



## Associations between clinical characteristics and oncogene expression in patients with non-small cell lung cancer

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**ABSTRACT.** More than 40 oncogenes associated with non-small cell lung cancer (NSCLC) have been identified with varied gene expression. The correlations between specific clinical characteristics and oncogene expression in NSCLC patients were examined. From October 2011 to September 2012, a total of 60 patients with NSCLC (male:female, 34:24; mean age, 59.5 ± 10.6 years; age range, 31-81 years) were diagnosed and evaluated for treatment with radical resection at a single facility. Eligible patients exhibiting tumor node metastasis (TNM) stage I-III NSCLC confirmed by post-surgical pathology were included. mRNA expression was detected by branched DNA-liquidchip technology (bDNA-LCT) and mutations were detected at *EGFR* exons 18, 19, 20, and 21, *KRAS* exons 2 and 3, *BRAF* and *PIK3CA* exons 9 and 20. Correlations between gene expression at mutations and clinical characteristics of gender, age, histological type, degree of differentiation, smoking status, immunohistochemical

(IHC) evaluation of TTF-1, TNM staging, and discrete age (“age”) were examined. Significant associations were observed between IHC staining for TTF-1 and histological type ( $P = 0.00001$ ) and with *BRAC1*, *TYMS*, *RRM1*, and *TUBB3* expression ( $P = 0.0187$ ,  $0.0051$ ,  $0.024$ , and  $0.0238$ , respectively). Significant cross-correlations were observed between *TYMS*, *BRAC1*, *TOP2A*, *STMN1*, *TUBB3*, and *RRM1* expression ( $P < 0.05$ ), but not between *EGFR* exon 21, *KRAS* exon 2, and *PIK3CA* exon 9 expression and any other mutation expression ( $P > 0.05$ ). Relationships between clinical characteristics and oncogene expression in NSCLC, particularly those of TTF-1 level and smoking status, may be useful indicators of prognosis and development of anti-cancer drug resistance.

**Key words:** Non-small cell lung cancer; Oncogene; Mutation; Radical resection; Clinical characteristics

## INTRODUCTION

The predominant type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for as much as 85% of all lung cancer cases (Tsuboi et al., 2007). Of these, 30-60% of patients will still die during the 5-year period following treatment (Mountain, 1997). Successful strategies for the treatment of NSCLC depend on earlier and more accurate tumor identification, which is currently indicated primarily by tumor node metastasis (TNM) stage at the time of treatment (Mountain, 2002). Recently, it has been suggested the specific gene signature of NSCLC tumors can be used as a prognostic indicator superior to TMN staging alone (Boutros et al., 2009). Despite the identification of thousands of heterogeneous genes involved in NSCLC, including over 40 well-documented genetic mutations (Bhattacharjee et al., 2001; Larsen et al., 2007; Sun et al., 2008), the association between the clinical characteristics of NSCLC patients expressing these genes and these mutations remain poorly documented, limiting clinical assessments.

Important genetic and molecular changes, including autocrine growth loops, proto-oncogene activation, and tumor-suppressor gene loss or inactivation have been shown to affect the clinical characteristics and drug responsiveness in NSCLC tumors (Jackman and Johnson, 2005). There are several genes with notable importance in these abnormalities, which may be useful in indicating prognosis and designing successful therapeutic regimens. Excision repair cross-complementation group 1 (*ERCC1*) is regulated by *ERCC1* and involved in DNA strand excision and damage repair (Bepler et al., 2006), occurring in all tumor cell types (Lord et al., 2002; Olausson et al., 2006). Tubulin, beta 3 class III (*TUBB3*) is regulated by *TUBB3*. It consists of two core protein families (alpha and beta tubulins) that heterodimerize to form microtubules (Sève and Dumontet, 2008). Thymidylate synthase (*TYMS*) regulated by *TYMS* is a rate-limiting enzyme important in tumor growth and DNA synthesis (Shirota et al., 2001). Topoisomerase II (Topo II), observed at high levels during malignant cell proliferation, is regulated by *TopoIIa* (Nitiss, 2009). These genes are critical in the clinical presentations of NSCLC tumors.

Additionally, some other genes have been recently linked to NSCLC tumor presentation. Expression of breast cancer type 1 (*BRCA1*) regulated by *BRCA1* is elevated in most

tumor tissues, including lung cancers (Boukovinas et al., 2008). Cell permeability, a consideration in the design of targeted treatments, is affected by epidermal growth factor receptor (EGFR), regulated by *EGFR* (Vastag, 2005). Furthermore, the rat sarcoma (*RAS*) gene family members *HRAS*, *KRAS*, and *NRAS* have been linked to lung cancer tumor occurrence and development (Larsen et al., 2007). Serine-threonine protein kinases encoded by the *BRAF* gene affect mitogen-activated protein kinases (MAPK) pathways, thereby impacting cellular function regulation in tumor tissues (Ikenoue et al., 2004). Phosphatidylinositol 3-kinases (PI3Ks) are a group of protein polymers regulated by the *PIK3CA* gene, which affect cell function (Bachman et al., 2004). Cumulatively, *ERCC1*, *TUBB3*, *TYMS*, *TopoII $\alpha$* , *BRCA1*, *RAS* genes, *BRAF*, and *PIK3CA* each plays unique and important roles in the clinical presentation and prognosis of NSCLC patients, though the link between these genes and clinical presentations has not been fully established.

The expression of these genes has been linked to tumor sensitivity to conventional cancer therapies. *TUBB3* mRNA expression has been closely associated with the therapeutic efficacy of anti-microtubule drugs (i.e., paclitaxel, docetaxel, vinblastine, vincristine, and vinorelbine), which act on microtubules to inhibit mitosis (Johnston et al., 1995). Low *TYMS* mRNA expression has been linked to the therapeutic efficacy of 5-fluorouracil drugs (i.e., tegafur, carmofur, floxuridine, doxifluridine, and capecitabine) in numerous cancer types (Shirota et al., 2001), including lung cancer (Shintani et al., 2011). Gemcitabine, a deoxycytidine analogue, belongs to cell cycle-specific antimetabolite drugs. It primarily induces S phase (DNA synthesis phase) arrest in tumor cells, blocking the G1 (presynthetic phase) to S phase cell cycle transition. In the United States, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology indicate that *RRM1* expression is associated with the therapeutic efficacy of gemcitabine in NSCLC (2010). Etoposide, a cell cycle-specific anti-tumor drug, is an inhibitor of DNA topoisomerase II (Topo II), commonly used in NSCLC treatment (Uesaka et al., 2007). Low *BRCA1* expression has been shown to increase the effectiveness of anti-tumor platinum drugs (i.e., cisplatin, carboplatin, and oxaliplatin), while resistance is often observed in patients with high *BRCA1* expression (Boukovinas et al., 2008). Furthermore, the United States NCCN Clinical Practice Guidelines in Oncology recommend that *EGFR* expression might be assessed prior to treatment with EGFR-targeted drugs (2009). Currently, many EGFR-targeted drugs (i.e., gefitinib and erlotinib) have been developed on the basis of overexpression of *EGFR* in tumor cells (Sequist et al., 2008; Yang et al., 2008). However, drug resistance may occur in some patients at a later stage, despite the success of initial therapies (Suda et al., 2009).

The prevalence of poor outcomes due to ineffective treatment or resistance to treatment necessitates the development of a better understanding of the clinical characteristics associated with gene expression in NSCLC. Recently, transfection of genes into malignant cells has been used to examine cancer therapy resistance (Niimi et al., 1991). Despite a growing interest in the field, no practical clinical guidelines have been developed to allow assessment of NSCLC patients based on genetic risk by clinical characteristics.

The current study examined a range of variables, including clinical characteristics and gene expression of certain mutations in NSCLC patients to determine the correlation between gene expressions and clinical characteristics. These data can be used as a basis for the future development of clinically useful guidelines for assessment of prognosis and treatment strategy in NSCLC patients.

## MATERIAL AND METHODS

### Study design

A total of 60 NSCLC patients (male:female, 34:24; mean age,  $59.5 \pm 10.6$  years; age range, 31-81 years) were retrospectively studied following diagnosis and evaluation for treatment with radical resection at the Department of Surgery between October 8, 2011 and September 10, 2012. Detection of mRNA expression and mutations at *EGFR* exons 18, 19, 20, and 21, *KRAS* exons 2 and 3, *BRAF*, and *PIK3CA* exons 9 and 20 was conducted. The protocol was approved by the Ethics Committee of the Beijing Chest Hospital, and all patients provided written informed consent for participation.

### Patients

The patients included in the present study all met the following inclusion criteria: 1) positive NSCLC diagnosis; 2) eligible for radical resection of lung cancer; 3) positive confirmation of NSCLC by post-surgical pathology; 4) clinical staging of I-III; and 5) fresh tissue specimens successfully provided during surgery. Patients were excluded for following reasons: 1) exhibited small-cell lung cancer confirmed by pathological examination after resection of lung cancer; 2) were not eligible for radical resection of lung cancer based on intraoperative exploration; and 3) were diagnosed preoperatively or intraoperatively with stage IV lung cancer. All patients were diagnosed according to the guidelines provided by Mountain (1997, 2002). Due to limited data availability, patients with incomplete records were included, and data are presented for the maximum number of patients with data for each variable ( $N_i$ ) in the entire study cohort ( $N$ ).

### Data collection and variables

Of the 41 variables assessed in the preliminary screening, 22 variables were selected for use in the analysis. The genetic variables included expression of *ERCC1*, *BRCA1*, *TYMS*, *RRM1*, *TUBB3*, *TOP2A*, and *EGFR* and *EGFR* mutations in exons 19, 18, 20, and 21, *KRAS* mutations in exons 2 and 3, and *PIK3CA* mutations in exons 9 and 20. Patients were described as stage IA, IB, IIA, IIB, IIIA, IIIB, or IV, according to standard tumor node metastasis (TNM) staging, and the primary TNM classification was determined using Clinicopathological Staging System version 6 to generate the variable “stage”. Variables of gender (“sex”), age in years (“age”), histological type (“type”), degree of differentiation (“D”), smoking status (“smoking”), immunohistochemical evaluation of TTF-1 (“ICH”), tumor node metastasis (TNM) staging (“stage”), and discrete age (“nage”) were recorded for further analysis.

### Laboratory testing

As mentioned in Li et al. (2014), mRNA expression was detected by branched DNA-liquidchip technology (bDNA-LCT), including mRNA expression of *ERCC1*, *BRCA1*, *TYMS*, *RRM1*, *TUBB3*, *STMN1*, *TOP2A*, *EGFR*, *PDGFR $\beta$* , *VEGF1*, *VEGF2*, *VEGF3*, *KIT*, *HER2/*

*NEU*, and *IGF1R*. A novel multiplex branched DNA liquidchip (MBL) technology which integrates the branch-DNA (b-DNA) and liquidchip technology was developed for quantitative measurement of gene mRNA level in FFPE slides. MBL is a non-PCR-based technology involving a sandwich nucleic acid hybridization platform in which targets are captured through cooperative hybridization of multiple probes and then conjoined with a fluorescence signal amplification system

### Mutation detection

Mutation detection of *EGFR* exons 18, 19, 20, and 21, *KRAS* exons 2 and 3, *BRAF* and *PIK3CA* exons 9 and 20 was conducted. Tumor genomic DNA from each FFPE slide was extracted with the Maxwell system (Promega, USA) and the concentration was determined using the NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). *EGFR*, *KRAS*, *BRAF*, and *PIK3CA* mutations were detectable in all samples analyzed. The mutation status of *EGFR* (exons 18 to 21), *KRAS* (exons 2 and 3), *BRAF* (exon 15) and *PIK3CA* (exons 9 and 20) were analyzed simultaneously by xTAG liquidchip technology.

### Immunohistochemistry

Expression of TTF-1 was assessed by immunohistochemistry (IHC).

### Statistical analysis

All data were analyzed using SPSS version 18.0 (IBM, USA). The degree of differentiation (D), gender (sex), smoking status, *EGFR* mutation in exon 21, *EGFR* mutation in exon 19, *BRAF* mutation belonged to two-class variables, whose theoretical frequencies were estimated prior to the evaluation of between-variable associations. Pearson correlation analysis was then performed between sex and IHC, sex and smoking status, and IHC and smoking status. Spearman's correlation analysis was employed for all other variables. Gene expression, as ranked data, was also analyzed by Spearman's correlation analysis.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Patient demographics and clinical data

A total of 60 patients underwent radical resection, though complete data were not available for each variable from all patients ( $N = 60$ ;  $N_1 = 60$  - frequency missing). Patient demographic and clinical data are shown in Table 1. More patients exhibited either low moderate (39.58%) or moderate differentiation (39.58%) types, and positive IHC staining of TTF 1 (54.55%). At one-year follow-up, the mortality rate of included patients was 20%.

### TNM staging

In the 57 patients with staging data, staging was IA in 7 patients (12.28%), IB in 21

patients (36.84%), IIA in 1 patient (1.75%), IIB in 4 patients (7.02%), IIIA in 14 patients (24.56%), IIIB in 3 patients (5.26%), and IV in 7 patient (12.28%). Notably, IB staging was significantly more frequent than all other stages, followed by IIIA ( $P > 0.05$ ). Staging data are shown in Table 2.

**Table 1.** Demographic and clinical parameters of the study cohort.

Variable	N (%)	Total N <sub>1</sub> (%)
Gender		
Male	34 (58.62%)	58 (100%)
Female	24 (41.38%)	
Age (years)		
30-50	8 (14.29%)	56 (100%)
50-60	23 (41.07%)	
>60	25 (44.64%)	
Histological type		
Adenocarcinoma	34 (59.65%)	57 (100%)
Squamous cell carcinoma	22 (38.60%)	
Adenosquamous carcinoma	1 (1.75%)	
Smoking status		
Non-smoking	31 (53.45%)	58 (100%)
Smoking	27 (46.55%)	
Differentiation degree		
Low	5 (10.42%)	48 (100%)
Low-moderate	19 (39.58%)	
Moderate	19 (39.58%)	
Moderate-high	4 (8.33%)	
High	1 (2.08%)	
IHC staining of TTF-1		
Negative	25 (45.45%)	55 (100%)
Positive	30 (54.55%)	

\*N<sub>1</sub> = number of patients examined; N = 60 (total study cohort).

**Table 2.** Pathological staging (TNM staging) in the study cohort.

Staging	N (%) (N <sub>1</sub> = 57)*
IA	7 (12.28%)
IB	21 (36.84%)
IIA	1 (1.75%)
IIB	4 (7.02%)
IIIA	14 (24.56%)
IIIB	3 (5.26%)
IV	7 (12.28%)

TNM = tumor node metastasis. \*N<sub>1</sub> = number of patients with staging data; N = 60 (total study cohort).

## Gene expression

Low, low-moderate, moderate, moderate-high, and high expression of *ERCC1*, *BRCA1*, *TYMS*, *RRM1*, *TUBB3*, *STMN1*, *TOP2A*, and *EGFR* are shown in Table 3. These genes were expressed in 39-60 of patients examined. Notably, high expression was most commonly observed in the *RRM1* (27 patients) and *TYMS* (17 patients) genes. Low expression of the *ERCC1* gene was common (25 patients).

**Table 3.** Gene expression in the study cohort.

Gene expression	Total (N <sub>i</sub> , %)	Low	Low-moderate	Moderate	Moderate-high	High
<i>ERCC1</i>	59 (100%)	24 (40.68%)	12 (20.34%)	11 (18.64%)	7 (11.86%)	5 (8.47%)
<i>BRCA1</i>	39 (100%)	9 (23.08%)	4 (10.26%)	9 (23.08%)	7 (17.95%)	10 (25.64%)
<i>TYMS</i>	60 (100%)	15 (25.00%)	8 (33.33%)	14 (23.33%)	6 (10.00%)	17 (28.33%)
<i>RRM1</i>	60 (100%)	13 (21.67%)	5 (8.33%)	11 (18.33%)	4 (6.67%)	27 (45%)
<i>TUBB3</i>	59 (100%)	14 (23.73%)	19 (32.2%)	10 (16.95%)	6 (10.17%)	10 (16.95%)
<i>STMN1</i>	39 (100%)	12 (30.77%)	6 (15.38%)	7 (17.95%)	5 (12.83%)	9 (23.08%)
<i>TOP2A</i>	39 (100%)	6 (15.38%)	6 (15.38%)	10 (25.64%)	8 (20.51%)	9 (23.08%)
<i>EGFR</i>	60 (100%)	19 (31.67%)	11 (18.33%)	11 (18.33%)	5 (8.3%)	14 (23.33%)

\*N<sub>i</sub> = number of patients with expression data; N = 60 (total study cohort).

### Comparison of clinical characteristics

In comparisons of sex and IHC, sex and smoking, and IHC and smoking, the number of cells with theoretical frequencies of other variables <5 was more than 1/5 of total cells. Notably, there was a significant association between IHC staining for TTF-1 and histological types (P = 0.00001) (Table 4). Correlations between clinical characteristics are shown in Table 4.

**Table 4.** Correlations between clinical characteristics.

	Gender	Age	Type	D	Smoking	IHC	Stage	Nage
Gender	-	0.0014	0.0001	0.0192	0.00001	0.0022	*	0.0028
Age	0.0014	-	0.0243	*	*	*	*	0.00001
Type	0.0001	0.0243	-	*	0.0019	0.00001	*	*
D	0.0192	*	*	-	*	*	*	*
Smoking	0.00001	*	0.0019	*	-	0.0017	0.015	*
IHC	0.0022	*	0.00001	*	0.0017	-	*	*
Stage	*	*	*	*	0.015	*	-	*
Nage	0.0028	0.00001	*	*	*	*	*	-

IHC = immunohistochemistry. \*P > 0.05. D = differentiation; Nage = discrete age.

### Correlations between clinical variables and gene expression at mutations

Significant associations were found between IHC staining for TTF-1 and *BRCA1*, *TYMS*, *RRM1*, and *TUBB3* expression (P = 0.0187, 0.0051, 0.024, and 0.0238, respectively). Also, a significant relationship between smoking and *BRCA1*, *TYMS*, *RRM1*, *STMN1*, *TOP2A*, and KRAS exon 2 was observed. Correlations between gene expression at mutations and clinical variables are detailed in Table 5.

**Table 5.** Correlations between gene mutations and clinical characteristics.

	Gender	Age	Type	D	Smoking	IHC	Stage	Nage
<i>ERCC1</i>	*	*	*	0.0486	*	*	*	*
<i>BRCA1</i>	0.0027	*	*	*	0.0079	0.0187	*	*
<i>TYMS</i>	0.0036	*	0.001	*	0.0256	0.0051	*	*
<i>RRM1</i>	*	*	0.0143	*	0.0228	0.024	*	*
<i>TUBB3</i>	*	*	0.0158	*	*	0.0238	*	*
<i>STMN1</i>	0.0261	*	*	*	0.0135	*	*	0.0135
<i>TOP2A</i>	0.0261	*	*	*	0.0135	*	*	*
<i>EGFR</i>	*	*	*	0.0011	*	*	*	*
<i>EGFR19</i>	*	*	*	*	*	*	*	*
<i>EGFR21</i>	*	*	*	0.0428	*	*	*	*
<i>KRASE2</i>	*	*	*	*	0.0428	*	*	*
<i>PIK3CAE9</i>	*	*	0.0127	*	*	0.0495	*	*

IHC = immunohistochemistry. \*P > 0.05. D = differentiation; Nage = discrete age.

### Correlations between gene expressions at mutations

Significant correlations were observed between *TYMS*, *BRAC1*, *TOP2A*, *STMN1*, *TUBB3*, and *RRM1* expression ( $P < 0.05$ ). Cross-correlations were observed in some pairs of genes. Notably, no significant associations were found between *EGFR* exon 21, *KRAS* exon 2, *PIK3CA* exon 9 expression and any other mutation expression ( $P > 0.05$ ). The significance levels of these associations are shown in Table 6.

**Table 6.** Correlations between gene mutations.

	ERCC1	BRCA1	TYMS	RRM1	TUBB3	STMN1	TOP2A	EGFR	EGFR19
ERCC1	-	*	*	0.0115	0.0059	0.0049	*	0.0082	*
BRCA1	*	-	0.00001	0.0006	*	0.00001	0.00001	*	*
TYMS	*	0.00001	-	0.00001	*	0.00001	0.00001	*	0.0261
RRM1	0.0115	0.00001	*	-	*	0.00001	0.00001	0.008	*
TUBB3	0.0069	*	*	*	-	*	*	*	*
STMN1	*	0.00001	0.00001	0.00001	*	-	0.00001	0.0098	*
TOP2A	*	0.00001	0.00001	0.00001	*	0.00001	-	*	*
EGFR	0.0082	*	*	0.008	*	0.0098	*	-	*
EGFR19	*	*	0.0261	*	*	*	*	*	-

\* $P > 0.05$ . No significant associations were found between EGFR21, KRASE2, PIKAE9 and any other mutations ( $P > 0.05$ ).

### Special considerations for the use of TTF-1 IHC determination for NSCLC identification

Variable histological type may be a confounding factor, necessitating further stratified analyses of *BRAC1*, *TYMS*, *RRM1*, and *TUBB3* expression (data not shown). If this was performed, changes in the significance levels for the associations of IHC determination of TTF-1 with *BRAC1*, *TYMS*, *RRM1*, and *TUBB3* were observed. If the variable “type” was defined as 1 (histological type = adenocarcinoma), weakly significant associations were detected between IHC determination of TTF-1 and *BRAC1*, *TYMS*, *RRM1* and *TUBB3* expression. If the variable “type” was defined as 2 (histological type = squamous cell carcinoma), highly significant associations were observed between IHC determination of TTF-1 and *BRAC1*, *TYMS*, *RRM1*, and *TUBB3* expression. In all analyses, however, the associations between IHC determination of TTF-1 and *BRAC1*, *TYMS*, *RRM1*, and *TUBB3* expression were significant ( $P < 0.05$ ).

### DISCUSSION

Clinical characteristics and expression of oncogenes associated with NSCLC were investigated, demonstrating that certain clinical factors may indicate higher risk of gene-associated abnormalities. Most notably, significant cross-correlations were found between *TYMS*, *BRAC1*, *TOP2A*, *STMN1*, *TUBB3*, and *RRM1* mRNA expression, which may provide clues to the mechanisms of clinical phenomenon, such as anti-cancer drug resistance development. Furthermore, TTF-1 expression indicated by IHC can potentially be used to identify both primary and secondary NSCLC in adenocarcinoma patients. These observations have potential use in prognostic assessment and identification of NSCLC patients at risk for development of treatment resistance, allowing for improved prognostic assessment and treatment selection.



Variant levels of gene expression were observed in NSCLC patients in the current study, indicating moderate or high levels of *BRCAl*, *TYMS*, *RRM1*, and *TOP2A* mRNA expression and low or moderate levels of *ERCC1*, *TUBB3*, *STMN1*, and *EGFR* mRNA expression. These findings are consistent with a number of previous reports indicating that the expression of these genes varies greatly in cancer patients, including those with lung cancer (Lowy and Willumsen, 1993; Shirota et al., 2001; Lord et al., 2002; Bachman et al., 2004; Ikenoue et al., 2004; Vastag, 2005; Bepler et al., 2006; Olaussen et al., 2006; Boukovinas et al., 2008; Sève and Dumontet, 2008; Nitiss, 2009). Furthermore the cross-correlations between these gene expressions indicated that several genes may be involved in similar mechanistic processes that impact prognosis, tumor growth, and development of anti-cancer drug resistance.

These findings may also be used to make general observations about NSCLC patient risks for developing resistance to certain conventional anti-cancer therapies. On the basis of the current indications that most NSCLC patients exhibit low *ERCC1* expression, these patients are likely to be predominantly sensitive to platinum drugs (2010). Conversely, high *BRCAl* suggests that NSCLC patients may be resistant to platinum drugs. Notably, no significant associations were found between *ERCC1* and *BRCAl* expression levels ( $P > 0.05$ ), suggesting that these two mechanisms for resistance to platinum-containing anticancer drugs, such as cisplatin, may be independent. It has been theorized that platinum drug resistance is related to accumulation of the drug, affecting gated-ion channels (Gately and Howell, 1993). Further studies, however, are needed to determine whether these genes are related to platinum drug accumulation and ion channel deficiency.

Notably, moderate to high expression levels of *TYMS* were observed. Because *TYMS* encoded by this gene is the major target of 5-fluorouracil, with the metabolite of 5-fluorouracil binding to *TYMS* and inhibiting its normal function in DNA synthesis (Johnston et al., 1995), alterations in the responses of many NSCLC patients to fluorouracil drugs can be expected. Notably, survival time has been shown to be longer in patients with low *TYMS* expression levels, which may also be useful as a prognostic indicator (Yasumatsu et al., 2009). Similarly, longer median survival times have been observed in lung cancer patients treated with paclitaxel or vinblastine, if they exhibited low *RRM1* (Rosell et al., 2004) and *TUBB3* (Sève et al., 2005) expression levels. Most NSCLC patients in the present study, however, exhibited high expression of *RRM1* and low levels of *TUBB3*. Notably, these gene expressions were not significantly related ( $P > 0.05$ ), suggesting independent mechanisms of action. These correlations may be useful in determining the mechanistic action of these genes in producing certain clinical characteristics, though further study is required.

Low to moderate expression was observed in the *STMN1* gene among current NSCLC patients, indicating that therapeutic efficacy of anti-microtubule drugs, such as vinorelbine or cisplatin, against multiple tumor cells is likely to produce good outcomes in a large number of NSCLC patients (Rosell et al., 2003). The *TOP2A* gene encoding TopoII $\alpha$ , which mediates DNA unwinding vital to cellular metabolic processes, was shown to be expressed at moderate to high levels in NSCLC patients, consistent with previous studies (Uesaka et al., 2007). Notably, low expression of *EGFR* (19.9%) was found in NSCLC patients in the current study, consistent with previous reports of only 10-20% *EGFR* expression in these patients (Vastag, 2005). Thus, the current gene expressions are very consistent with previous reports. Further collection of clinical data is, however, required to fully evaluate gene expression profiles and therapeutic efficacy to make specific clinical recommendations based on these findings.

It has been demonstrated that TTF-1 expression can be used to identify primary or secondary lung cancers (Kitamura et al., 2009). In fact, lung cancer cells almost always express mammalian achaete-scute homolog-1 (MASH1) and thyroid transcription factor-1 (TTF-1), a marker used to identify adenocarcinomas in distal airway and pulmonary cells as well as in extrapulmonary neuroendocrine cancers (Kitamura et al., 2009). In the current study, both histological type and IHC staining for TTF-1 correlated strongly with gene expression, supporting previous reports that TTF-1 is a marker of NSCLC that may have a strong genetic basis.

Recently, other studies have also investigated the biological factors associated with NSCLC. Berghmans et al. (2008) reported a study of 84 patients with stage III NSCLC in which they found a significant association between TTF-1 levels and *EGFR* as well as TTF-1 levels and *MDM2*. Notably, no such association was observed between TTF-1 and *EGFR* in the current study, which might have been due to the staging of patients or selection of the study group. Clinical and demographic parameters of age, performance status, gender, weight loss, serum lactate dehydrogenase level, white blood cells, and neutrophil counts have been previously associated with NSCLC prognosis (Kanters et al., 1995; Sculier et al., 1997). Additionally, current or history of smoking has been widely accepted to positively correlate with lung cancer development and poor prognosis (Kitamura et al., 2009). The current study, however, showed a significant relationship between smoking and many genes related to NSCLC, including *BRCA1*, *TYMS*, *RRM1*, *STMN1*, *TOP2A*, and *KRASE2*. These observations may indicate both greater likelihood of lung cancer developing as well as increased likelihood of other clinical complications, such as development of anti-cancer drug resistance in smokers.

While mortality at one year was assessed at 20% in the present study, further analysis is needed to determine the relationship between gene expression and survival in NSCLC patients. As suggested by Berghmans et al. (2008), the Cox multivariate model will be useful in providing detailed relationships between clinical characteristics and biological factors on NSCLC patient survival. Additionally, this study may be limited by the relatively small sample size and primarily Chinese cohort. Furthermore, the significance level of the correlation matrix should be adjusted to 0.0025 due to the use of multiple hypotheses in the present study. Therefore, the findings of the current analysis can only provide a reference, and should be cautiously applied. Further study is needed before broad clinical recommendations can be made on the basis of these correlations.

The associations between clinical characteristics and gene expression of oncogenes associated with cancer progression and anti-cancer drug resistance were investigated in NSCLC patients. Significant cross-correlations were found between the mRNA expression of the genes *TYMS*, *BRAC1*, *TOP2A*, *STMN1*, *TUBB3*, and *RRM1*. Additionally, TTF-1 expression indicated by IHC was associated with NSCLC, indicating that it may be useful in prognostic assessment of these patients. Furthermore, consistent with previous studies, clinical parameters, such as smoking, were shown to be positively correlated with abnormal gene expression in certain genetic mutations associated with NSCLC.

### Conflicts of interest

The authors declare no conflict of interest.

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