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ORIGINAL ARTICLE

Molecular Alterations and Expression Dynamics of *LATS1* and *LATS2* Genes in Non-Small-Cell Lung Carcinoma

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Abstract Large tumor suppressor (*LATS*) is an important member of the Hippo pathway which can regulate organ size and cell proliferation. However, very little is known about the expression and clinical significance of *LATS* in lung cancer especially from this part of the world. We elucidated the frequency of *LATS1* &*LATS2* promoter hypermethylation (by methylation-specific PCR) and expression (by real-time PCR) in sixty nine (n = 69) Non-Small Cell Lung Cancer (NSCLC) patients and their corresponding normal lung tissue samples. We found promoter hypermethylation frequencies of *LATS1* & *LATS1*to be 66.66% (46/69) and 71% (49/69) in NSCLC tissues. Decreased *LATS1* & *LATS2* mRNA expression was found in 55% and 66.66% of NSCLC patients. The *LATS1* mRNA expression was significantly higher in normal lung tissues. Also, the mRNA levels of *LATS1* and *LATS2* NSCLC tissues with hypermethylation were significantly lower. Multivariable analysis confirmed that *LATS1* under expression increased the hazard of death after adjusting for other clinicopathological factors. Importantly, the loss of *LATS1* mRNA expression was associated with overall short survival. *LATS1* is an independent prognostic factor and may play an important role in NSCLC progression and may serve as a novel therapeutic target of NSCLC.

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Introduction

Lung cancer is a leading cause of death worldwide and majority of which are NSCLC's [1–3]. Although the targeted molecular therapies on NSCLC have demonstrated great potential, the incidence of NSCLC is growing and the long term survival rate is still not satisfied [4]. In US, 2.2 million new cases of lung cancer emerged in 2015 and in the same year 1.58 million died from the disease, accounting for around 27% of all cancer deaths occurred due to that year. Lung cancer is the most common cancer amongst men in India with approximately 33,000 new cases ever year. Lung cancer constitutes 6.8% deaths in India [5]. Asper the study done by Arshad et al. Lung cancer is the second most common cancer type in men and third among all (14.6%) in Kashmir valley [6]. NSCLC arise by a stepwise acquisition of genetic and epigenetic alterations concomitantly with morphological changes as well that gives rise to the transformation of benign bronchial epithelium into neoplastic tissue. Recently, Hippo signal pathway was discovered in *Drosophila melanogaster* which controls organ size by regulating the expression of genes that promote cell survival, stemness and cell proliferation [7, 8]. The core effector molecules of this pathway are highly conserved in mammals that include *Mst1/2*; *WW45; LATS1/2; Mob1; YAP; TAZ; NF; FRMD6*; and *Fat4*. Two mammalian homologs of fly *LATS* genes; *LATS1* and *LATS2* were shown functionally as tumor suppressors because of their regulation of cellular homeostasis and cellular apoptosis [9]. Loss of function of either *LATS1* or *LATS2* leads to a variety of tumor types including soft tissue sarcomas, leukemia, as well as breast, prostate, lung and esophageal cancers. Mechanistic studies concerning

LATS1 revealed that it is a tumor suppressor gene and might control tumorigenesis by negatively regulating the cell cycle [10, 11]. Reduced expression of *LATS1* is connected with deregulation of SWH, thus activating the *YAP* or *TAZ* oncogene [12, 13].*LATS2* has a role in the maintenance of mitotic fidelity and genomic stability, since *LATS*−/− mutant embryonic cells exhibit an increased frequency of cytokinetic defects, accumulation of micronuclei, supernumerary centrosomes and aneuploidy [14, 15]. *LATS2* also functions as an inducer of apoptosis through down-regulation of anti-apoptotic proteins of the Bcl-family [16]. Both *LATS1* and *LATS2* are hypermethylated in astrocytoma [17] as well as acute lymphoblastic leukemia [18], NSCLC [19], breast cancer [20] and associated with poor prognosis.

A number of studies have shown the diagnostic and prognostic potential of the central kinases *LATS1* and *LATS2* and their role in patient survival in relation to lung cancer patients and all the research findings strongly suggest hypermethylation as most common cause for under expression of *LATS1* and *LATS2* genes [21]. To our knowledge no such study focusing on alteration of *LATS1* and *LATS2* genes has been carried out in Kashmir valley (Northern part of India) on lung cancer. So, we pursued this study for the first time from this part of the world to elucidate the methylation of promoter regions and expression status of the of *LATS1* and *LATS2* genes inNSCLC patientsand to derive a more precise association between the epigenetic alterations/expression of *LATS1* and *LATS2* genes with overall lung cancer development, progression and patient survival.

Material and Methods

Tissue Samples

A total of sixty nine (n = 69) freshly diagnosed NSCLC patients who underwent complete resection between January 2013 to August 2015 at Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar were included in the study. The tumor tissue and their adjacent normal tissues were taken and properly stored for further processing. The study was approved by the Ethics Committee of SKIMS, Soura, India. Informed consent was obtained from all patients for specimen collection.

DNA Extraction

Total genomic (g) DNA was extracted from tissue specimens using quick gDNA Miniprep kit (Zymo Research, USA) according to the manufacturer's protocol. DNA concentrations were calculated using a Nano Drop 2000c Spectrophotometer (Thermo Scientific, Asheville, NC, USA).

Methylation-Specific PCR (MSP)

Sodium bisulphite modification of gDNA was performed using EZ DNA Methylation-Gold Kit (Zymo Research, USA). Methylation status in the CpG islands of *LATS1* and *LATS2* promoter regions was determined by methylation-specific PCR [22]. Bisulfite-modified DNA was amplified with primers specific for methylated or unmethylated sequences. The methylated DNA of *LATS1* was amplified using methylated (M) set of primers, 5′-GGAGTTTCGTTTTGTC-3 (sense), 5-CGACGTAATAACGAACGC CTA-3 (antisense), and the unmethylated DNA of *LATS1* was amplified using unmethylated (U) set of primers, 5-TAGGTTGG AGTGTGGTGGT-3 (sense), 5-CCCAACATAATAAC AAACACCT-3 (anti-sense); the methylated DNA of *LATS2* was amplified using M set primers, 5-ATTTCGGTTTATTG TAATTTTC-3 (sense), 5-AACCAACATAATAAAACCCC G-3 (antisense), and the unmethylated DNA of *LATS2* was amplified using U set primers, 5-TTTGTTTTTTGGGT TTAAGT-3 (sense), 5-CCAACATAATAAAACCCC A-3 (antisense). The Universal Human methylated and non-methylated DNA (Zymo Research, USA) were used as positive and negative control respectively. Two microliters of bisulfite-modified DNA was amplified in a total volume of 25 μL containing 10× PCR buffer 2.5 μL (Fermentas, USA), 10 mmol/L dNTP 0.5 mL, 10 mmol/L of each primer 0.5 mL, and 5 U/mL Taq DNA polymerase0.1 μL (Fermentas, USA). Methylation-specific PCR reaction conditions of *LATS1* and *LATS2* were as follows: initial denaturation at 95 °C for 5 min, 40 cycles of amplification at 95 °C for30 s, 58 °C (methylated) or 50 °C (unmethylated) for 30 s, and 72 °C for 30 s, followed by a final extension of 72 °C for 10 min.

RNA Extraction and Reverse Transcription

Total RNA was extracted from snap-frozen tissues using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. RNA treated with DNase (*NEBS*, USA) was purified by using RNAeasy mini kit (Quiagen, Hilden, Germany). Subsequently, RNA was eluted in 30 μl of the RNA storage solution (Ambion, USA) and stored at −80 °C. The total RNA was quantified by a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Asheville, NC, USA).cDNA synthesis was carried out using the Revert-Aid H minus cDNA synthesis kit (Fermentas, USA).

Real-Time RT-PCR

For real time PCR analysis, the following primer sequences were used: 5′-GACTGACTTTGGCCTCTGCA-3′(sense), 5′-CCACATCGACAGCTTGAGGG-3′ (antisense) for *LATS1*; 5′-TAGAGCAGAGGGCGCGGAAG-3′ (antisense), 5′-

CCAACACTCCACCAGTCACAGA-3′ (antisense) for *LATS2*; 5′-AGAAGGCTGGGGCTCATTTTG-3′ (sense), 5′-AGGGGCCATCCACAGTCTTC-3′ (antisense) for endogenous control, beta actin. Two (02) μL of the dilution of the reverse transcriptase reaction was amplified in the 20 μL reaction including the 2× SYBR Green ROX Mixes 10 μL (Fermentas, USA), 20 μM of each primer 0.25 mL and H_2O 7.5 μL. The reaction was run in the ABI 7500 Real time PCR Systems (Applied Biosystems, USA). Reaction conditions included a hot start (15 min, 95 °C), followed by 40 cycles of 95 °C, 15 s and 60 °C, 60 s). Melt curve analysis was performed to ensure a single product species. Parallel reactions were performed using primers to Beta Actin as an internal control.

Statistical Analysis

Correlation between promoter hypermethylation, expression and clinicopathological parameters was analyzed by χ^2 test. Multivariate survival analysis was performed on all parameters using the Cox regression model. Survival curves were assessed by the Kaplan-Meier method and compared by the log-rank test. Two-sided $P \leq 0.05$ age and sex adjusted were considered statistically significant. All analysis were performed with the SPSS 16.0 software package.

Table 1 Clinicopathological characteristics of NSCLC patients undertaken for study

Characteristic	Subgroup	No. of Cases n (%)
Sex	Female (Reference)	26 (37.68)
	Male	43 (62.38)
Age	≤60 years (Reference)	33 (47.82)
	>60 years	36 (52.18)
Smoking	Non-smoker (Reference)	42 (60.87)
	Smoker	27 (39.13)
Dwelling	Urban (Reference)	30 (43.48)
	Rural	39 (56.52)
LN metastasis	No (Reference)	42 (60.87)
	Yes	27 (39.13)
Clinical stage	I & II (Reference)	53 (76.81)
	III	16 (23.19)
Histological Grade	WD (Reference)	39 (56.52)
	MD & PD	30 (43.48)
Histological type	ADC and others[a] (Reference)	26(37.70)
	SCC	43 (62.30)
Tumor size	≤ 3 cm (Reference)	45 (65.22)
	>3 cm	24 (34.78)

WD Well differentiated tumors, *MD* & *PD* Moderately and Poorly differentiated tumors, *ADC* Adenocarcinoma, *SCC* Squamous cell carcinoma, *LN* Lymph node

[a] ADC & others include adenocarcinoma, large cell carcinoma and bronchogenic carcinoma

Results

Patient Characteristics

The baseline characteristics of the patients with NSCLC are shown in Table 1.The cases included 43(62.32%) males and 26(37.68%) females with age distribution of 47.82% (33 of 69) ≤ 60 years of age and52.18% (36 of 69) > 60 years of age having mean age of 57.80 ± 10.97. Patients were followed up from 13 to 55 months. The study was approved by Ethical Clearance Committee of SKIMS, Soura, Srinagar-190,011. All the patients were informed about the study and gave written consent for participation in the study.

Methylation Status of LATS1 and LATS2 Genes and their Correlation with Clinicopathological Parameters

Methylation status of *LATS1* and *LATS2* genes were analyzed in sixty nine ($n = 69$) NSCLC tissues by methylation-specific PCR. Of all NSCLC samples, 46(66.66%), and 49(71%) of *LATS1* and *LATS2* promoter regions were found hypermethylated as compared to normal tissue where hypermethylation was not found in any tissue sample. Among various Clinicopathological characteristic, *LATS1* promoter hypermethylation was statistically significant only with smoking [Adj. OR = 4.24 (1.35–13.27), $P = 0.013$]. However, *LATS2* promoter hypermethylation was statistically significant with large tumor size [Adj. OR = 4.08 (1.234–16.50), $P = 0.038$], smoking [Adj. OR = 5.88 (1.71–20.23), $P = 0.011$] and positive lymph node metastasis [Adj. OR = 6.73, (1.54–29.35), $P = 0.011$] (Table 2).

mRNA Expression Status of LATS1 and LATS2 Genes and their Correlation with Clinicopathological Parameters

Furthermore, real time expression analysis revealed decreased expression of *LATS1* in 55% of the cases and the *LATS1* mRNA expression was significantly lower in cases with lymph node metastasis [Adj. OR = 10.20 (2.53–41.11), $P = 0.001$], smoking history [Adj. OR = 4.09 (1.33–12.54), $P = 0.014$] and large tumor size [Adj. OR = 4.02, (1.31–12.38), $P = 0.015$]. Also, *LATS2* mRNA expression was decreased in 66.66% of the lung cancer cases. A statistical significance was observed in cases with lymph node metastasis [Adj. OR = 8.34 (2.00–34.64), $P = 0.003$] and tumor size [Adj. OR = 3.89 (1.12–13.52), $P = 0.032$] for the decreased expression of *LATS2* mRNA in tumor tissues as compared to the normal (Table 3).

Table 2 Association of LATS1 and LATS2 promoter hypermethylation with various Clinicopathological characteristics of NSCLC patients

Variable	Subgroup	LATS1 M+	LATS1 M-	P* Value	OR* (95%CI)	LATS2 M+	LATS2 M-	P* Value	OR* (95%CI)
Age	≤60 years	20	13	–	–	22	11	–	–
	>60 years	26	10			27	09		
Sex	Male	29	14	–	–	30	13	–	–
	Female	17	9			19	07		
Dwelling	Rural	28	11	0.162	2.22 (0.72–6.84)	31	08	0.051	3.30 (1.00–10.87)
	Urban	18	12			18	12		
Smoking	Non-smoker	13	14	**0.013**	4.24 (1.35–13.27)	14	13	**0.005**	5.88 (1.71–20.23)
	Smoker	33	9			35	07		
LN metastasis	No	24	18	0.062	3.27(0.94–11.38)	25	17	**0.011**	6.73(1.54–29.35)
	Yes	22	5			24	03		
Clinical stage	I & II	34	19	0.562	1.49 (0.38–5.76)	37	16	0.740	1.26 (0.32–4.94)
	III	12	4			12	04		
Tumor grade	WD	23	16	0.204	2.10 (0.66–6.63)	26	13	0.448	1.57 (0.48–5.09)
	MD & PD	23	7			23	07		
Tumor histology	SCC	30	13	0.177	2.37 (0.67–8.33)	32	11	0.219	2.23 (0.62–8.04)
	ADC /others	16	10			17	09		
Tumor size	≤ 3 cm	27	18	0.130	2.44 (0.76–7.81)	28	17	**0.038**	4.23 (1.08–16.50)
	>3 cm	19	5			21	03		

LATS1M+ LATS1 Methylation positive, *LATS1M-* LATS1 Methylation negative, *LATS2M+* LATS2 Methylation positive, *LATS2M-* LATS2 Methylation negative, *P* Value P* Value adjusted for age and sex, *OR** Adjusted odds ratio for age and sex, *CI* Confidence interval, *LN* Lymph node, *WD* Well differentiated tumors, *MD & PD* Moderately and Poorly differentiated tumors, *SCC* Squamous cell carcinoma, *ADC* Adenocarcinoma

Bold represents the parameters with statistical significance

Relationship of Methylation of LATS1 and LATS2 with their mRNA Expression

LATS1 and *LATS2* mRNA expression was compared between tumors with and without hypermethylation. Both *LATS1* and *LATS2* mRNA expression was significantly lower in NSCLC tissues with hypermethylation than those without hypermethylation ($P = 0.005$ for *LATS1* and $P = 0.001$ for *LATS2*) (Table 4).

LATS1 and LATS2 mRNA Expression and Survival

To find clinically useful prognostic factors, we performed univariate analysis for the effect of *LATS1* and *LATS2*

Table 3 Association of LATS1 and LATS2 mRNA expression with various clinico-pathological characteristics of NSCLC patients

Variable	Subgroup	LATS1LE	LATS1NE	P* Value	OR* (95%CI)	LATS2LE	LATS2NE	P* Value	OR* (95%CI)
Age	≤60 years	20	13	–	–	22	11	–	–
	>60 years	18	18			24	12		
Sex	Male	25	18	–	–	27	16	–	–
	Female	13	13			19	07		
Dwelling	Rural	23	16	0.455	1.48 (0.52–4.18)	26	13	0.823	0.88 (0.30–2.60)
	Urban	15	15			20	10		
Smoking	Non-smoker	10	17	**0.014**	4.09 (1.33–12.54)	15	12	0.056	3.03 (0.97–9.51)
	Smoker	28	14			31	11		
LN metastasis	No	17	25	**0.001**	10.20 (2.53–41.11)	23	19	**0.003**	8.34 (2.00–34.64)
	Yes	21	06			23	04		
Clinical stage	I & II	28	25	0.445	1.62 (0.46–5.62)	35	18	0.646	1.35 (0.37–4.88)
	III	10	06			11	05		
Tumor grade	WD	21	18	0.626	1.30 (0.44–3.78)	25	14	0.471	1.50 (0.49–4.60)
	MD & PD	17	13			21	09		
Tumor histology	SCC	25	18	0.637	1.31 (0.42–4.05)	31	12	0.229	2.08 (0.62–6.93)
	ADC/others	13	13			15	11		
Tumor size	≤ 3 cm	20	25	**0.015**	4.02 (1.31–12.38)	26	19	**0.032**	3.89 (1.12–13.52)
	>3 cm	18	06			20	04		

LATS1 LE LATS1 Low mRNA expression, *LATS1 NE* LATS1 Normal mRNA expression, *LATS2 LE* LATS2 Low mRNA expression, *LATS2 NE* LATS2 Normal mRNA expression

Bold represents the parameters with statistical significance

Table 4 Association of LATS1 promoter hypermethylation with LATS1 mRNA expression in NSCLC patients

	LATS1LE	LATS1NE	*P* Value	OR (95% CI)
LATS1M+	32	14	**0.001**	6.46 (2.10–19.90)
LATS1M-	6	17		
	LATS2LE	LATS2NE		
LATS2M+	42	7	**0.001**	24.00 (6.18–93.20)
LATS2M-	4	16		

LATS1M+ = LATS1 methylation positive; *LATS1M-* = LATS1 methylation negative; *LATS2M+* = LATS2 methylation positive; *LATS2M-* = LATS2 methylation negative; *LATS1LE* = LATS1 low expression; *LATS1NE* = LATS1 normal expression; *LATS2LE* = LATS2 low expression; *LATS2NE* = LATS2 normal expression

Bold *P* values predict the statistical significance between the two parameters

hypermethylation and their mRNA expression and various clinicopathological characteristics on survival. Decreased *LATS1* mRNA, clinical stage, lymph node metastasis, age, sex and smoking were the prognostic factors affecting the survival significantly (Table 5). The crude hazard ratio (HR) of low *LATS1* mRNA expression compared with high mRNA expression was 2.82 (95% CI = 1.57–5.07), which indicates that the low mRNA expression may increase the hazard of lung cancer-related deaths by almost three times that of high *LATS1* expression. In case of *LATS2* mRNA expression, there was a direction effect towards poor overall survival with low *LATS2* mRNA but it was not statistically significant. We then performed the multivariate analysis for those factors whose presence significantly affected prognosis and for demographic factors like age and sex (Table 5). The results showed that low *LATS1* mRNA expression were significantly related to

Table 5 Univariate and multivariate analysis for the effect of LATS1 & LATS2 hypermethylation and their mRNA expression on the overall survival

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* Value	*Adj. HR	*(95% CI)	P* Value
Age						
> 60 years vs ≤60 years	1.77	1.00–3.13	**0.048**	2.08	1.02–4.24	**0.042**
Sex						
Male vs Female	1.79	1.00–3.20	**0.048**	2.01	0.99–4.06	0.052
Dwelling						
Rural vs Urban	0.87	0.49–1.52	0.628	–	–	–
Smoking						
Smoker vs Non-smoker	2.05	1.15–3.67	**0.014**	1.11	0.57–2.14	0.751
Lymph node metastasis						
Yes (N+) vs No (N_o)	9.50	4.25–21.21	**0.001**	4.30	1.57–11.77	**0.005**
Clinical stage						
III vs I &II	1.39	1.79–11.19	**0.002**	1.40	0.50–3.94	0.519
Tumor grade						
MD/PD vs WD	1.37	0.77–2.45	0.275	–	–	–
Tumor histology						
SCC vs ADC & Others	0.67	0.37–1.19	0.174	–	–	–
Tumor size						
≤ 3 cm vs >3 cm	1.68	0.95–2.980	0.073	–	–	–
LATS1 hypermethylation						
Methylated vs Unmethylated	1.42	0.80–2.55	0.225	–	–	–
LATS2 hypermethylation						
Methylated vs Unmethylated	1.69	0.92–3.11	0.089	–	–	–
LATS1 mRNA expression						
Low vs Normal	2.82	1.57–5.06	**0.001**	4.29	1.96–9.35	**0.001**
LATS2 mRNA expression						
Low vs Normal	1.59	0.88–2.88	0.126	–	–	–

WD Well differentiated tumors, *MD & PD* Moderately and Poorly differentiated tumors, *SCC* Squamous cell carcinoma, *ADC* Adenocarcinoma, *HR* Hazard ratio, *CI* Confidence interval, *Adj HR* Adjusted hazard ratio for age and sex (multivariate analysis), **(95% CI)* Adjusted CI (multivariate analysis), *P* Value P* value on multivariate analysis

Bold represents the parameters with statistical significance

prognosis and were independent of age, sex and lymph node metastasis [Adj. HR = 4.29 (1.96–9.35), $P = 0.001$].

Kaplan-Meier Estimate of Survival for Surgically Resected NSCLC Patients

We performed the Kaplan-Meier estimate of survival of resected NSCLC patients with or without *LATS1/LATS2* hypermethylation and normal or low *LATS1/LATS2* mRNA expression. Only *LATS1* under-expression had a profound effect of decreasing the patient survival significantly ($P < 0.001$). The mean overall survival was 50 months for patients with normal *LATS1* mRNA expression and for patients with low mRNA *LATS1* it was 39 months, respectively (Fig. 1).

Discussion

In this study, the promoter region of *LATS1* was hypermethylated as high as 66.66% (46/69). This was quite comparable to the frequencies reported in astrocytoma (63.66%) and NSCLC (79.8%) [17, 19]. However, in colorectal cancer, breast cancers [20] and in head and neck cancers the methylation frequencies were reported to be 57%, 56.70% and 24% respectively [23] which is less than the frequency found in NSCLC. In addition, among the various Clinicopathological characteristics only the patients with smoking history showed statistical significance with *LATS1* promoter hypermethylation in NSCLC's and results are in concordance with the study carried out by Sasakiet al. who reported the promoter hypermethylation to be statistically significant with SCC [19]. The *LATS2* promoter was hyper-methylated in 71% of the cases and the promoter hypermethylation frequencies were quite comparable to those reported in astrocytoma (71.50%) [17] and NSCLC (78.80%) [19]. Also, in breast cancers [20], head and neck cancers [23] and ALL [18] the *LATS2* methylation frequencies were reported to be 50%, 24% and 8%, respectively which is less than the frequency reported in our study. *LATS2* promoter hypermethylation showed a strong significant association with smoking, lymph node metastasis and large tumor size ($P < 0.05$) which is not in agreement with those reported earlier [19] and the reason for this discrepancy might be a different ethnic background of our population. However, we observed that *LATS2* hypermethylation was independent of age and sex and the results are in line with the earlier studies [17–20, 23]. The reason for higher frequency of promoter hypermethylation of *LATS1* and *LATS2* might be the different kind of tissue, nature of disease and the different level of assault by smoking and various air pollutants as the lungs are the only organs which have a direct contact with the environmental air pollutants. Further, the hypermethylation of *LATS1* and *LATS2* was not observed in any of the normal lung tissues, showing that *LATS1* and *LATS2* hyper-methylation might be involvedin the pathogenesis of NSCLC and hence, may be used as a tool in diagnosis of NSCLC. As we had a proper follow-up of the patients, we tried to find some association of *LATS1* and *LATS2* hypermethylation with the survival. Despite the fact that the *LATS1* and *LATS2* hypermethylation increased the adjusted hazard ratio by a factor of 1.4 and 1.69 but they were not statistically significant, this is in agreement with the earlier reports [19].

Since promoter hypermethylation has direct effect on mRNA expression, we compared the mRNA expression in tumour and their adjacent normal tissues. We observed a strong decrease of 55.06% (38 of 69) and 66.66% (46 of 69) in *LATS1*and *LATS2 mRNA* expression respectively in NSCLC tissues as compared to normal. These results are quite comparable to those observed in NSCLC [24], Soft tissue sarcomas [12], breast cancers [20], astrocytomas [16], gliomas [25] colorectal cancers [26], gastric cancers [27] and ALL [18]. Since, *LATS1* is reportedly involved in the control of proliferation, invasion, apoptosis and/or differentiation of various types of cells [28, 29] we also compared the *LATS1* mRNA expression with various clinicopathological characteristics. Our study showed that *LATS1* mRNA expression significantly correlated with positive lymph node status ($P = 0.001$) which is in agreement with the earlier reported studies [20, 24, 27]. In addition, tumor size was significantly associated with *LATS1* expression ($P = 0.015$) and results are in concordance with those reported in breast cancer [20] but in contradiction with those reported in NSCLC [24]. However, this discrepancy might be due to low sample size. Furthermore, *LATS1* mRNAexpression showed a significant four times decrease in smokers [Adj. OR 4.09(1.33–12.54), $P = 0.014$] but has not been reported elsewhere. We also compared the *LATS2* mRNA expression with

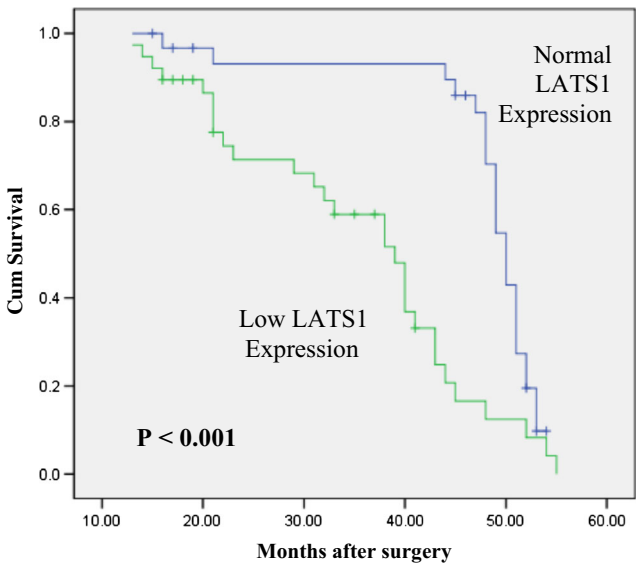

Fig. 1 Overall survival curve with LATS1 Expression

various clinicopathological characteristics and found a significant correlation with positive lymph node status ($P = 0.003$). Our results are in line with the previously reported studies for breast cancer and colon cancer [20, 30] but in contradiction with a single study pertaining to lung cancer [31]. This discrepancy might be due to the inclusion of only one subtype of NSCLC patients (ADC) by Luo et al. in his study, whereas our study included all type of NSCLC subtypes. In addition, tumor size significantly correlated with *LATS2*mRNA expression ($P = 0.032$) and results are in concordance with those reported for breast cancer [20] but in contradiction with reported for lung cancer [31]. However, no correlation was found for low *LATS2* mRNA expression with smoking, pathological grade, clinical stage, dwelling, histological type, age and sex. The results are in line with those observed by Luo et al. [31].

Some studies showed that the decreased mRNA expression of *LATS1* & *LATS2* might be related to *LATS1* gene mutation [32], *LATS2* gene polymorphic change [32] or hypermethylation of gene promoters [17, 18, 20] which could induce down-regulated mRNA expression. However, in our study, a significant decrease in *LATS1* and *LATS2* mRNA expression was observed in NSCLC tissues with *LATS1* and *LATS2* hypermethylation respectively, than those without hypermethylation ($P < 0.001$) suggesting that hypermethylation of the promoter region down-regulates its expressionand is in consistence with the reports on ALL [18], breast cancers [20], astrocytomas [7], soft tissue sarcomas [12], colorectal cancers [26], and gastric cancers [27], which showed that hypermethylation of *LATS1* and *LATS2* genes was associated with a decreased mRNA expression.

Our study also shows that the *LATS1* under expression increase the hazard ratio by a factor of 2.82 which was statistically significant ($P = 0.001$). Also, the *LATS2* underexpression did not correlate with patient survival irrespective of the fact that its under-expression increased the adjusted hazard ratio by a factor of 1.59 ($P = 0.1265$). Multivariate analysis further confirmed the role of decreased *LATS1* mRNA expression as an independent prognostic factor for survival in resected NSCLC tissues and the results are in agreement with those reported earlier [20, 24, 25]. It is important to mention that the loss of *LATS1* expression was associated with overall shorter survival in NSCLC patients (log rank test: $P < 0.001$). These results are in agreement with the previous reports that patients with lower *LATS1* mRNA expression had a significantly shorter overall survival time than patients with higher *LATS1* mRNA expression [24, 25, 33]. Since the role of *LATS1* in lung cancer has been implicated in cell migration and invasion, loss of *LATS1* expression may reveal its significance with lymph node metastasis and poor prognosis [24]. These results indicate that loss of *LATS1* expression clubbed with the implication of *LATS1* as a tumor suppressor could be a useful tool for predicting the poor prognosis of NSCLC patients.

Since, promoter hypermethylation plays an important role in the down regulation of *LATS1* & *LATS2* expression, the reversal of epigenetic silencing that could reactivate the normal transcriptional mechanism and allow production of normal proteins that might induce the regression of the malignant phenotype offers novel targets for therapy. The availability of de-methylating agents gives our results additional importance because decreased *LATS1* expression might represent a direct therapeutic target in patients with NSCLC as its underexpresssion decreases the patient survival significantly. Further studies investigating *LATS1* activation in patients with NSCLC are waned to be carried out to gain more insight into the potential clinical usefulness of this therapeutic approach.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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