tissues was higher than that in adjacent normal lung tissues. LAC, lung adenocarcinoma; STIP1, stress-inducible phosphoprotein 1; Hsp90, heat shock protein 90; 16HBE, 16 human bronchial epithelial cells.

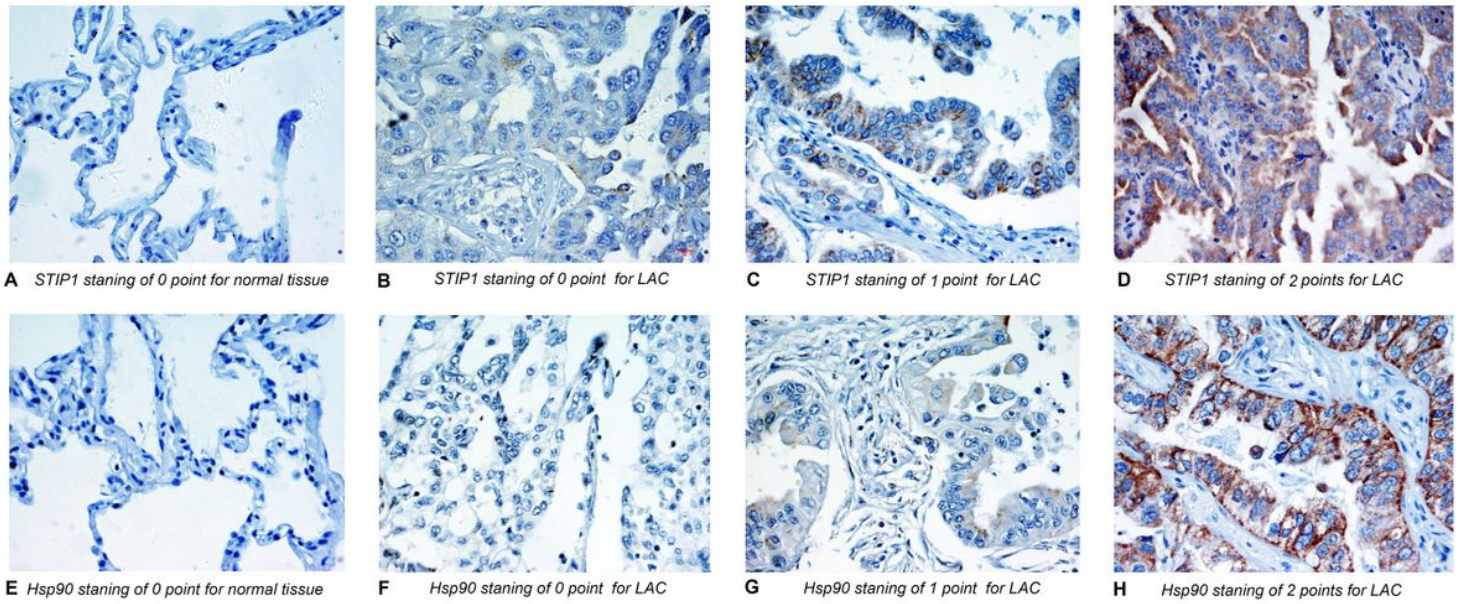

Figure 2

Protein expressions of STIP1 and Hsp90in LAC tissues (×400). (A) Low expression of STIP1 in adjacent normal lung tissues. (B) Low expression of STIP1 in well differentiated LAC tissues. (C) Moderate expression of STIP1 in moderate differentiated LAC. (D) High expression of STIP1 in poor differentiated LAC. (E) Low expression of Hsp90 in adjacent normal lung tissues. (B)Low expression of Hsp90 in well differentiated LAC tissues. (F) Moderate expression of Hsp90 in moderate differentiated LAC. (G) High expression of Hsp90 in poor differentiated LAC.LAC, lung adenocarcinoma; STIP1, stress-inducible phosphoprotein 1; Hsp90, heat shock protein 90; 16HBE, 16 human bronchial epithelial cells.

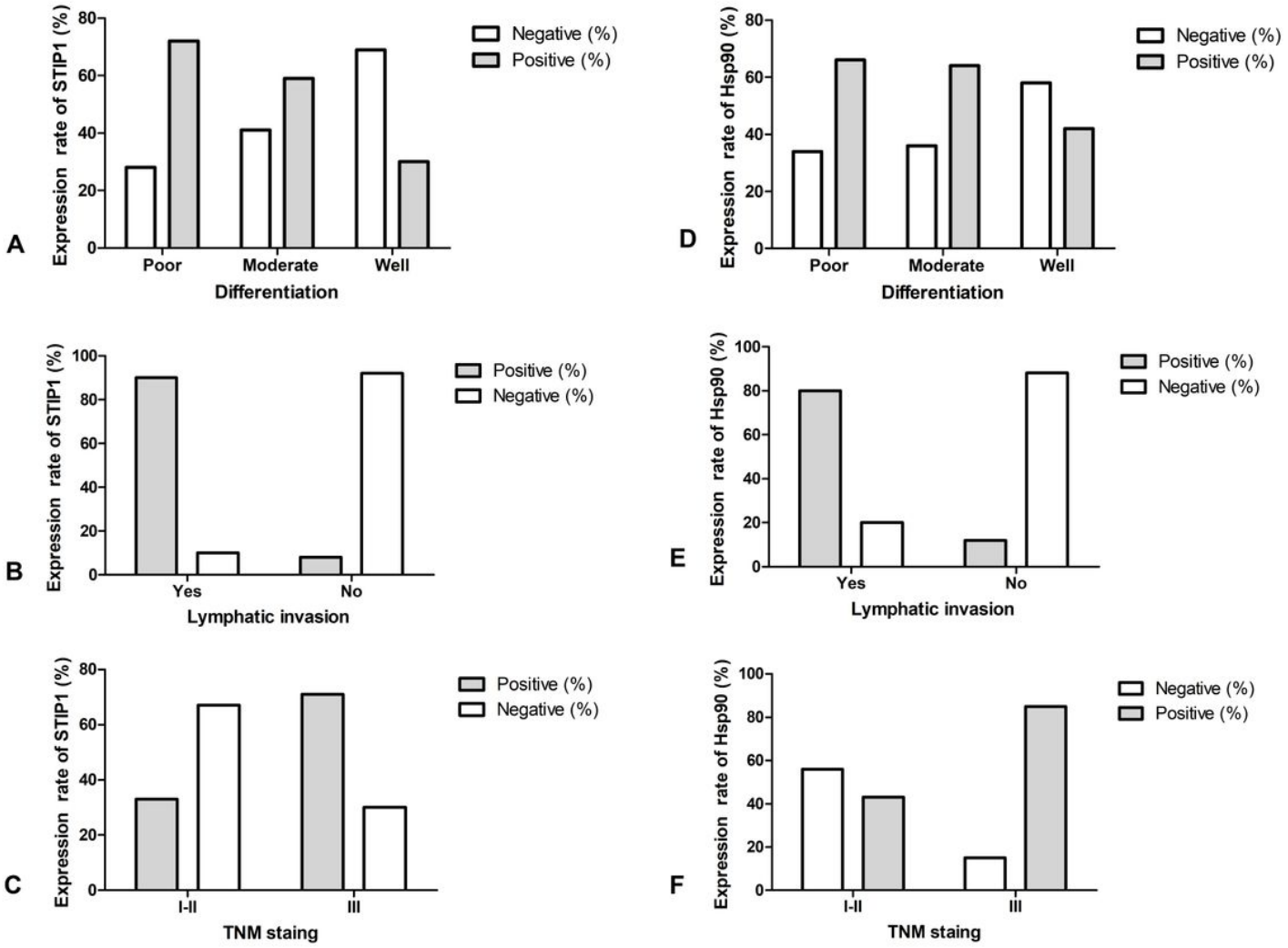

Figure 3

Correlation between expressions of STIP1 and Hsp90 with clinicopathologic factors of LAC patients. (A) Increased STIP1 was observed in poorly differentiated LAC compared with the moderately and well differentiated LAC($p<0.05$). (B) LAC tissues with lymphnode metastasis revealed an increased STIP1 expression compared with those without lymph node metastasis ($p<0.05$). (C) Tissues at stage III had a higher STIP1expression than those at stages I-II ($p<0.05$).(D) Increased Hsp90 was observed in poorly differentiated LAC compared with the moderately and well differentiated LAC($p<0.05$). (E) LAC tissues with lymphnode metastasis had the sameexpression levelcompared with those without lymph node metastasis ($p>0.05$). (F) Tissues at stage III showeda higherHsp90expression than those at stages I-II ($p<0.05$). pTNM (pathologic TNM classification); NSCLC, non-small cell lung cancer; LAC, lung adenocarcinoma; STIP1, stress-inducible phosphoprotein 1; Hsp90, heat shock protein 90; 16HBE, 16 human bronchial epithelial cells.

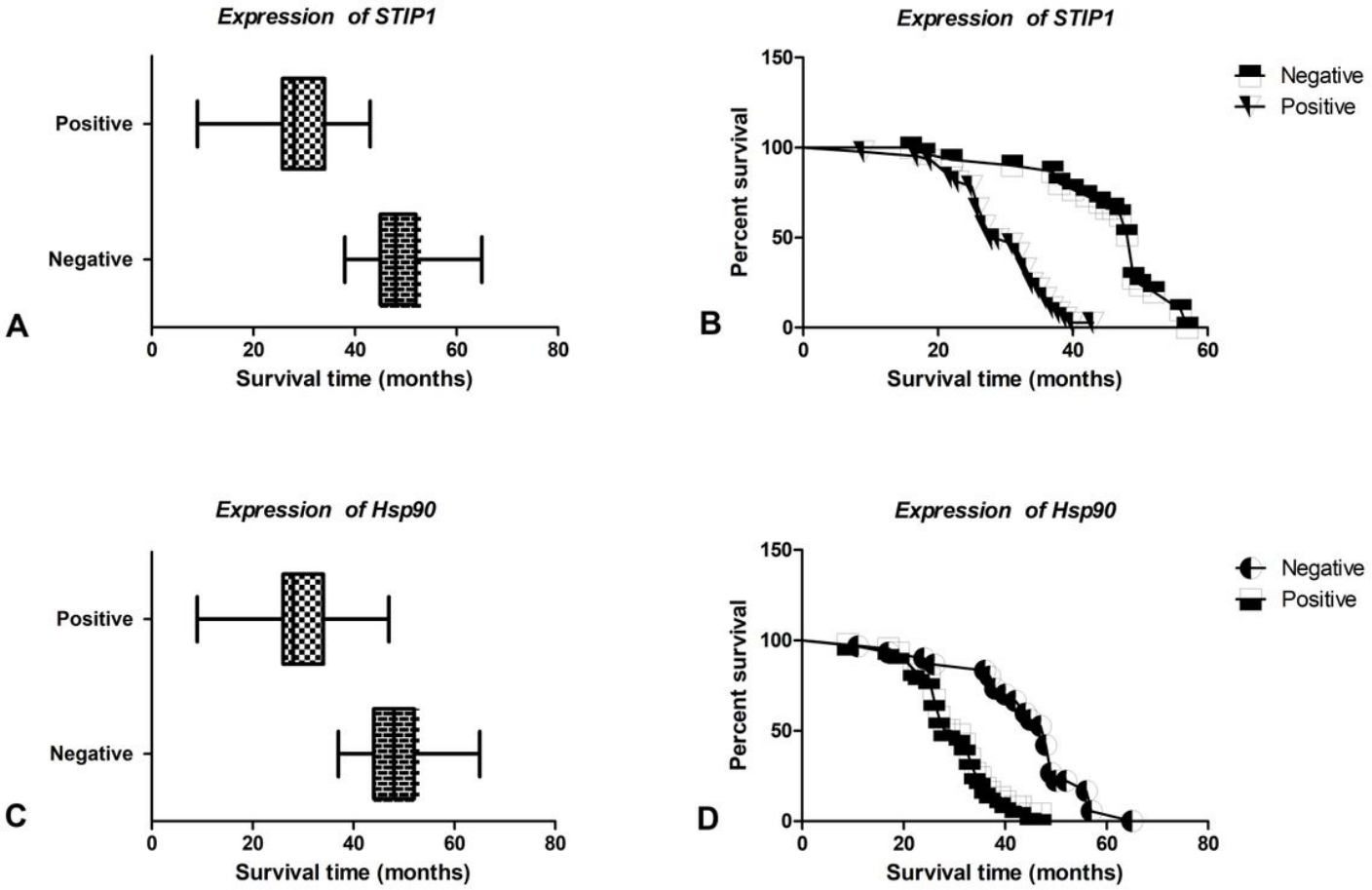

Figure 4

Relationships between the expressions of STIP1 and Hsp90 and prognosis of LAC patients. (A and B)Survival analysis showed that higher expression of STIP1 was associated with shorter survival of LAC patients ($p<0.05$). (C and D) Survival curve showed that higher expression of Hsp90 was associated with shorter survival of LAC patients($p<0.05$). LAC, lung adenocarcinoma; STIP1, stress-inducible phosphoprotein 1; Hsp90, heat shock protein 90; 16HBE, 16 human bronchial epithelial cells.