Original Article
Thymidine kinase 1 is a better prognostic marker than Ki-67 for pT1 adenocarcinoma of the lung

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Abstract: Objectives: The sensitivity and reliability of the biomarkers thymidine kinase 1 (TK1) and Ki-67 were studied in relation to clinical features and prognosis of survival for pathological-T1 (pT1) lung adenocarcinoma patients. Methods: TK1 and Ki-67 expression was determined in 80 patients with pT1 adenocarcinoma of the lung and in 20 specimens from normal lung tissues, using immunohistochemistry. Results: TK1 was found in most lung tumor cells both in the cytoplasm and the nuclei. The positive labelling index (LI) for total TK1 was significantly higher than that for Ki-67. There was a significant correlation between the LI of total TK1 and lymph node metastasis, degree of tumor invasion and pathologic stages, which was not found for Ki-67. In addition, the overall 5-year survival of patients was statistically significant different between low and high levels of TK1 expression, but not in cases of Ki-67. A multivariate analysis revealed that expression of TK1, lymph node involvement and TNM pathology staging could serve as independent prognostic factors for the disease progression of pT1 lung adenocarcinoma patients. Conclusions: Compared with Ki-67, TK1 is a more reliable proliferation index in pT1 adenocarcinoma of lung, which can evaluate the invasion and the prognosis of tumor.

Keywords: Lung neoplasms, adenocarcinoma, pT1, thymidine kinase 1, TK1, Ki-67, survival

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death globally. In males, the highest lung cancer incidence rates are found in Eastern and Southern Europe, North America, Micronesia and Polynesia, and Eastern Asia. Among females, it is the fourth most commonly diagnosed cancer and the second leading cause of cancer death. Lung cancer is increasing in countries in Asia, for example in China, and in Africa [1]. In recent years, tomography scanning and imaging technology in combination with pathological TNM staging improved the survival of lung carcinoma patients. The pathologic T stage appeared to have significant impact on predicting patient survival. Patients with pT1-2 type of lung tumor behaved statistically significantly better (*P* = 0.007) compared to patients with pT3-4 tumor [2]. Microscopic vascular invasion (MVI) is a

stronger prognostic indicator than T size in T1a-T2b categories. pT1 lung carcinoma tumor is defined as ≤ 3.0 cm in the greatest dimension. Despite complete resection, the prognosis of patients with pT1 lung adenocarcinoma is not consistent. In some cases, the 5-years survival is 100%, while other patients show short-term recurrence and metastasis, and thus poor survival [3]. In a cohort of 20,461 patients with lung cancer, 3,152 patients were identified as non-small lung cancer (NSCLC). The mortality hazard ratios of these patients were calculated during three consecutive time periods following surgery (0-1 month, 1 month-1 year and > 1 year), according to Charlson comorbidity score (CCS 0, 1, 2, 3+). 5-year survival in the NSCLC patients with severe comorbidity was significantly lower (38%, TP1+ CCS 3+) than in patients without comorbid disease (69%, TP1+ CCS 0). Thus, the pT1 classification should be combined with the CCS comorbidity score in

Table 1. Clinical and pathological data

Type	Number of patients
Total number	80
Mean age	59.3, 28-81
Sex	
Men	41
Female	39
Smoker	22
Non-smoker	58
Lung lobectomy	70
Lobes wedge or segment resection	10
Tumor diameter	0.5-3 cm, mean 2.3 cm
Histological types:	
Adenocarcinoma in situ	7
Micro-invasive adenocarcinoma	10
Invasive adenocarcinoma	63
Acinar predominant	34
Lepidic predominant	11
Papillary predominant	9
Micropapillary predominant	2
Solid predominant with mucin production	6
Invasive mucinous adenocarcinoma	1
Lymphatic/vascular invasion	17
Lymph node metastasis	18
Pathological stage:	
IA	60
IIA	15
IIIA	3
IV	2
Proliferation biomarker (LI > 5%)	
TK1	73
TK1 cyto/nuclei	27
TK1 in cyto only + cyto/nuclei	46
Ki-67	40

order to identify the patients which are most likely to benefit from surgery and adjuvant therapy [4]. Furthermore, it is also important to evaluate the biological characteristics of pT1 lung tumor and to identify factors related to lymph node metastasis, microscopic vascular invasion and stromal invasion, as well as the invasion of the inner and outer visceral pleura to improve the assessment of prognosis. A multivariate analysis showed that lymph node metastasis is a significant predictor of poor prognosis [5].

Tumor proliferation biomarkers have also been used for determining of the prognosis of lung cancer patients. Proliferation markers reflect the growth rates of a tumor, specifically in relation to un-control regulation of the cell cycle, which is a hallmark of cancer [6]. However, only Ki-67 and TK1 have been shown to be of independent prognostic values.

In this study we compared the usefulness of TK1 and Ki-67 in relation to pathological characteristics and over-all 5-years survival of pT1 lung carcinoma patients. TK1 was found to be an independent prognostic factor and more reliable than Ki-67. Although TK1 is classically known as a cytosolic enzyme, several recent studies have demonstrated existence of a nuclear form of TK1 [7, 8] and determinations of both nuclear and cytosolic TK1 in tumor cells were done in this study.

Patients and methods

Patients

The clinical and pathological information about the patients data are summarized in **Table 1**. pT1 lung adenocarcinoma patients (n = 90) were recruited for this study during 1997~2007 at Jinling Hospital, Nanjing University School of Medicine, China. These patients underwent surgery, and complete clinical and pathological characterization and follow-up results are available. The patients were classified according to the pTNM classification of the International Union Against Cancer (UICC) [9], i.e. having a tumor ≤ 3.0 cm in the greatest dimension, being surrounded by lung tissues or visceral pleura, but not involving the main bronchus. Some of these patients (n = 10) who had a medical history of pre-operative radiotherapy and chemotherapy was excluded from this study. The final number of patients investigated in this present study was 80 cases. Specimens from normal lung tissues from 20 patients with trauma (14 male and 6 female; median age, 55 years; range 22-74) served as non-malignant controls.
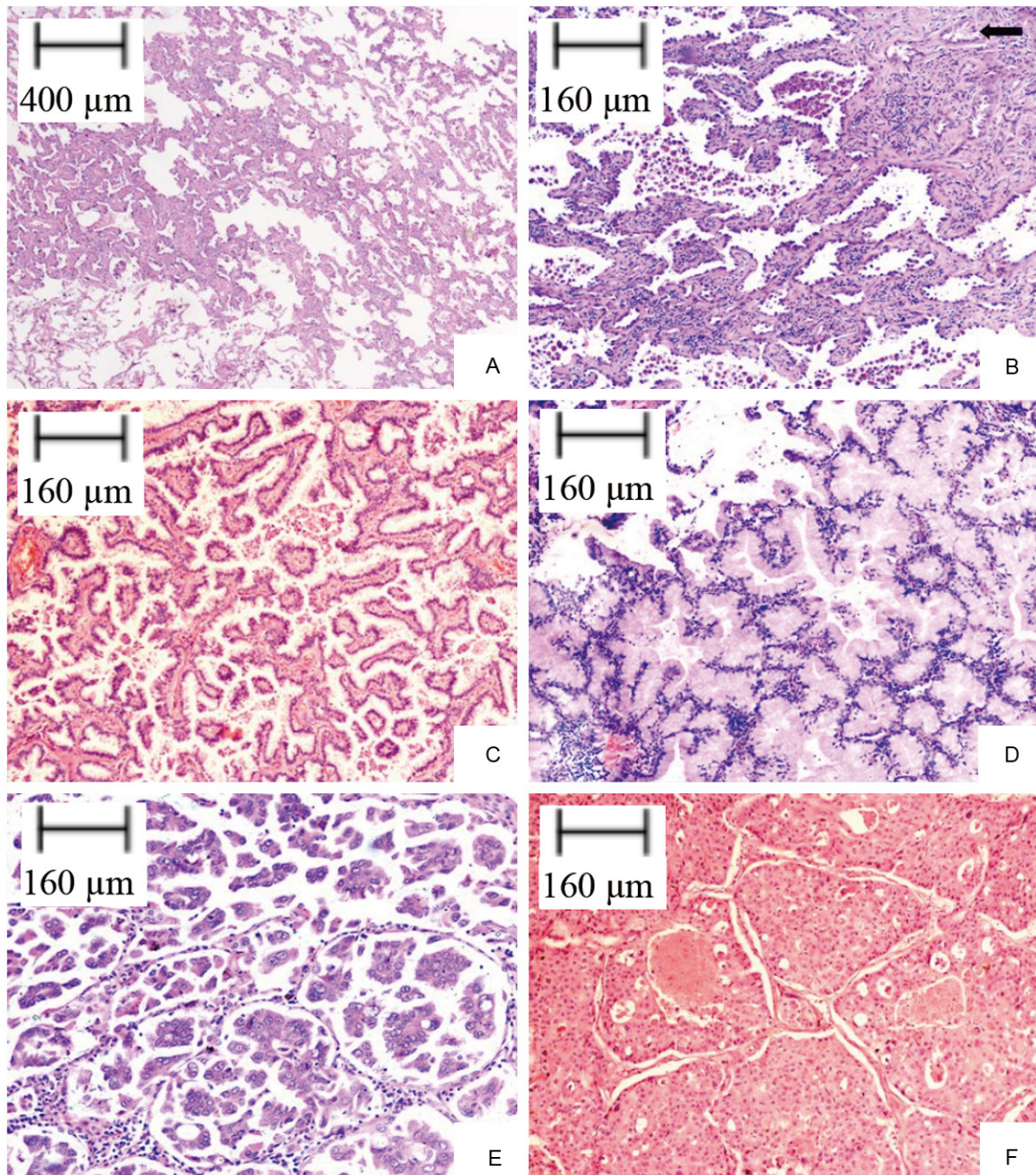
Figure 1. Classification of the lung adenocarcinoma according to the new classification. A. Adenocarcinoma in situ: this tumor grows purely with a lepidic pattern. No foci of invasion or scarring are seen (× 40). B. Micro-invasive adenocarcinoma: This tumor consists mostly of lepidic growth, but there is a scar with small foci of invasion (arrow) at the edge of the scar (× 100). C-F. Invasive adenocarcinoma: C. Papillary predominante (× 100). D. Invasive mucinous adenocarcinoma (× 100). E. Micro-papillary predominant (× 100). F. Solid predominant with mucin production (x 100). All sections were hematoxylin and eosine counterstained.

Histological and pathological assessment

Surgical specimens were fixed in 4% neutral buffered formaldehyde solution. Depending on the size of the tumor, they were embedded in three to six blocks of paraffin from which four-micrometer-thick slices were cut and stained with hematoxylin-eosine (HE), followed by light microscope pathology assessment. The histological type were classified according to the International Society for the Study of Lung Cancer (IASLC), the American Thoracic Society
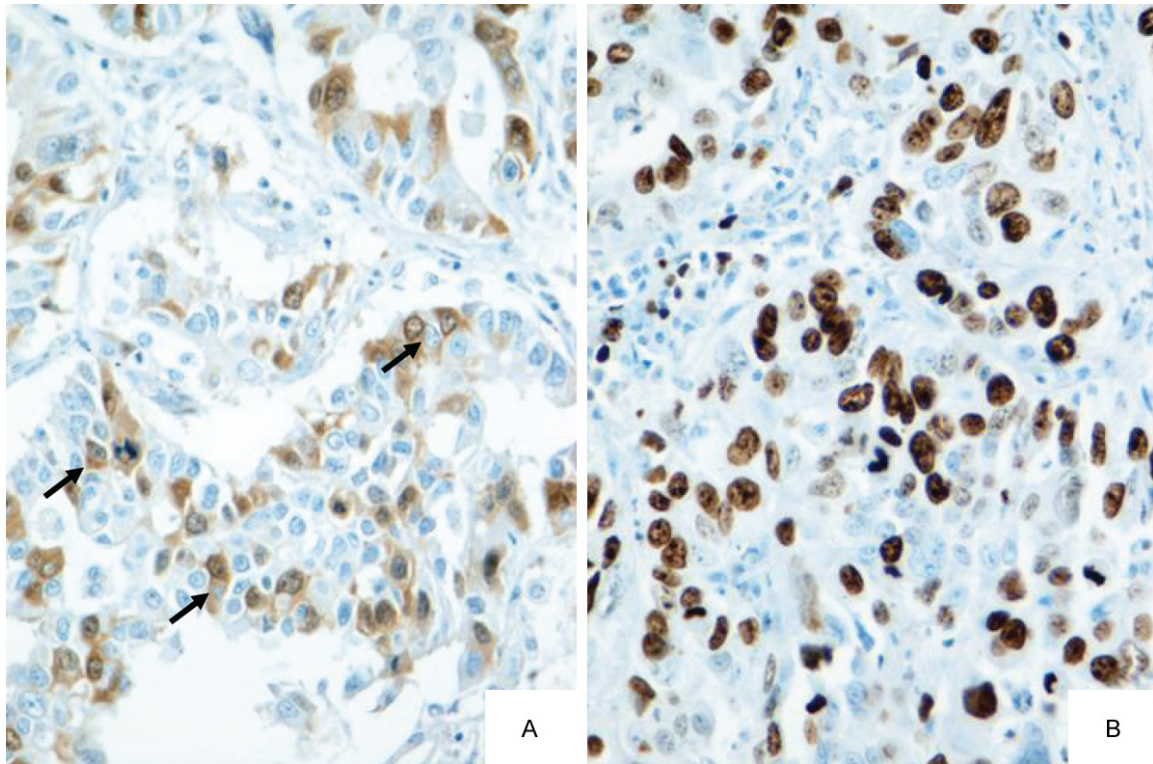
Figure 2. A. TK1 is simultaneously expressed in cytoplasm and nucleus of the tumor cells, but is also expressed only in the cytoplasm of some tumor cells (black arrows). (× 200, EnVision). B. Ki-67 is expressed in the nuclei of the tumor cells. (× 400, EnVision).

(ATS) and the European Respiratory Society (ERS) lung cancer multidisciplinary classification scheme published in 2011 (referred to as the "new classification") [10, 11]. Based on the degree of invasion, the tumors were classified as adenocarcinoma in situ (AIS), micro-invasive adenocarcinoma (also called minimally invasion adenocarcinoma, MIA) and invasive adenocarcinoma (**Figure 1**). The histopathological characteristics are shown in **Table 1**.

Follow-up

The follow-up information was obtained from the medical records of the clinic, by telephone or written contacts with the patients. The follow-up time was from the date of surgery to death or last follow-up. The follow-up investigation was closed 15th of July, 2012.

Immunohistochemistry

TK1 and Ki-67 immunohistochemistry were performed on 4 μm thick slices of paraffin blocks. The slices were incubated with antibodies against TK1 and Ki-67 using the EnVision immunohistochemical procedure (DAKO, Denmark). The TK1 Ab was a gift from SSTK Ltd., (Shenzhen, China), Ki-67 Ab was purchased from DAKO, Denmark. TK1 expression was found in both cytoplasm and nuclei (**Figure 2A**). TK1 expression in some tumor cells was unique in the cytoplasm (**Figure 2A**). The percentage of these cells varied from about 1% to 10%. For the purpose of this study tumor cells expressing TK1 simultaneously in the cytoplasm and in the nuclei as well as cells where TK1 was found only in the cytoplasm were combined and denoted as "total TK1". The tumor cells expressing TK1 in cytoplasma and nuclei simultaneously were denoted "cytoplasma/nuclei TK1". Ki-67 was exclusively expressed in the nuclei of the tumor cells (**Figure 2B**).

The numbers of antibody stained tumor and normal cells were counted among 100 cells at × 400 magnification and classified into four groups depending on the number of antibody stained cells: ≤ 5% "-"; 6-25% "+"; 26-50% "++"; ≥ 50% "+++". The percentages of labeled TK1 and Ki-67 cells were denoted as labeling index (LI). LI index above 5% was denoted as "positive LI".

Table 2. Expression of TK1 and Ki-67 in relation to clinicopathological features in pT1lung adenocarcinoma. The statistical analysis was done by the non-parametric Wilcoxon rank-sum test

Parameters	n	TK1				P	Ki-67				P
		-	+	++	+++		-	+	++	+++	
Sex						0.431					0.192
M	41	5	13	18	5		16	16	6	3	
F	39	7	14	13	5		21	16	1	1	
Age						0.908					0.608
≤ 60 years	38	7	10	18	3		19	14	3	2	
> 60 years	42	5	17	13	7		18	18	4	2	
Smoke						0.115					0.348
yes	22	2	5	12	3		7	8	5	2	
no	58	10	22	19	7		30	24	2	2	
Tumor size						0.133					0.709
≤ 2 cm	39	7	16	13	3		18	17	2	2	
> 2 cm	41	5	11	18	7		19	15	5	2	
Lymphatic/vascular invasion						0.061					0.733
yes	17	0	4	12	1		10	7	0	0	
no	63	12	23	19	9		27	25	7	4	
Lymph node metastasis						0.009					0.577
yes	18	0	3	13	2		11	7	0	0	
no	62	12	24	18	8		26	25	7	4	
Histo. New classification						< 0.001					0.341
AIS + MIA	17	9	8	0	0		10	5	0	2	
Invasive adenocarcinoma	63	3	19	31	10		27	27	7	2	
Path. Stage						0.004					0.451
IA	60	12	24	16	8		26	23	7	4	
> 1A	20	0	3	15	2		11	9	0	0	

Statistical analysis

The SPSS17.0 analysis software was used for the statistical calculations, including Chi-square test for comparison of the rate between the two groups, the non-parametric Wilcoxon rank-sum test for comparison of the level of TK1 and Ki-67 expression between the two groups, Kaplan-Meier method and log-rank test when comparing survival rate, COX proportional hazard for multivariate analysis. A *P*-value less than 0.05 was regarded as statistically significance.

Results

The positive LI of total TK1 and Ki-67

The positive LI of total TK1 and Ki-67 was 91.3% (73/80) and 50.0% (40/80), respectively, which was a statistically significant difference (*P* = 0.011). There were no significant differences between men and women.

TK1 and Ki-67 LIs in relation to clinicopathological features

Of the clinicopathological features included, a statistically significant higher total TK1 LI_was found in patients with lymph node metastasis (*P* = 0.009), in patients with invasive adenocarcinoma compared to AIS and MIA (*P* < 0.001) and in patients with stage above IA compared to stage IA (*P* = 0.004) (**Table 2**), which was not found for Ki-67. There was no statistically significant difference in both total TK1 LI and Ki-67 LI according to gender, age, smoking history or vascular invasion (**Table 2**).

Survival analysis

The 5-year overall survival (OS) of AIS, MIA and invasive adenocarcinoma were 100%, 90% and 53.7%, respectively (**Figure 3A**). Log rank analysis showed a statistically significant difference of OS between MIA and invasive adenocarcinoma (*P* = 0.045). Since no death was found
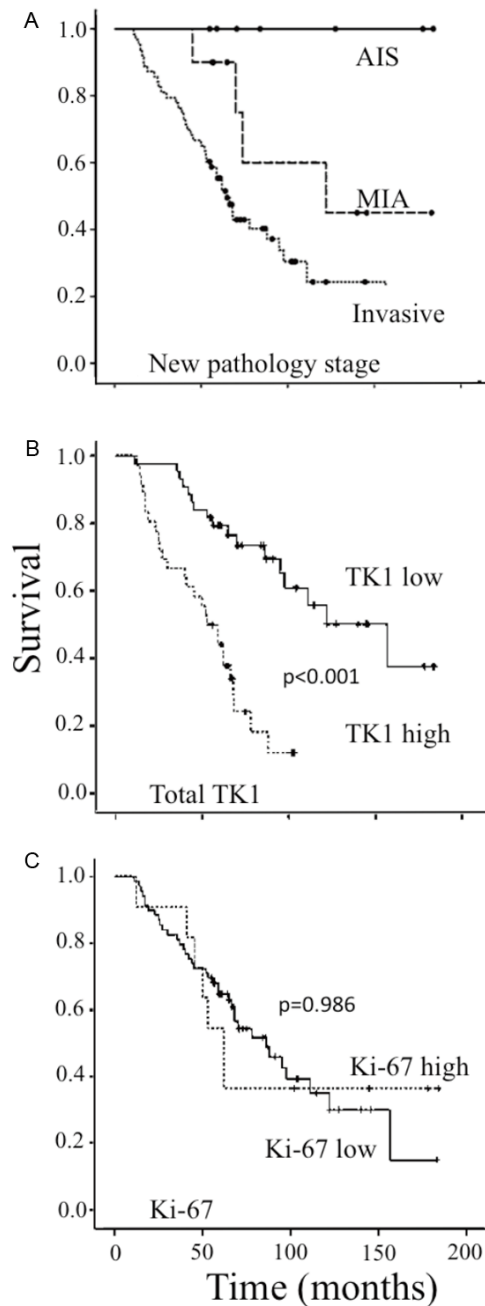
Figure 3. Over-all 5-year survival in relation to: (A) the new pathological stages, (B) total TK1, (C) and Ki-67.

among the AIS patients, no log rank analysis could be done. The mortality of AIS, MIA and invasive adenocarcinoma were 0%, 40% and 65.1% during follow-up, respectively. There was a statistically significant difference of mortality between the AIS and invasive adenocarcinoma group (P = 0.001), while no statistically difference was found between the AIS and MIA group. The patient prognosis of AIS and MIA is

similar, whereas better than that of invasive adenocarcinoma.

There was a significant difference in the 5-year over-all survival between pathology stage IA and above IA (P < 0.001), with and without lymph node metastasis (P < 0.001) and with and without vascular invasion (P = 0.001) (data not shown).

LI of TK1 and Ki-67 in relation to 5-year over-all survival

Patients were divided into two groups regarding LI, i.e. below 25% and above 25%, denoted as low and high LI, respectively.

Mortality

The mortalities during the 5-year follow-up of patients with low and high TK1 expression were 35.9% and 73.2%, respectively, which was significantly different (P = 0.003). The corresponding values for low and high Ki-67 were 53.6% and 63.6%, respectively, which was also significantly different (P < 0.001). The differences in mortality levels between TK1 and Ki-67 concerning low (35.9% versus 53.6%) and high (73.2% versus 63.6%) LI groups were also significantly different (P < 0.001). Thus, TK1 is a more sensitive marker for mortality compare to Ki-67.

Survival

The 5-year survival rates for low and high TK1 LI was 83.5% and 38.8%, respectively. There was a significant difference between patients with low and high TK1 LI regarding the 5-year survival rates, both in the total TK1 group and in the cytoplasma/nuclei TK1 group of patients (total TK1 χ^2 = 20.47, P < 0.001, **Figure 3B**; cytoplasm/nuclei χ^2 = 22.05, P < 0.001, data not shown). The corresponding values for low and high Ki-67 LI were 65% and 55%, i.e. no significant difference in the survival was found between low and high Ki-67 LI (**Figure 3C**).

Multivariate COX analyses

A COX multivariate analysis was performed with total TK1 LI, invasiveness based on the new classification, pathology stages, lymph node metastasis and lymphatic/vascular invasion. Of these factors, only total TK1 LI, lymph node

Table 3. COX multi-variate analysis

Variables	p-values	Hazard risk	95% CV
Total TK1	0.016	1.637	1.097-2.443
Pathology stage	< 0.001	68.560	10.625-442.376
Lymphnode metastasis	0.001	23.201	3.512-153.246
lymphatic/vascular invasion	0.136	-	-
New pathology stage	0.084	-	-

metastasis and pathology stage showed significance. Thus, these three parameters are independent prognostic factors for overall survival (**Table 3**).

Discussion

Thymidine kinase (EC 2.7.1.21) (TK) is a key enzyme in the pyrimidine salvage pathway, catalysing transfer of the terminal phosphate from ATP to 5'-hydroxyl group of thymidine (dTdR), producing dTMP, which is subsequently incorporated into DNA. There are two forms cellular TK enzymes, one cytoplasmic form, thymidine kinase 1 (TK1), and one mitochondrial form, thymidine kinase 2 (TK2). TK2 is cell-cycle independent, while TK1 protein level is tightly correlated to S-G2 phases of the cell cycle and thus to overall cell proliferation. TK1 plays a role in regulating the intracellular thymidine pools throughout the cell cycle, but is probably also involved DNA repair process [12]. In proliferating normal cells and tumor cells, the synthesis of TK1 starts at the latter stage of the G1 phase and significantly increases until the late S-G2 phases [7]. TK1 in non-proliferating cells and in serum of healthy persons is minimal or undetectable [7]. TK1 also provides information on recurrence, on 5-years survival of cancer patients and for monitoring the outcome of tumor therapy in lung, rectal, breast and cervical cancer patients [7, 12]. Thus, elevated TK1 is an important risk factor indicating a high proliferation capacity of tumors, leading to shorter survival.

Ki-67 is a frequently used proliferation biomarker expressed in all stages of the cell cycle, except in the G0 phase [13, 14]. Several studies show that Ki-67, but not PCNA, is a good prognostic factor for recurrence in patients with NSCLC [15]. A multivariate analysis showed that EGFR and Ki-67 were independent negative prognostic factors for survival of squamous cell lung cancer patients [15], while lymph-node metastasis and Ki-67 were independent prog-

nostic parameters for overall survival in patients with NSCLC [16, 17]. Multivariate COX regression analysis also showed that the Mini-chromosome maintenance factor (MCM7) is an independent prognostic marker, but not MCM2 or Ki-67, in pT1 lung adenocarcinoma patients [18].

TK1 was recently shown to increase earlier during the G1 to S transition of the cell cycle than Ki-67 [6], suggesting that TK1 is a very efficient proliferating marker [6]. In a privious study we also found that TK1 was a more reliable and sensitive proliferating marker than Ki-67 in lung adenocarcinoma patients [19].

In our previous study on pT1 lung carcinoma patients [20], we showed for the first time that TK1 expression in tumor tissues correlated positively to 5-year survival. TK1 also improved the stromal invasion grading system for prognostic evaluation and gave a further reliable assessment for prognosis. Even when the tumor reaches grade 3 of stromal invasion in patients with pT1 lung adenocarcinoma, as long as the TK1 expression still is low, these patients have a good prognosis. This explain why some patients with grade 3 stromal invasion show better survival than others, i.e. their lung tumor may still have lower proliferation rates. In a multivariate analysis, TK1 expression was also found to be an independent prognostic factor. Here we extended the previous study by comparing TK1 with Ki-67 and found that TK1 has some advantages over this ususal used proliferation marker. Total TK1-LI correlated to lymph node metastasis, tumor invasion, pathological stage and 5-year survival, whereas these was not observed for Ki-67. Multivariate analysis showed that total TK1 LI, lymph node metastasis and pathology stage were independent prognostic factors, but not Ki-67.

In a recent study on patients with cervical carcinoma we found that TK1 expression in the nuclei correlated to decreased 5-year survival [12]. Tumor cells with almost no TK1 expression in the nuclei (Cervical intraepithelial neoplasias type) showed good survival, while tumor cells with high TK1 expression in the nuclei (invasive carcinoma type) showed worse survival [12]. However, the results in the present study on the pT1 lung carcinoma did not con-

firm that observation. The physiological significance of TK1 in these two compartments, i.e. cytoplasm/nuclei, is the subject of a separate investigation. It is possible that TK1 re-localized into the nuclei directly participates in repair of DNA, which could improve the survival of the tumor cells and consequently reduces the survival of the patients [12]. However, it is also known that TK1 accumulates in late S and G2 cells, particularly, when cells are subjected to DNA damage [21]. A fraction of TK1 may constantly be transferred into the nuclei through co-transport processes. This may be is not linked to any functional role for nuclear TK1, but simply allow the detection of this form of TK1 by immunohistochemical methods once it exceeds a certain threshold level in the cytoplasma. Further studies are needed to clarify this interesting question.

Disclosure of conflict of interest

YX, BL, QS, PH, XZ, HM, ZL, YB and SE do not declare any conflicts of interest, EH and SS are consulters to SSTK Ltd, Shenzhen, China.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Collaud S, Stahel R, Inci I, Hillinger S, Schneiter D, Kestenholz P, Weder W. Survival of patients treated surgically for synchronous single-organ metastatic NSCLC and advanced pathologic TN stage. Lung Cancer 2012; 78: 234-238.

[3] Kawakami T, Nabeshima K, Makimoto Y, Hamasaki M, Iwasaki A, Shirakusa T, Iwasaki H.Micropapillary pattern and grade of stromal invasion in pT1 adenocarcinoma of the lung: usefulness as prognostic factors. Mod Pathol 2007; 20: 514-521.

[4] Lüchtenborg M, Jakobsen E, Krasnik M, Linklater KM, Mellemgaard A, Møller H. The effect of comorbidity on stage-specific survival in resected non-small cell lung cancer patients. Eur J Cancer 2012; 48: 3386-3395.

[5] Hamasaki M, Kato F, Koga K, Hayashi H, Aoki M, Miyake Y, Iwasaki A, Nabeshima K. Invasion of the inner and outer layers of the visceral pleura in pT1 size lung adenocarcinoma measuring ≤ 3 cm: correlation with malignant aggressiveness and prognosis. Virchows Arch 2012; 461: 513-519.

[6] Gasparri F, Wang N, Skog S, Galvani A, Eriksson S. Thymidine kinase 1 expression defines an activated G1 state of the cell cycle as revealed with site-specific antibodies and Array-Scan assays. Eur J Cell Biol 2009; 88: 779-785.

[7] Zhou J, He E, Skog S. The proliferation marker thymidine kinase 1 (TK1) in clinical use (Review). Mol Clin Oncol 2013; 1: 18-28.

[8] Brockenbrough JS, Morihara JK, Hawes SE, Stern JE, Rasey JS, Wiens LW, Feng Q, Vesselle H. Thymidine kinase 1 and thymidine phosphorylase expression in non-small-cell lung carcinoma in relation to angiogenesis and proliferation. J Histochem Cytochem 2009; 57: 1087-1097.

[9] Sobin LH, Gospodarowicz MK, Wittekind CH. International Union Against Cancer (UICC): TNM Classification of Malignant Tumours. 7th ed. New York: Wiley-Liss Press; 2009. pp. 138-146.

[10] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Petersen I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M, Yankelewitz D. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thoracic Oncol 2011; 6: 244-285.

[11] Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, Park BJ, Rusch VW, Travis WD. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol 2011; 24: 653-664.

[12] Chen G, He C, Li L, Lin A, Zheng X, He E, Skog S. Nuclear TK1 expression is an independent prognostic factor for survival in pre-malignant and malignant lesions of the cervix. BMC Cancer 2013; 13: 249-258.

[13] Scholzen T and Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000; 182: 311-322.

[14] Ciancio N, Galasso MG, Campisi R, Bivona L, Migliore M, Di Maria GU. Prognostic value of p53 and Ki67 expression in fiberoptic bronchial biopsies of patients with non small cell lung cancer. Multidiscip Respir Med 2012; 14: 29.

[15] Oka S, Uramoto H, Shimokawa H, Iwanami T, Tanaka F. The Expression of Ki-67, but Not Proliferating Cell Nuclear Antigen, Predicts Poor Disease Free Survival in Patients with Adenocarcinoma of the Lung. Anticancer Res 2011; 31: 4277-4282.

[16] Niemiec J, Kolodziejski L, Dyczek S. EGFR LI and Ki-67 LI are independent prognostic parameters influencing survivals of surgically treated squamous cell lung cancer patients. Neoplasma 2005; 52: 231-237.

[17] Sun JG, Wang Y, Chen ZT, Zhuo WL, Zhu B, Liao RX, Zhang SX. Detection of lymph angiogenesis in non-small cell lung cancer and its prognostic value. J Exp Clin Cancer Res 2009; 28: 21.

[18] Fujioka S, Ito H, Shomori K, Nishihara K, Yamaga K, Nosaka K, Araki K, Haruki T, Taniguchi Y, Nakamura H, Ito H. Expression of minichromosome maintenance 7 (MCM7) in small lung adenocarcinoma (pT1): Prognostic implication. Lung Cancer 2009; 65: 223-229.

[19] Mao Y, Wu J, Skog S, Eriksson S, Zhao Y, Zhou J, He Q. Expression of cell proliferating genes in patients with non-small-cell lung cancer (NSCLC) by immunohistochemistry and cDNA profiling. Oncology Report 2005; 13: 837-846.

[20] Xu Y, Shi QL, Ma H, Zhou H, Lu Z, Yu B, Zhou X, Eriksson S, He E, Skog S. High thymidine kinase 1 (TK1) expression is a predictor of poor survival in patients with pT1 of lung adenocarcinoma. Tumor Biol 2012; 33: 475-83.

[21] Skog S, Tribukait B. Analysis of cell flow and cell loss following X-irradiated using sequential investigation of the total number of cells in the various parts of the cell cycle. Cell Tissue Kinet 1985; 18: 427-444.