

# Tyrosylprotein sulfotransferase 1 expression is negatively correlated with c-Met and lymph node metastasis in human lung cancer

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Received October 6, 2014; Accepted June 23, 2015

DOI: 10.3892/mmr.2015.4096

**Abstract.** The present study aimed to test the expression of tyrosylprotein sulfotransferase 1 (TPST-1) in human lung cancer and to analyze the correlation with clinicopathologic features and c-Met expression levels. Expression levels of TPST-1 and c-Met were analyzed by immunohistochemistry in 50 lung cancer tissues. Non-neoplastic tissues 5 cm from the cancer tissues were collected as controls. The association between TPST-1 and c-Met expression and TPST-1 and clinicopathologic parameters was then analyzed. TPST-1 was expressed in all normal tissue samples, but only in 60% of lung cancer tissues. In tumor tissues, they appeared to be significantly lower than those in matched control lung tissues. The expression of TPST-1 was significantly correlated with the tumor-node-metastasis (TNM) stage and lymph node metastasis and was significantly inversely associated with c-Met expression. In conclusion, the present study demonstrated that TPST-1 expression was associated with the TNM stage and lymph node metastasis in patients with lung cancer. TPST-1 was significantly negatively correlated with the expression of c-Met in lung cancer and may be a negative prognostic biomarker of lung cancer.

## Introduction

Lung cancer has been and remains the most common malignancy in the world, with an estimated 1.6 million novel cases per year (1). Despite the great progress made in several areas of oncology, the treatment and outcome of lung cancer have not significantly improved (2,3). Its high mortality rate is attributed to a high incidence of metastases, thereby making systemic

therapies the mainstay for treatment. As chemotherapy against metastatic lung cancer has yet to be shown effective (4,5), molecular targets are required to be established to design appropriate pharmacologic agents to provide novel treatment modalities. In recent years, targeted therapies, including those directed towards epidermal growth factor receptor, anaplastic lymphoma kinase, mesenchymal-epithelial transition factor and angiogenesis, have been increasingly used (6-9). However, other pathways or molecular biomarkers may be identified in lung cancer.

Tyrosylprotein sulfotransferase (TPST) is a 54-to 50-kDa integral membrane glycoprotein of the trans-Golgi network found in essentially all tissues investigated, catalyzing the tyrosine O-sulfation of soluble and membrane proteins passing through this compartment (10). Two different TPSTs (TPST-1 and TPST-2) have been identified (11,12) and are broadly co-expressed in human tissues (13,14). The levels of TPST-1 and TPST-2 expression vary among different tissues, which may imply distinct physiological functions of TPST-1 and TPST-2 (14). Several studies have found that TPST-1 is highly expressed in breast carcinoma (15), oral squamous cell carcinoma (16) and soft-tissue sarcoma (17) compared with expression levels in normal tissues. In addition, a recent study of human nasopharyngeal carcinoma (NPC) found that the expression of TPST-1 was directly and clinically correlated with NPC and was associated with metastasis (18).

To the best of our knowledge, little has been uncovered regarding the involvement of TPST genes in lung cancer. TPST expression may be associated with treatment efficacy or prognosis in patients with lung cancer; however, the knowledge concerning TPST expression in lung cancer is currently insufficient. Overexpression of c-Met has been described in lung cancer and a multitude of other malignant human neoplasms (19-21). The present study was designed to clarify the TPST-1 expression in lung cancer by using a number of consecutive cases of primary tumors with complete histopathologic and clinical data.

## Materials and methods

**Patients and tumors.** The present study was approved by The Third Xiangya Hospital Institutional Review Board of

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*Abbreviations:* TPST, tyrosylprotein sulfotransferase

*Key words:* tyrosylprotein sulfotransferase 1, lung cancer, c-Met

Central South University (Changsha, China). All patients with stage I-IV lung cancer who were undergoing a tumor resection or biopsy procedure at the Third Xiangya Hospital between March 2010 and October 2012 were included. None of the patients received pre-operative chemotherapy, and all were treated with routine chemotherapy after the operation. Fixed in formaldehyde and embedded in paraffin, the specimens from 50 patients (16 women, 34 men) who were pathologically diagnosed with lung cancer were available for the present study. Clinical data of patients were obtained through a retrospective analysis of the reports. Clinical staging was based on the 7th edition of the tumor-node-metastasis (TNM) classification for lung cancer (22). In addition, surgically removed non-neoplastic tissues 5 cm from the cancer tissues were used as controls. Written informed consent was obtained from all the participants involved in the present study.

**Antibodies.** A rabbit polyclonal antibody against human TPST-1 (cat no. SAB1300286) and a mouse monoclonal antibody against human c-Met (cat. no. SAB4501869) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

**Immunohistochemistry.** Immunohistochemistry staining was performed using the two-step EnVision™ method (Dako, Glostrup, Denmark). Briefly, 5-μm tissue sections were cut from each of the selected 50 paraffin-embedded tumor and control specimens, and they were dried at 65°C for 30 min. The sections were de-paraffinized with xylene and re-hydrated with a graded ethanol series. Endogenous peroxide blocking was performed with 3% hydrogen peroxide (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 10 min, and antigen retrieval was performed at 100°C for 30 min in a citrate buffer (10 mmol/l; pH 6.0; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China). After the sections were washed three times with phosphate-buffered saline (PBS; Sigma-Aldrich) for 3 min each, they were incubated with 10% normal goat serum (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) to block the non-specific binding. Then, the sections were incubated with the anti-human TPST-1 (1:150 dilution), and c-Met (1:500 dilution) monoclonal antibodies at 4°C overnight. After the sections were washed with PBS, the secondary antibody, peroxidase-conjugated goat anti-rabbit/mouse immunoglobulin G (no. K5007; Bottle A; Dako REAL™ EnVision™; Dako, Glostrup, Denmark) was applied for 15 min. The peroxidase reaction was developed for 3 min at room temperature with 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) with 0.03% hydrogen peroxide. Counterstaining was performed with Mayer's hematoxylin. The negative control was prepared with omission of the primary antibody and the use of normal serum instead of the primary antibody.

**Evaluation of immunostaining.** Two independent pathologists who were blinded to the clinical data evaluated the staining results. Any cases where inter-observer discrepancy occurred were reviewed at the double-head microscope (Olympus, Tokyo, Japan), and an agreement was reached. Immunohistochemical scoring was evaluated in a semi-quantitative fashion according to the similar method described by Jiang *et al.* (23). It was based on the percentage of positive cells and the intensity of

Table I. Demographic characteristics of the lung cancer patients.

Parameter	Patients (n)	%
Mean age (years)	50	59.84±9.59
Gender		
Female	16	32
Male	34	68
Histological type		
Squamous	20	40
Adeno	25	50
Other	5	10
Differentiation		
Well	10	20
Moderate	27	54
Poor or undifferentiated	13	26
TNM stage		
I	9	18
II	11	22
III	23	46
IV	7	14
Lymph node metastasis		
Yes	27	54
No	23	46
Surgery		
Lobectomy/pneumonectomy	43	86
Biopsy	7	14
TNM, tumor-node-metastasis.		

Table II. Expression of tyrosylprotein sulfotransferase in the tumor group and in the control group.

Group	Positive, n (%)	Negative, n (%)
Tumor	30 (60.0)	20 (40.0)
Control	50 (100.0)	0 (0.0)
P=0.001, tumor vs. control group.		

staining in their cytoplasm in five randomly visual fields under the optical microscope. The intensity of staining was scored as follows: 0, without stain; 1, straw yellow; 2, brown; and 3, dark brown. According to the percentage of tumor cells stained positive, the extent of staining was scored as follows: 0, ≤5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; and 4, >75%. The product of the intensity and extent of staining yielded final scores: ≤1, negative; 2-3, weakly positive (1+); 4-5, moderately positive (2+); and ≥6, strongly positive (3+).

**Statistical analyses.** The data were processed and statistically analyzed using SPSS for Windows XP (Version 13.0; SPSS, Inc., Chicago, IL, USA). The significance of the association

Table III. Association between TPST-1 expression and clinicopathologic variables.

Variable	Patients, n (%)	TPST-1 expression		P-value
		Positive, n (%)	Negative, n (%)	
Histological type				
Squamous	20 (40)	13 (65.0)	7 (35.0)	0.913
Adeno	25 (50)	14 (56.0)	11 (44.0)	
Other	5 (10)	3 (60.0)	2 (40.0)	
Differentiation				
Well	10 (20)	6 (60.0)	4 (40.0)	0.925
Moderate	27 (54)	17 (63.0)	10 (37.0)	
Poor or undifferentiated	13 (26)	7 (53.8)	6 (46.2)	
TNM stage				
I	9 (18)	7 (77.8)	2 (22.2)	0.002
II	11 (22)	9 (81.8)	2 (18.2)	
III	23 (46)	14 (60.9)	9 (39.1)	
IV	7 (14)	0 (0)	7 (100)	
Lymph node metastasis				
Yes	27 (54)	10 (37.0)	17 (63.0)	<0.001
No	23 (46)	20 (87.0)	3 (13.0)	

TPST, tyrosylprotein sulfotransferase; TNM, tumor-node-metastasis.

Table IV. Association of TPST-1 expression with c-Met expression in patients with lung cancer [n (%)].

c-Met expression	TPST-1 expression				r	P-value
	Negative	Positive (1+)	Positive (2+)	Positive (3+)		
Negative	2 (16.7)	1 (8.3)	2 (16.7)	7 (58.3)	-0.470	0.001
Positive (1+)	5 (41.7)	1 (8.3)	2 (16.7)	4 (33.3)		
Positive (2+)	5 (45.5)	3 (27.3)	2 (18.2)	1 (9.1)		
Positive (3+)	8 (53.3)	6 (40.0)	1 (6.7)	0 (0.0)		

TPST, tyrosylprotein sulfotransferase.

between immunohistochemical expression and clinical variables was evaluated by using the  $\chi^2$  test or Fisher's exact test, as appropriate. Spearman's rank correlation analysis was used to analyze the association between TPST-1, and c-Met expression levels.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Patients' demographic data.** A total of 50 patients (16 women, 34 men) with stage I-IV lung cancer were enrolled in the present study. The mean age of the patients was  $59.84 \pm 9.59$  years (mean  $\pm$  standard deviation; range, 18-77 years). Among the 50 patients with lung cancer, 9 (18%) were stage I, 11 (22%) were stage II, 23 (46%) were stage III and 7 (14%) were stage IV. All patient characteristics are summarized in Table I.

*Expression of TPST-1 is decreased in lung cancer tissues.* Positive expression of TPST-1 was identified as a brownish yellow stain in the cytoplasm of lung cancer cells (Fig. 1). Immunohistochemical analysis demonstrated that TPST-1 was expressed in all matched control lung tissues (100%) and in 30 out of 50 (60%) tumor tissues. The expression of TPST-1 in tumor tissues appeared to be significantly lower than that in matched control lung tissues ( $P = 0.001$ ). Table II shows the results of the expression of TPST-1 in the tumor group and the control group.

*TPST-1 expression is associated with clinical tumor stage, TNM stage and lymph node metastasis.* The association of clinicopathologic characteristics with TPST-1 expression in lung cancer tissues is summarized in Table III. The expression of TPST-1 in clinical stage IV cases was lower than that in clinical stage I-II cases (Fig. 2). In addition, TPST-1 expression

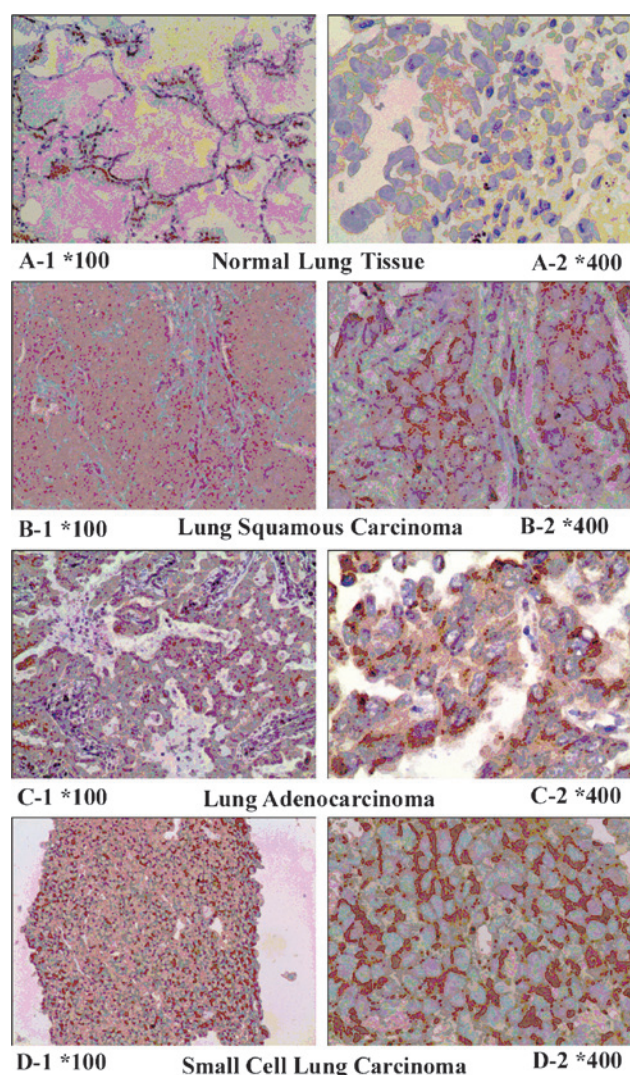


Figure 1. Immunohistochemical analysis of tyrosylprotein sulfotransferase in (A) normal lung tissue, (B) lung squamous carcinoma, (C) lung adenocarcinoma and (D) small cell lung carcinoma (magnification, x100 and x400 in the left- and right-hand images, respectively). Positive expression is indicated by a brown stain.

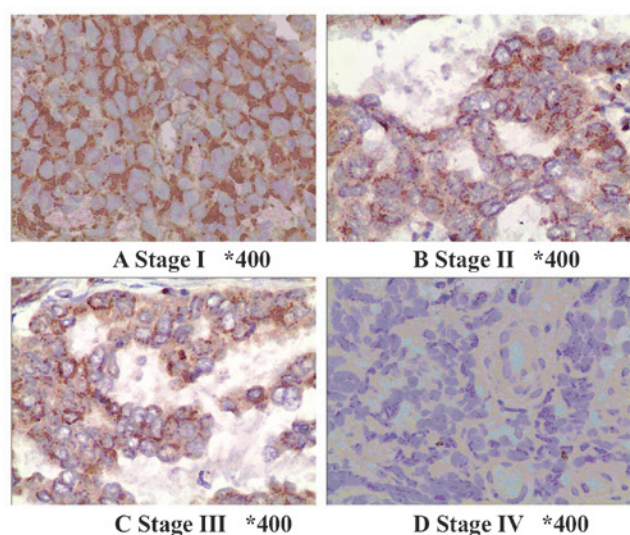


Figure 2. Expression of tyrosylprotein sulfotransferase in different clinical stages: (A) Stage I (B) Stage II (C) Stage III and (D) Stage IV (magnification, x400). Expression in stage IV cases was lower than in clinical stage I-II cases.

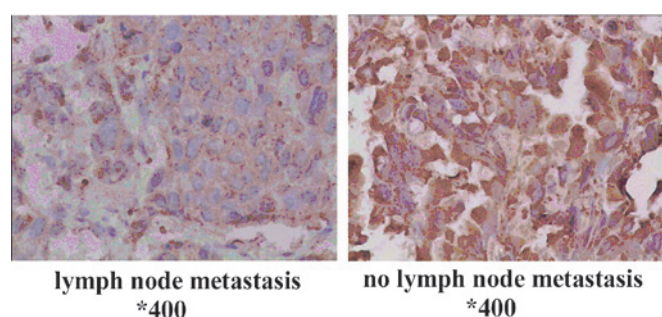


Figure 3. The expression of TPST-1 in tumor samples from lung cancer patients without lymph node metastasis (right) was higher than that in patients with lymph node metastasis (left). The expression of TPST-1 was significantly associated with lymph node metastasis in lung cancer tissue. TPST-1, tyrosylprotein sulfotransferase.

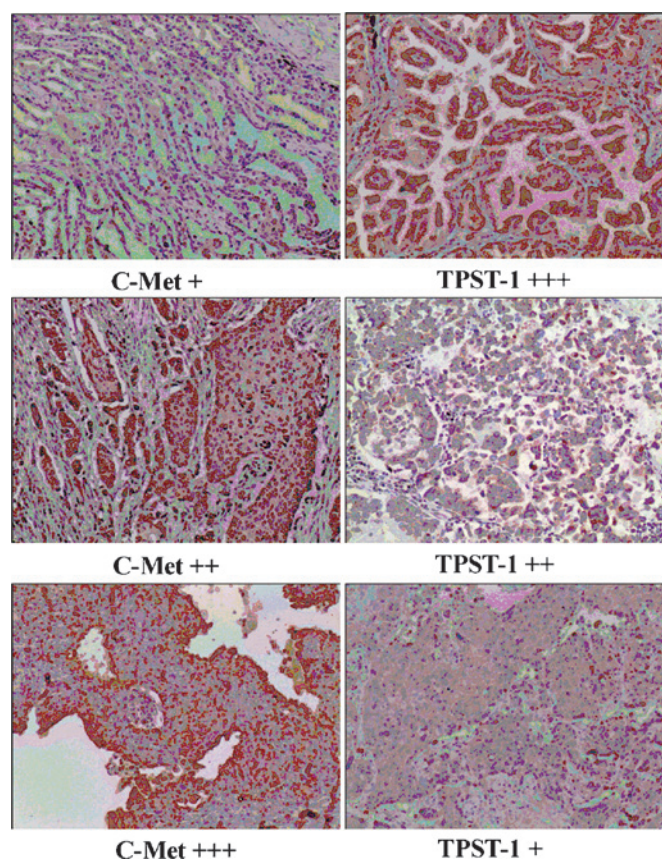


Figure 4. Immunohistochemical detection of the expression of TPST-1 and c-Met in tumor tissues. A negative association was observed between TPST-1 expression and c-Met expression in tumor tissues. TPST-1, tyrosylprotein sulfotransferase.

was highly associated with the TNM stage ( $P=0.002$ ) and lymph node metastasis ( $P<0.001$ ) (Fig. 3). However, statistical analysis revealed no significant correlations between the expression of TPST-1 and the histological type or tumor differentiation.

*TPST-1 expression is inversely correlated with c-Met expression in lung cancer tissues.* The present study further investigated the association between TPST-1 and c-Met expression in tumor tissues by immunohistochemical scoring. A significant association between TPST-1 and c-Met expression

levels was identified ( $r=-0.470$ ,  $P=0.001$ ) (Fig. 4). Table IV shows the negative association between TPST-1 expression and c-Met expression in tumor tissues.

## Discussion

Individual patients with lung cancer respond differently to chemotherapy and have different survival rates. This variability is associated with the histological lung cancer sub-type and its individual biological characteristics (24). Therefore, it is important to enhance the current knowledge of the pathophysiology and molecular profiles of the different histological types of lung cancer, thus allowing for personalization of the available therapies.

TPST is an enzyme responsible for protein tyrosine sulfation (25), which enhances protein-protein interactions, thereby having an important functional role. The present study hypothesized that a change in tyrosine sulfation of human trypsinogens may alter the risk for numerous types of disease. This notion was based on the observation that human trypsinogens undergo post-translational sulfation modification in peptides and proteins synthesized through the secretory pathway of most eukaryotes (13). In fact, several studies have proved that TPST-1 deficiency results in a series of dysfunction. Among these studies, Westmuckett *et al* (26) found that TPST deficiency results in early post-natal pulmonary failure in mice. Another study found that a loss-of-function mutant of the Arabidopsis TPST displayed a markedly abnormal phenotype including severely stunted growth and early senescence (27). In addition, double knockout of TPST-1 and TPST-2 was found to severely disrupt the integrity of the retina, resulting in abnormal disc morphology (28). In summary, the previous studies demonstrated that protein-tyrosine sulfation is a key determinant in the development and maintenance of tissue function.

To the best of our knowledge, there have been no previous studies regarding TPST-1 expression in lung cancer tissue and their possible roles in conjunction with the clinical outcome of lung cancer patients by means of any modalities. The present study identified TPST-1 expression in all normal control lung tissues. These data confirmed once again that TPST-1 is expressed in normal tissues, in line with a study by Mishiro *et al* (14). However, the present study also observed that TPST-1 was expressed in only 30 out of 50 (60%) tumor tissue samples. It has not yet been determined why TPST-1 expression is reduced in lung cancer. It is possible that tumors may be caused by insufficient protein-tyrosine sulfation. Of note, a recently published study on NPC showed that TPST-1 was up-regulated at the mRNA as well as the protein level in NPC cells, and this up-regulation was associated with metastasis (18). This result is contrary to the observation of the present study. This phenomenon may be explained by the fact that different tumor types have different signal transduction pathways and different mechanisms. Another possible explanation is that the authors of the aforementioned study was performed using NPC cells cultured *in vitro*, whereas the present study assessed tumor samples from patients.

The present study observed a tendency toward a lower expression rate of TPST-1 when the lung cancer TNM stage was advanced. Furthermore, TPST-1 expression in patients

with lymph node metastasis was significantly lower than that in patients without lymph node metastasis. All these results demonstrated that TPST-1 may be a useful prognostic biomarker. Detection of TPST-1 expression in tumor tissue may provide a more exact prognosis for patients with lung cancer.

C-Met, a high-affinity receptor for hepatocyte growth factor, is usually considered as an oncogene (29). Abnormalities regarding C-Met, including protein overexpression, gene mutation and gene amplification frequently occur in cancer (30). The overexpression of c-Met has been detected in several types of human cancer, including liver, lung, colorectal, stomach, and colon cancer (31-35), and is usually associated with poor outcome. Consistent with these studies, the present study also found that c-Met protein was overexpressed in lung cancer tissues. Furthermore, TPST-1 was significantly negatively correlated with c-Met expression in lung cancer. In addition to the other results of the present study, this result implied that TPST-1 may be a prognostic biomarker in lung cancer.

It should be noted that the present study has several limitations. First, the sample size was limited; further studies with larger samples may provide a more accurate prediction. Second, the expression of TPST-1 was examined only by immunohistochemistry, and no molecular studies or RNA analyses of TPST-1 were conducted. Finally, without a survival analysis, it was not possible to determine whether TPST-1 was an independent predictor of survival. Further studies are required to clarify the impact of TPST-1 expression on the survival of patients with lung cancer.

In conclusion, the expression of TPST-1 in lung cancer tumor tissues was significantly reduced compared to that in matched control lung tissues. TPST-1 expression was associated with lung cancer TNM stage and lymph node metastasis. The results of the present study also showed that TPST-1 was significantly negatively correlated with c-Met expression in lung cancer, suggesting that it may be a prognostic biomarker for lung cancer.

## Acknowledgements

This work was supported by the National Natural Scientific Foundation (grant no. 81472774) and the Research Programme of the Science and Technology Department of Hunan Province (grant nos. 2012FJ4076 and 2012TT2011).

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