Original Article

Prognostic Significance of Tissue Inhibitor of Matrix Metalloproteinase-2 [TIMP-2] Expression in Non-Small Cell Carcinoma of Lung

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Background: Non-small cell lung cancer [NSCLC] has a poor prognosis. Therefore, knowing the factors to predict the prognosis is necessary. Tissue inhibitors of matrix metalloproteinases [TIMPs] are the enzymes that reduce the destruction of the extracellular matrix, which in turn reduces the stromal invasion by a tumor and may cause a better prognosis in lung cancer. TIMP-2 is the major type in the TIMP family that inhibits stromal destruction. The p27 is a tumor-suppressor gene theoretically controlled by the TIMP-2 gene.

Objective: To evaluate the expression of both TMP-2 and p27 as prognostic factors.

Materials and Methods: The present study used immunohistochemical staining to detect the expression of TIMP-2 and p27 in 106 and 91 cases of NSCLC, respectively. The survival analysis of each group was calculated by the Kaplan-Meier curve, log rank test, and Cox's proportional hazard regression model.

Results: The results showed no correlation between the expression of TIMP-2 and p27 (p = 0.621). The log rank test showed no difference between the survival period of TIMP-2 positive and the negative cases (p = 0.17). However, multivariate analysis demonstrated that TIMP-2 positive was an independent prognostic factor. Furthermore, TIMP-2 expression indicated good prognosis in patients who received chemotherapy (p = 0.0079). The expression of p27 immunostaining did not correlate with the survival period (p = 0.30).

Conclusion: The expression of TIMP-2 is an independent prognostic factor to predict the prognosis in NSCLC patients who receive chemotherapy. The expression of p27 does not correlate with the prognosis.

Keywords: Non-small cell lung cancer, Tissue inhibitors of matrix metalloproteinases [TIMPs], p27, Prognosis

J Med Assoc Thai 2018; 101 (10): 1311-7
Website: http://www.jmatonline.com

Lung cancer is the most common cause of cancer death worldwide and is the second common cancer of males in Thailand[1,2]. Lung cancer has two major types, small cell lung cancer and non-small cell lung cancer [NSCLC]. The most common subtype of NSCLC in Thailand is adenocarcinoma[2]. Unfortunately, about 45% of all lung cancer patients come to the hospital in the late stage. The aim of treatment in the late state is to prolong life by using chemotherapy, radiation, and epidermal growth factor receptor-targeted therapy[3]. The medial survival time after treatment of late state is 10 months.

Matrix metalloproteinases [MMPs] are a group of zinc ion dependent proteolytic enzymes that degrade extracellular matrix. MMPs are produced by many normal cells such as fibroblasts, macrophages, and neutrophils. This enzyme is also secreted by tumor cells that promote the invasion into the surrounding stromal matrix. MMPs are controlled by the inhibition enzymes, which are called tissue inhibitors of matrix metalloproteinases [TIMPs][4].

The TIMP family has four members (TIMP-1 to TIMP-4). TIMP-2 is a protein that is synthesized by a gene on chromosome 17q25. The main function of this protein is inhibition of MMPs. The TIMP-2 also activates α3β1 integrin receptor to produce p27 protein that stops the cell cycle in the G1 phase. Therefore, TIMP-2 may prevent invasion by a tumor[5,6]. Many studies demonstrated that the over-expression of TIMP-2 was associated with the prognosis in many

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How to cite this article: Kanjanapradit K, Thongsuksai P, Phukaoloun M, Geater SL. Prognostic significance of tissue inhibitor of matrix metalloproteinase-2 [TIMP-2] expression in non-small cell carcinoma of lung. J Med Assoc Thai 2018;101:1311-7.

cancers; however, some studies showed no correlation[7-14]. Therefore, the prognostic significance of TIMP-2 expression in human cancer is still controversial.

P27kip1 is a member of the KIP family that binds and inhibits cyclin D-CDKs in the cell cycle. The increased expression of p27 decreases cellular proliferation and inhibits tumor cell growth[15,16]. Many research studies showed a correlation between the decreased expression of p27 and the aggressive behavior of many cancer. It related to the poor prognosis in NSCLC patients. However, some articles did not show a definite correlation[17-25].

The objective of the present study was to determine the relationship between the prognosis of NSCC and expression of TIMP-2 and p27. Correlation between expression of TIMP-2 and p27 was also evaluated.

Materials and Methods

Study population

The present study was retrospectively collected data from NSCLC patients treated in Songklanagarind Hospital between January 2006 and December 2007. All patients were diagnosed by tissue biopsy and had paraffin-embedded tissue block. All patients received complete treatment and had complete medical records. One hundred six patients met the criteria. The study protocol was approved by the Human Ethics Research Committee of the Faculty.

Immunohistochemistry

TIMP-2 and p27 immunohistochemistry staining were performed in paraffin-embedded tumor block. The staining procedure was done by an automatic immunostaining machine (Leica BOND-MAX, Leica Biosystems, Newcastle, UK). First, one block of formalin-fixed paraffin-embedded tissue was sectioned in two-micrometer thicknesses onto a glass slide. Second, the glass slide was baked in a microwave oven for about 10 minutes and then baked in a hot air oven (60°C) for about 15 minutes. Third, the slide was inserted into the automatic immunostaining machine to perform deparaffinization, antigen retrieval, and immunostaining with TIMP-2 antibody (clone sc-21735, diluted 1:200, Santa Cruz Biotechnology, Inc Dallas, TX, USA), and p27 (clone sc-528, diluted 1:100, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Then the slide was stained with diaminobenzidine tetrachloride as a chromogen to develop the peroxidase reaction. Finally, the slide was counter stained with Mayer hematoxylin.

TIMP-2 immunostaining was initially performed and then p27 was performed later. Unfortunately, some cases had insufficient tissue and only 91 cases were available for p27 staining. TIMP-2 positive cases showed cytoplasmic staining in the tumor cells and p27 showed nuclear staining. The case was scored as positive when 10% or more of tumor cell showed positive staining (Figure 1).

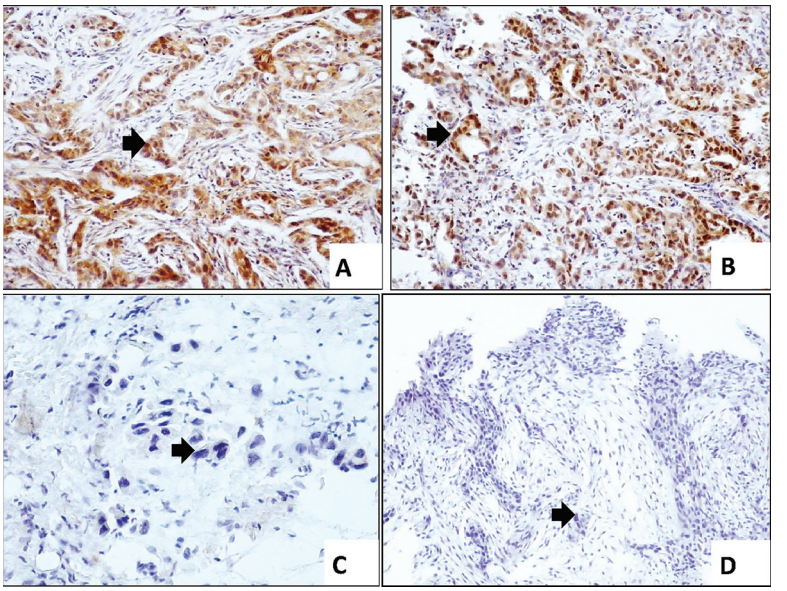

Figure 1. The immunostaing of TIMP-2 shows positive staining in the cytoplasm of the tumor cells (arrow) (A). The immunstaining of p27 is positive in the nucleaus of tumor cells (arrow) (B). The negative immunstaining of TIMP-2 and p27 are demonstrated in C and D (arrow), respectively.

Statistical analysis

The associations between the expressions of TIMP-2 and p27 with the clinical parameters (age, sex, staging, chemotherapy, and surgery) were tested using the Chi-square test, t-test, and Fisher's exact test. The Kaplan-Meier curve was used to identify the survival of patients with TIMP-2 and p27 expression. The log-rank test and Cox's proportional hazard regression model were used to reveal the prognostic significance of TIMP-2 and p27 expression. All statistical analyses were performed using Stata software version 13.

Results

In 106 cases of TIMP-2 immunostaining, the results showed 82 positive cases and 24 negative cases. In 91 cases of p27 immunostaining, the result showed 84 positive cases and 7 negative cases. The statistical analysis showed no correlation between expression of TIMP-2 and p27 ($p = 0.621$).

The clinical data of patients regarding TIMP-2 expression are shown in Table 1. The mean ages of the patients with TIMP-2 positive and negative were 64.46 and 64.85 years, respectively. The results revealed no statistically significant association between the expression of TIMP-2 and the age of the patients ($p = 0.13$), sex ($p = 0.17$), performance status of the patients ($p = 0.31$), radiation therapy ($p = 0.57$), or surgical status ($p = 0.41$). Most patients were in tumor stage 4 (64%) and there was no association between tumor stage and TIMP-2 expression ($p = 0.71$).

The only parameter that had marginally associated with TIMP-2 expression was chemotherapy treatment ($p = 0.09$). The results showed 46 cases (45%) received chemotherapy and 32 cases (31%) had TIMP-2 positive. In patients who received chemotherapy, 24 cases received mitomycin C with vinblastine and cisplatin, 17 cases received paclitaxel with carboplatin, four cases received gefinib, and only one case received cisplatin with etoposide.

The demographic data of the patients with a statistical analysis of the correlations of p27 expression are shown in Table 2. In the p27 immunostaining group, the mean ages of the patients with p27 positive and negative were 60.48 and 64.73 years, respectively. The results revealed no statistical significance between

Table 1. Demographic data of patients with the results of TIMP-2 immunostaining and correlation between clinical data and results of TIMP-2 immunostaining

Data	TIMP-2 positive case	TIMP-2 negative case	*p*-value
Age (year), mean ± SD	64.46±13.2	64.85±12.6	0.13*
Sex, n (%)			0.17
Male	58 (54.7)	18 (17.0)	
Female	24 (22.6)	6 (5.7)	
Stage, n (%)			0.71#
I-II	3 (2.8)	0 (0.0)	
III	22 (20.9)	9 (8.5)	
IV	54 (50.9)	14 (13.2)	
Unknown	3 (2.8)	1 (0.9)	
Performance status, n (%)			0.31#
0	5 (4.7)	2 (1.9)	
1	20 (18.9)	11 (10.4)	
2	27 (25.5)	4 (3.8)	
3	19 (17.9)	3 (2.8)	
4	3 (2.8)	1 (0.9)	
Unknown	8 (7.6)	3 (2.8)	
Chemotherapy, n (%)			0.09
Yes	32 (30.2)	14 (13.2)	
No	50 (47.2)	10 (9.4)	
Radiation, n (%)			0.57
Yes	29 (27.4)	10 (9.4)	
No	53 (50.0)	14 (13.2)	
Surgery, n (%)			0.41+
Yes	1 (0.9)	1 (0.9)	
No	80 (76.2)	23 (22.0)	

TIMP = tissue inhibitors of matrix metalloproteinase

* t-test, # Logistic regression test, + Fisher's exact test

Table 2. Demographic data of patients with the results of p27 immunostaining and correlation between clinical data and results of p27 immunostaining

Data	p27 positive	p27 negative	*p*-value
Age (year), mean ± SD	60.48±13.1	64.73±11.3	0.83*
Sex, n (%)			1.00
Male	61 (67.0)	5 (5.6)	
Female	23 (25.2)	2 (2.2)	
Stage, n (%)			0.68#
I-II	3 (3.3)	0 (0.0)	
III	22 (24.2)	3 (3.3)	
IV	55 (60.4)	4 (4.4)	
Unknown	4 (4.4)	0 (0.0)	
Performance status, n (%)			0.72#
0	4 (4.4)	1 (1.1)	
1	25 (27.4)	1 (1.1)	
2	24 (26.4)	2 (2.2)	
3	19 (20.9)	1 (1.1)	
4	3 (3.3)	1 (1.1)	
Unknown	9 (9.9)	1 (1.1)	
Chemotherapy, n (%)			1.00
Yes	33 (36.3)	3 (3.3)	
No	51 (56.0)	4 (4.4)	
Radiation, n (%)			0.10
Yes	29 (31.9)	5 (5.5)	
No	55 (60.4)	2 (2.2)	
Surgery, n (%)			1.00+
Yes	1 (1.1)	0 (0.0)	
No	82 (91.1)	7 (7.8)	

* t-test, # Logistic regression test, + Fisher's exact test

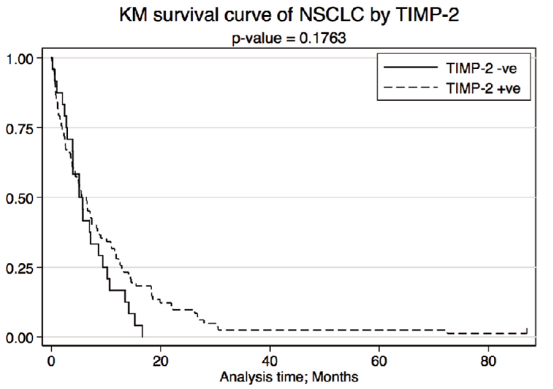

Figure 2. Kaplan-Meier survival curves of patients classified by TIMP-2 expression status.

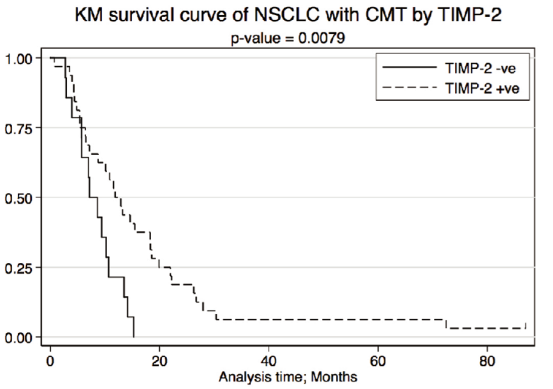

Figure 3. Kaplan-Meier survival curves of the patients who received chemotherapy according to TIMP-2 expression status.

the expression of p27 with age of the patients (p = 0.83), sex (p = 1.00), performance status (p = 0.72), chemotherapy status (p = 1.00), radiation therapy (p = 0.10), and surgical status (p = 1.00). Most patients were in tumor stage 4 (64%) and showed no statistical significance between staging of the patients and p27 immunostaining (p = 0.68).

Survival analysis

Kaplan-Meier survival curve with log-rank test analysis showed no statistical significance between the survival period of TIMP-2 positive and TIMP-2 negative cases (p = 0.17) (Figure 2).

Univariate and multivariate analysis results are shown in Table 3. The results demonstrated that the performance status, chemotherapy, and TIMP-2 positive expression were independent prognostic factors in NSCLC.

Table 3. Univariate and multivariate analyses of TIMP-2 status and other risk factors

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio	95% CI	Hazard ratio	95% CI
Age (year)	1.01	0.99 to 1.02	0.99	0.97 to 1.00
Tumor stage				
Local stage (I-III)	1		1	
Advance stage (IV)	1.14	0.76 to 1.71	1.13	0.74 to 1.71
Performance status	2.51	1.57 to 4.04	2.70	1.67 to 4.37
Radiation				
No	1		1	
Yes	0.73	0.49 to 1.09	0.81	0.54 to 1.21
Chemotherapy				
No	1		1	
Yes	0.40	0.27 to 0.60	0.60	0.25 to 0.57
TIMP-2				
Negative	1		1	
Positive	0.72	0.45 to 1.15	0.38	0.37 to 0.97

TIMP-2 = tissue inhibitor of matrix metalloproteinase-2

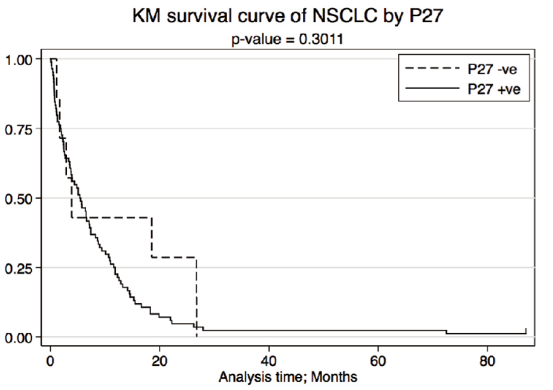

Figure 4. Kaplan-Meier survival curve of the patients who received chemotherapy according to p27 expression status.

The survival analysis by Kaplan-Meier curve and log-rank test in patients who received chemotherapy showed statistical significance between the survival of TIMP-2 positive and negative patients (p = 0.0079) (Figure 3). The patients having TIMP-2 positive had longer survival times compared to the TIMP-2 negative patients.

Kaplan-Meier survival curve and log rank test analysis showed no statistical significance between survival period of p27 positive and negative cases (p = 0.30) (Figure 4).

Discussion

The invasion by tumor cell has multiple pathways. The tumor cell can induce angiogenesis and express many proteins for degradation of the extracellular matrix. MMP is an important protein that destroys the interstitial stroma and extracellular matrix to promote

tumor invasion. MMP is synthesized by tumor cells and surrounding stromal cells. This protein is controlled by the expression of TIMP, which reduces the destruction of peritumoral stroma[4,5]. TIMP-2 is a member of TIMP gene family. Its major function is to inhibit MMP and stimulate the expression of p27 to inhibit the cell cycle[6]. Therefore, many studies suggested that over-expression of TIMP-2 may reduce the activity of MMP and the metastasis of the tumor, leading to a good prognosis[11,12].

In the present study, about 77% of the cases showed positive staining of TIMP-2. The statistical analysis showed no correlation between the expression of TIMP-2 and the clinical data (sex, age, stage, performance status, radiation, and surgery). Interestingly, the survival analysis shows a statistical significance of TIMP-2 expression in the patients who received chemotherapy.

In patients who received chemotherapy, most patients (89%) received combined drugs of ankylating agents (cisplatin and carboplatin) and mitotic inhibition drugs (vincristine and palitaxel). Both groups of chemotherapy inhibit the cell cycle and promote cell death. The over-expression of TIMP-2 affects cell cycle inhibition by activating α3β1 integrin receptor to produce p27 protein that stops the cell cycle in the G1 phase[6]. Therefore, the over-expression of TIMP-2 promotes the action of chemotherapeutic drug, inhibits tumor cell growth, and brings about a better prognosis. The results of the present study suggested that the expression of TIMP-2 in tumor cells could be used as an independent prognostic factor in NSCLC patients who receive chemotherapy.

However, the survival analysis showed no correlation between TIMP-2 expression and the overall survival of all patients. This finding is similar to the previous study by Eren et al[14]. The over-expression of TIMP-2 may reduce the local destruction of tumor cells in the surrounding stroma. However, the activity of TIMP-2 is not enough to prevent tumor metastasis and prolong the survival of the patients.

The results of p27 immunostaining showed positive staining in most cases (92%). No correlation was demonstrated between p27 expression and the clinical data (sex, age, stage, performance status, radiation, chemotherapy, and surgery). The results showed no statistical significance between expression of p27 and the survival time of the patients. In theory, cell injury and DNA damage increase the expression of p27 to reduce the growth of the cells by activating cyclinD-CDKs to stop the cell cycle. However, the result of over-expression of p27 in the present study indicates that the tumor cell has abnormal DNA and the cell try to stop the proliferation. This suggested that the expression of p27in tumor cell could not be used for independent prognostic factor in NSCLC patient. The result also showed no correlation between expression of TIMP-2 and p27. This finding suggested that the expression of p27 was not fully controlled by TIMP-2 expression.

Further study in patient who receive chemotherapeutic drug to identify the correlation between TIMP-2 expression and prognosis may be useful. If the result showed strong correlation, it will have benefit for using the TIMP-2 immunostaining for predicting the prognosis in patient who received chemotherapy.

Conclusion

The result of the present study shows no correlation between expression of TIMP-2 and p27 immunostaining in NSCLC. The expression of TIMP-2 is the independent prognostic factor that indicates the better prognosis in patient who receives chemotherapy. The expression of p27 immunostaining does not correlate with prognosis of NSCLC.

What is already known on this topic?

Previous research reveals controversy of the prognostic significance of TIMP-2 in NSCLC.

What this study adds?

The result of the present study shows the prognostic significance of TIMP-2 expression in NSCLC patients who receive chemotherapy.

Funding

The funding of this research is supported by Faculty of Medicine Prince of Songkla University.

Potential conflicts of interest

The authors declare no conflict of interest.

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