

## Original Article

# Detection of serum uroplakin 1A, cyclin D1 and MMP7 levels in patients with non-small cell lung cancer and their clinical significance

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Received December 30, 2015; Accepted March 17, 2016; Epub May 1, 2016; Published May 15, 2016

**Abstract:** To investigate the clinical significance of serum levels of uroplakin 1A (UPK 1A), cyclin D1 and matrix metalloproteinases 7 (MMP7) in patients with Non-Small Cell Lung Cancer (NSCLC). 92 NSCLC patients were selected for this study from January 2013 to April 2014. The ELISA test was performed to detect serum UPK 1A, cyclin D1 and MMP7 levels and compare them in patients with different type, clinical stage and degree of NSCLC. There was no significant difference in UPK 1A, cyclin D1 and MMP7 levels in lung squamous carcinoma patients compared to lung adenocarcinoma patients ( $P > 0.05$ ). With an increase in severity of NSCLC, the level of UPK 1A significantly ( $P < 0.05$ ) decreased. The levels of cyclin D1 and MMP7 significantly ( $P < 0.05$ ) increased with an increase in the clinical stage of NSCLC. The UPK 1A level in poorly differentiated NSCLC was significantly ( $P < 0.05$ ) lower than that in moderately and highly differentiated cancer. Cyclin D1 and MMP7 levels in poorly differentiated NSCLC were significantly ( $P < 0.05$ ) higher than those in moderately and highly differentiated cancer. The UPK 1A level was correlated negatively with cyclin D1 and MMP7 levels of NSCLC ( $r = -0.351$ ,  $P = 0.000$ ;  $r = -0.398$ ,  $P = 0.000$ ). The cyclin D1 was positively correlated with the MMP7 level ( $r = 0.367$ ,  $P = 0.000$ ). The levels of UPK 1A, cyclin D1 and MMP7 are closely related to the clinical stage and degree of differentiation of NSCLC. The UPK 1A level is negatively correlated with the levels of cyclin D1 and MMP7.

**Keywords:** Non-small cell lung cancer, uroplakin, cyclin, matrix metalloproteinases, expression level

## Introduction

Primary lung cancer is the leading cause of cancer-related death worldwide [1]. In 2008, lung cancer replaced liver cancer as the number one cause of death among people with malignant tumors in China, which imposes an enormous burden on patients, health-care professionals, and the society [2]. Most lung cancer cases (85%) are categorized as Non-Small Cell Lung Cancer (NSCLC), with the remaining cases constituting Small Cell Lung Cancer (SCLC), which has a 5-year survival of only 10% [3]. Although there have been significant progresses in clinical treatment of NSCLC in recent years, the overall survival duration of NSCLC patients has not improved dramatically. An important reason behind this finding is the lack of molecular biomarkers. Recently studied micro-RNAs 125b [4] and CYFRA 21-1 [5] are the tumor

markers commonly used as diagnostic or prognostic biomarkers for advanced NSCLC. Serum MMP-7 levels seem to be capable of distinguishing IPF patients from those with other lung diseases [6]. However, these tumor markers have limited utility in early detection of NSCLC due to lack of sufficiently high diagnostic sensitivity and specificity. Therefore, the significance of exploring novel highly sensitive and specific biomarkers for early detection of NSCLC should be emphasized.

Uroplakins (UPs) play a key role in tumorigenesis [7, 8]. UPs have been detected at lower levels and/or have been reported absent in invasive carcinomas during tumorigenesis [9]. The uroplakin 1A (UPK 1A) gene belongs to the transmembrane 4 super family (TM4SF) [10, 11], which is known to be highly specific to normal urothelium [12] and can be found inside a

**Table 1.** UPK 1A, cyclin D1 and MMP7 levels of different types of NSCLC

Pathological type	Case number	UPK 1A (µg/mL)	Cyclin D1 (µg/mL)	MMP7 (µg/mL)
Lung squamous carcinoma	51	19.18±3.61	39.62±3.97	49.26±4.27
Lung adenocarcinoma	41	22.76±3.17	36.92±3.65	47.19±4.05
t value		1.3261	1.2689	1.1672
P value		0.0976	0.0981	0.1029

**Table 2.** UPK 1A, cyclin D1 and MMP7 levels in patients with different clinical stage of NSCLC

Clinical stage	Case number	UPK 1A (µg/mL)	Cyclin D1 (µg/mL)	MMP7 (µg/mL)
I-II	37	23.75±3.69	35.71±3.59	46.39±4.29
III	55	17.52±3.09	42.89±3.71	52.76±4.32
t value		4.2781	5.0278	5.0061
P value		0.0019	0.0014	0.0017

normal genitourinary tract, uterus and prostate [13]. Recently, accumulated evidence indicates that UPK 1A is a significant tumor suppressor in Esophageal Squamous Cell Carcinoma (ESCC), where it has a role in inhibiting cell proliferation, clonogenicity, cell motility, and tumor formation [14]. In addition, UPK 1A can also be used as a molecular biomarker of prognostic and clinicopathological significance in human gastric carcinoma (GC) [15]. Furthermore, the cell cycle arrest elicited by UPK 1A at the G(1)-S checkpoint is associated with downregulation of cyclin D1, whereas metastasis suppression is associated with a reduction of MMP7 [14]. However, it is largely unknown whether UPK 1A can be used as a serum biomarker to detect early lung cancer, or to identify the type, progression and differentiation of lung cancer. In this study, we examined the expression levels of UPK 1A, cyclin D1, and MMP7 in plasma, and evaluated their potential use as tumor markers of NSCLC. We hypothesized that these NSCLC-related proteins might be released into the circulation during NSCLC initiation and progression and that they may be utilized to detect NSCLC.

## Materials and methods

### Clinical data

The study analyzed 92 pathological tissue samples from patients who were diagnosed as NSCLC and who underwent surgery in our hospital from January 2013 to April 2014, including 67 male and 25 female patients. The

patients were between 41 and 81 years old, with a mean age of 56.2±3.12 years. 51 cases of lung squamous carcinoma and 41 cases of lung adenocarcinoma were included in the study. 37 cases were in stage I-II of the disease and 55 cases were in

stage III. 61 cases had moderately and highly differentiated cancer and 31 cases had poorly differentiated cancer. Elimination rules: the study was approved by our hospital ethics committee and through informed consents given by patients.

### Blood sample

5 mL of morning fasting venous blood was obtained from all subjects. The serum was obtained using centrifugal separation to determine UPK 1A, cyclin D1 and MMP7 levels.

### ELISA analysis

ELISA analysis was performed to detect serum UPK 1A, Cyclin D1 and MMP7 levels, and the data were recorded and compared. The correlations between UPK 1A, cyclin D1 and MMP7 levels were analyzed.

### Statistical analysis

All statistical analyses were performed with SPSS 18.0 software. The data were presented as mean ± standard deviation ( $\bar{x} \pm s$ ) and analyzed using independent two-tailed t test.  $P < 0.05$  was considered as statistically significant.

## Results

### Baseline information

The study analyzed 92 cases pathological tissue samples from patients who were diagnosed as NSCLC and who underwent surgery in our hospital from January 2013 to April 2014. 67 cases male and 25 female patients were included in the study. The patients averaged 56.2±3.12 years. The cases included 51 cases with lung squamous carcinoma and 41 cases with lung adenocarcinoma. 37 cases were in stage I-II and 55 cases were in stage III of the disease. 61 patients had moderately or highly differentiated cancer and 31 cases had poorly

**Table 3.** UPK 1A, cyclin D1 and MMP7 levels in NSCLC with varying degree of differentiation

Differentiation degree	Case number	UPK 1A (µg/mL)	Cyclin D1 (µg/mL)	MMP7 (µg/mL)
Moderately and highly differentiated	31	23.82±3.71	35.60±3.56	46.29±4.19
Poorly differentiated degree	61	17.40±3.07	42.98±3.75	52.85±4.37
t value		4.3612	5.1256	5.0072
P value		0.0017	0.0012	0.0015

differentiated cancer. The baseline information of patients from the same clinical stage and degree of differentiation, such as age, gender, etc. was similar and not statistically different ( $P > 0.05$ ). These cases were comparable.

*UPK 1A, cyclin D1 and MMP7 levels of patients with different type of NSCLC*

No significant ( $P > 0.05$ ) differences in UPK 1A, cyclin D1 and MMP7 levels were observed between lung squamous carcinoma and lung adenocarcinoma patients (Table 1). The mean concentrations of UPK 1A, cyclin D1 and MMP7 in lung squamous carcinoma patients were 19.18±3.61 µg/mL, 39.62±3.97 µg/mL and 49.26±4.27 µg/mL, respectively, while the mean concentrations of UPK 1A, cyclin D1 and MMP7 in lung adenocarcinoma patients were 22.76±3.17 µg/mL, 36.92±3.65 µg/mL and 47.19±4.05 µg/mL, respectively.

*UPK 1A, Cyclin D1 and MMP7 levels in patients with different clinical stage of NSCLC*

The UPK 1A level decreased significantly with an increase in the clinical stage of NSCLC ( $P < 0.05$ ), from 23.75±3.69 µg/mL in stage I-II to 17.52±3.09 in stage III µg/mL. The cyclin D1 and MMP7 levels increased significantly with an increase in the clinical stage of NSCLC ( $P < 0.05$ ) (Table 2).

*UPK 1A, cyclin D1 and MMP7 levels in NSCLC of varying degree of differentiation*

The UPK 1A level in poorly differentiated NSCLC was significantly lower ( $P < 0.05$ ) than that in moderately and highly differentiated NSCLC. The cyclin D1 and MMP7 levels in poorly differentiated NSCLC were significantly higher ( $P < 0.05$ ) than those in moderately and highly differentiated NSCLC (Table 3).

*Correlation between UPK 1A and cyclin D1 and MMP7 levels*

The UPK 1A level was negatively correlated with cyclin D1 and MMP7 levels of NSCLC ( $r=-$

0.351,  $P=0.000$ ;  $r=-0.398$ ,  $P=0.000$ ), while the cyclin D1 level was positively correlated with the MMP7 level ( $r=0.367$ ,  $P=0.000$ ).

**Discussion**

Lung cancer is by far the leading cause of cancer death among both men and women, because most lung cancers have already progressed to an advanced stage by the time they are detected. These cancers are very hard to cure. In recent years, serum biomarkers have been used to screen for lung cancer in people at high risk for the disease. This test can help detect some of these cancers early, which can help lower the risk of dying from this disease. This study investigated serum UPK 1A, cyclin D1 and MMP7 levels of NSCLC. The results have shown that these levels are not related to the pathological type of NSCLC, but are related to the clinical stage and differentiation degree of NSCLC. The UPK 1A level decreased with an increase in the clinical stage of NSCLC while the cyclin D1 and MMP7 levels increased. The UPK 1A level decreased with a decrease in the differentiation degree of NSCLC but cyclin D1 and MMP7 levels increased. The UPK 1A level was positively related with the cyclin D1 and MMP7 levels. This study revealed for the first time that serum UPK 1A level was related to the clinical stage and degree of differentiation of NSCLC. It suggested that the serum UPK 1A level could serve as a biomarker of the clinical stage and differentiation degree of NSCLC.

It has been found that the changes in UPK 1A, cyclin D1 and MMP7 levels were related with the clinical stage and differentiated degree of NSCLC in this study. It suggested that UPK 1A, cyclin D1 and MMP7 levels had a potential diagnostic value for determining the clinical stage and differentiation degree of NSCLC but not for the pathological type of NSCLC. Matrix metalloproteinases-7 (MMP-7) is overexpressed in Non-Small Cell Lung Cancer (NSCLC) [16, 17]. MMP-7 is also expressed in

bronchiolization of alveoli (BCA), a state preceding lung cancer but not in normal epithelia, where it promotes proliferation, migration, and attenuation of apoptosis [18]. The overexpression of MMP-7 is associated with tumor proliferation and chemoresistance and constitutes a prognostic factor in several solid tumors [16, 19, 20], suggesting an independent positive prognostic factor in NSCLC patients [21]. The levels of MMP7 were increased in colorectal tumor tissues with prevalence in stage I/II [22], suggesting that MMP2 is related to the stage of tumorigenesis. Furthermore, the MMP7 serum levels were significantly elevated in cholangiocarcinoma patients ( $P < 0.001$ ). The area underneath the curve (AUC) from the Receiver Operating Characteristic (ROC) curve analysis for the diagnosis of cholangiocarcinoma using MMP7 is  $AUC=0.84$ , 95% CI: 0.778-0.903. The sensitivity and specificity of serum MMP7 (cut-off value of 5.5 ng/mL) were 75% and 78%, respectively [23]. Generally speaking, genetic polymorphisms in MMP7 and reduced MMP7 serum levels are associated with the development of bronchiolitis obliterans, a syndrome that appears after lung transplantation [24]. The above results suggest that serum MMP7 level is a potential biomarker for identifying the stage of NSCLC. Consistent with this hypothesis, the serum MMP7 level significantly increased in patients with stage III NSCLC, as well as in patients with highly differentiated NSCLC.

Increased expression of cyclin D1, which is a common characteristic of lung cancer, may prevent the induction of apoptosis in an oxidizing and growth factor-poor environment [25]. Serum cyclin D1 has proven to be a useful tumor marker in oral squamous cell carcinoma, which may be valuable in diagnosing oral squamous cell carcinoma [26]. In the present work, serum cyclin D1 increased in patients with stage III NSCLC, as well as in the patient with well-differentiated NSCLC. These results indicate that serum cyclin D1 may be a potential biomarker for identifying the stage and the degree of differentiation of NSCLC.

This study has confirmed that serum UPK 1A level in NSCLC patients is positively correlated with cyclin D1 and MMP7 levels. Given that the cell cycle arrest elicited by UPK 1A at the G(1)-S checkpoint was associated with downregulation of cyclin D1, whereas metastasis suppression was associated with reduction of MMP7

[14], we speculated that serum UPK 1A, cyclin D1 and MMP7 levels might be closely related with those in solid tumor. The specific molecular mechanism to explain this observation needs to be researched further. In addition, it is necessary to further investigate how UPK 1A, as a transmembrane protein, is secreted into the serum and its role in diagnosis of NSCLC.

The outlined study has several limitations. The results should be confirmed in a larger population of NSCLC patients. In addition, the sensitivity and specificity of serum UPK 1A should also be the subject of future research. The existence of a direct or indirect relationship between serum UPK 1A, cyclin D1 and MMP7 also needs to be studied in the future.

Serum levels of UPK-1A, cyclin D1, and MMP7 are useful biomarkers for diagnosing the stage and differentiation degree of NSCLC.

## Disclosure of conflict of interest

None.

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