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Original Article

TAZ expression in three distinct circulating tumor cells of NSCLC patients

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Abstract: Non-small cell lung cancer (NSCLC) causes many cancer related deaths worldwide. The migration ability of circulating tumor cells (CTCs) is enhanced by TAZ. In this study we aimed to investigate the correlation of TAZ expression with clinicopathological features of different phenotypical CTCs from NSCLC patients. Thirty-nine patients with newly diagnosed NSCLC who could tolerate radical resection were included. Blood samples were obtained from patients during the operations. RNA in situ hybridization (RNA-ISH) was applied to identify CTCs and detect TAZ expression. CTC-positive rate reached 89.5% in all patients. We found that higher TNM stage was significantly correlated with higher number of epithelial CTCs. TAZ-positive rate in CTCs from EGFR mutation-negative patients was higher than that in EGFR mutation-positive patients (60.0% vs. 46.2%, *P*=0.016), and these patients displayed positive mediastinal lymph nodes (49.1% vs. 29.8%, *P*=0.047), high TNM stage (49.1% vs. 29.8%, *P*=0.047), and high CEA (60.0% vs. 22.7%, *P*=0.014) and NSE levels (63.8% vs. 47.0%, *P*=0.042). TAZ expression in CTCs decreased after surgery. Kaplan-Meier survival analysis showed that higher TAZ expression was associated with a worse overall survival (OS, $P < 0.001$), worse first progression (FS, *P*=0.001) of NSCLC patients. In conclusion, our study suggests that TAZ expression in CTCs could be used as a potential predictor of survival of NSCLC patients.

Keywords: Circulating tumor cell, non-small lung cancer, TAZ, epithelial mesenchymal transition, molecular diagnosis

Introduction

Lung cancer caused approximately 158,040 deaths during 2015, which was the largest death toll of any cancer [1]. Lung cancer was the leading cause of cancer-related deaths in women (26%) and men (26%) during 2015 in the United States. Approximately 85-90% cases of lung cancer are non-small cell lung cancer (NSCLC). Tumor metastasis is the major cause of morbidity and mortality in patients with NSCLC [2]. Although the reasons for tumor metastasis remain a mystery, several studies have shown that circulating tumor cell (CTC) formation is crucial for metastasis [3]. Therefore, we could potentially monitor CTC to determine the state of NSCLC and its response to therapy [3, 4].

Numerous studies have shown that epithelial-mesenchymal transition (EMT) plays a critical role in both tumor metastasis and CTC origination [5, 6]. Recent studies have demonstrated that EMT can stimulate epithelial CTCs to transform into mesenchymal CTCs or biphenotypic CTCs. These changes weaken cell-cell cohesion, favor matrix-degrading abilities, and modify cytoskeleton, all of which facilitate cell motility [6]. The migration and invasiveness of CTCs were enhanced by EMT in breast cancer [7]. EMT is controlled by many transcription factors, signaling pathways, microRNAs, and cell microenvironmental factors. The Hippo pathway is a key pathway in EMT progression that consists of a large network of proteins that control the growth of different tissues during development and regeneration as well as in pathological states, such as cancer [8, 9]. TAZ (transcriptional coactivator with PDZ-binding domain, also known as WWTR1) and its paralog YAP (YES associated protein) are the two main downstream effectors in Hippo signaling pathway.

In this study we aimed to investigate the correlation of TAZ expression with clinicopathological features of different phenotypical CTCs from

NSCLC patients. We chose to use the *CanPatrol System* to better isolate CTCs with different phenotypes compared to the traditional *CellSearch System*. After capturing CTCs, we detected the expression of TAZ in CTC cells of different phenotypes by multiplex RNA in situ hybridization (RNA-ISH) assay. We analyzed the relationships between clinicopathological features of NSCLC patients and different phenotypical CTCs and TAZ expression. In addition, we examined the effects of surgery on CTCs with different phenotypes and TAZ expression.

Patients and methods

Patients

Between April 2015 and November 2015, 39 patients with newly diagnosed NSCLC at our institution were included in this study. The patients met all the following criteria: 1) patients were diagnosed with NSCLC; 2) imaging such as positron emission tomography-computed tomography (PET-CT) and chest-enhancement CT indicated that the patients could tolerate radical resection; 3) none of the patients received chemotherapy or radiotherapy before surgery; and 4) no postoperative complications occurred after the operation. Blood samples from the selected patients were acquired on the day before surgery and on the fifth day after surgery, and analyzed with a *CanPatrol* System (Surexam Biotech, Guangzhou, China).

CTC isolation using CanPatrolTM system

Peripheral blood samples (2 × 5 mL) were collected in EDTA tubes from each NSCLC patient by venipuncture. An 8-μm diameter pore calibrated membrane (Millipore, Billerica, MA, USA) was used to filter the blood samples over 4 hours. The required filtration system included a filtration tube containing the membrane (SurExam, Guangzhou, China), a manifold vacuum plate with valve settings (SurExam, Guangzhou, China), an E-Z 96 vacuum manifold (Omega, Norcross, USA) and a vacuum pump (Auto Science, Tianjin, China). Erythrocytes from these samples were removed using a red blood cell lysis buffer (Sigma, St. Louis, MO, USA). The cell suspension was transferred to the filtration tube. Filtration began when the valve pressure reached at least 0.08 MPa.

CTC classification and TAZ detection

A multiplex RNA-ISH assay based on branched DNA (bDNA) signal amplification technology was employed. Four groups of RNA-ISH assays were used to classify phenotypes and detect TAZ expression in CTCs, following the procedures previously described [10]. Group 1 probes were for four epithelial biomarkers: keratins (KRT) 8/18/19 and EpCAM. Group 2 probes were for two mesenchymal biomarkers (Vimentin and Twist). Group 3 probe was for a leukocyte CD45 biomarker to distinguish leukocytes and CTCs. Group 4 probe was for TAZ. The cells were incubated with the probes at 42°C for 20 min, then stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min. Finally, the cells were analyzed with a fluorescence microscope using a 100 × oil objective (Olympus BX53).

Statistical analysis

Follow-up data were obtained for 39 patients with NSCLC. Two patients were removed because of extreme values and distorted statistical data. We chose the postoperative data as the clinical baseline. Since the CTC number showed a skewed distribution, we used the median value to compare the overall differences. Mann-Whitney U and Kruskal-Wallis H tests were used to analyze the relationship between CTC counts and clinical data. The association between the CTC positivity and TAZ expression was analyzed by the chi-square test. Kaplan-Meier survival analysis for the relationship between survival and TAZ expression in NSCLC was performed using the online tool (http://kmplot.com/analysis/). Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows version 20.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

Patient demographics

A total of 39 patients with NSCLC were enrolled in this study between April 2015 and November 2015 at Nanfang Hospital, Southern Medical University. All the patients received radical resection. The tumors were staged according to the 7th edition of the international tumor-node-metastasis (TNM) system. Patient demograph-

Table 1. Characteristics of NSCLC patients

Characteristic	Number	%
Age, years		
≤ 60	17	45.9
> 60	20	54.1
Gender		
Male	26	70.3
Female	11	29.7
Histologic subtype		
Squamous cell carcinoma	5	18.5
Adenocarcinoma	29	78.4
Other	3	8.1
Smoking status		
Never-smoker	22	59.5
Current/Former smoker	15	40.5
Lymph nodes		
Negative	28	75.7
Positive	9	24.3
TNM stage		
IA	10	27.0
IB	18	48.6
IIIA	9	24.4
Pleura invasion		
No	22	59.5
Yes	15	40.5
EGFR mutations		
Negative	22	75.7
Positive	15	24.3
CEA		
< 5 ng/ml	29	78.4
≥ 5 ng/ml	8	21.6
Cyfra 21-1		
< 3.3 ng/ml	22	59.5
≥ 3.3 ng/ml	15	40.5
NSE		
< 13 μg/ml	17	45.9
≥ 13 μg/ml	20	54.1
SCC		
< 5 μg/L	29	78.4
≥ 5 μg/L	8	21.6

ics are listed in **Table 1**. After removing two extreme values, 22 patients (59.5%) had positive CTC counts at baseline with ≥ 3 CTCs in 10 mL of the blood.

CTCs number and clinicopathological features

According to our RNA-ISH assay results, the three phenotypes of CTCs were epithelial, mesenchymal and biphenotypic. The red dots and green dots corresponded to epithelial and mesenchymal CTCs, respectively. CTCs displaying both red and green dots were biphenotypic CTCs (**Figure 1**). TAZ expression in CTCs was detected as purple and divided into high, medium and low expression categories (**Figure 2**).

The positive CTC rate had no significant differences based on the different clinical characteristics. As shown in **Table 2**, there were no significant differences between the number of all CTCs based on the gender, age, tumor size, EGFR mutation status, histological type, smoking status, pleural invasion, and serum levels of CEA, CYFRA21-1 and SCC. We found that lower TNM stage corresponded to fewer number of all CTCs in the blood, although there was no significant difference (2.0 vs. 4.5 vs. 6.0, P=0.176). However, when the TNM stage was higher, there were significantly more epithelial CTCs (0.5 vs. 1.5 vs. 5.0, P=0.037). The numbers of all CTCs and epithelial-type CTCs in the blood were higher in patients with visceral pleural tumor invasion, although this was not statistically significant (2.0 vs. 6.0, P=0.053). The number of epithelial-type CTCs in patients with mediastinal lymph node metastasis and subject to radical resection was significantly higher than that in patients without mediastinal lymph node metastasis (1.0 vs. 5.0, P=0.026). This also occurred in individuals with abnormal NSE levels (1.0 vs. 3.0, P=0.023).

TAZ expression and clinicopathological features

TAZ expression in post-surgery samples and the associations with clinical characteristics are shown in **Table 3**. There was no significant difference in TAZ expression in CTCs from patients based on age, gender, tumor size, positive or negative mediastinal lymph node metastasis, TNM stage, pleural tumor invasion, smoking status, or serum CEA, CYFRA 21-1 and SCC levels. TAZ positive rate was higher in EGFR mutation-negative patients than in EGFR mutation-positive patients (60.0% vs. 46.2%, P= 0.016). Additionally, TAZ positive rate was significantly lower in patients with normal NSE levels than in patients with abnormal NSE levels (45.8% vs. 63.8%, P=0.027).

TAZ expression was analyzed according to the three CTC types. TAZ expression in epithelial

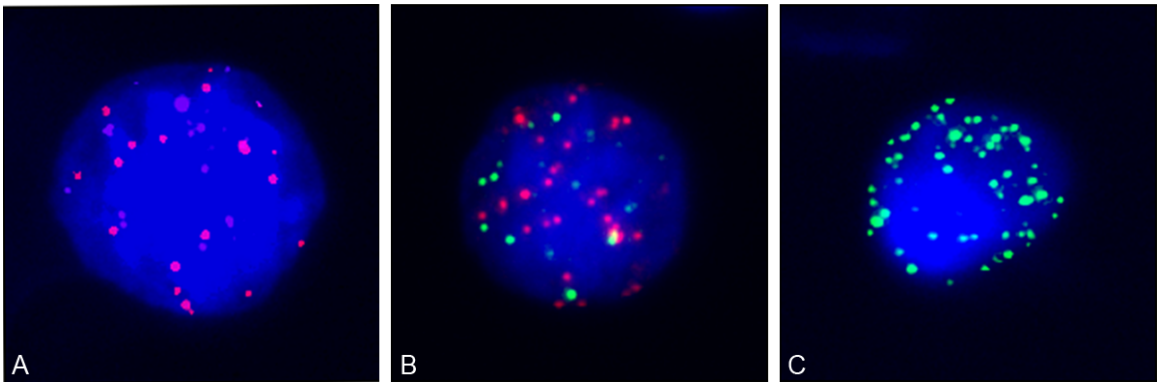

Figure 1. Detection of three types of CTCs. A: Epithelial CTCs; B: Biphenotypic CTCs; C: Mesenchymal CTCs.

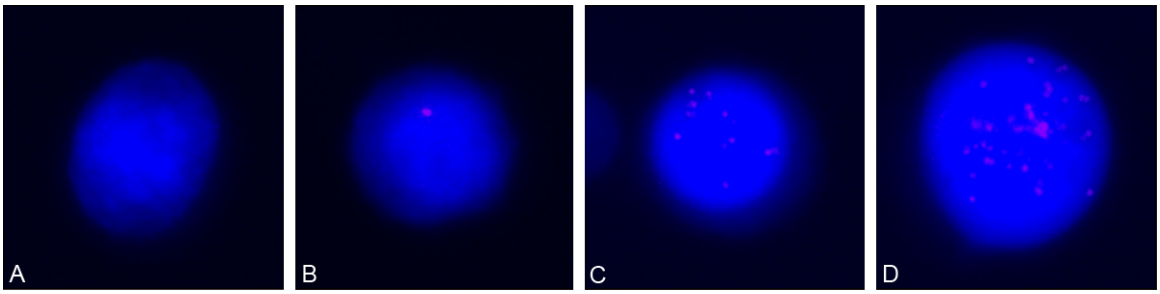

Figure 2. Different TAZ expression level in CTCs. A. Blank control; B. Low expression; C. Medium expression; D. High expression. TAZ was stained purple.

and mesenchymal CTCs displayed no significant differences in different clinical conditions. In biphenotypic CTCs, there was no significant difference between TAZ expression in CTCs from patients based on age, gender, tumor size, positive or negative mediastinal lymph node metastasis, TNM stage, pleural tumor invasion, smoking status, or serum CEA, CYFRA 21-1 and SCC levels. TAZ positive rate in biphenotypic CTCs from EGFR mutation-negative patients was higher than in EGFR mutation-positive patients (77.8% vs. 46.5%, P=0.05). TAZ positive rate in biphenotypic CTCs in patients with normal NSE levels was significantly lower than in patients with abnormal NSE levels (41.9% vs. 88.9%, $P < 0.01$).

The effects of surgery on CTCs and TAZ expression

We assessed the impact of surgery on the patients according to the changes in CTC-positive rate, mesenchymal CTC-positive rate, TAZ expression in all CTCs, TAZ expression in biphenotypic type CTCs and TAZ expression in mesenchymal CTCs. The effects of surgery on CTC positive rate and TAZ expression in mesenchymal-type CTCs had no significant differences ($P > 0.05$) compared with different clinical data. Mesenchymal CTCs in patients with visceral pleural tumor invasion (53.3% vs. 20.0%, P=0.058) and patients who smoked (53.3% vs. 20.0%, P=0.058) decreased significantly before and after surgery, although the differences were not statistically significant. TAZ expression in all CTCs after surgery decreased in patients with positive mediastinal lymph nodes (49.1% vs. 29.8%, P=0.047), higher TNM stages (49.1% vs. 29.8%, P=0.047), and higher CEA (60.0% vs. 22.7%, P=0.014) and NSE levels (63.8% vs. 47.0%, P=0.042).

We performed Kaplan-Meier survival analysis and found that higher TAZ expression was associated with a worse overall survival (OS, $P < 0.001$), worse first progression (FS, P=0.001) of NSCLC patients, while there was no significant difference in Post Progression Survival (PPS) of NSCLC patients (P=0.084) (**Figure 3**). These data suggest that TAZ could be used as a potential predictor of survival of NSCLC patients.

Table 2. Association of CTCs number with clinical characteristics

Characteristic	Total CTC	*P*	Epithelial CTC	*P*	Biphenotypic CTC	*P*	Mesenchymal CTC	*P*
Age, year								
≤ 60	3	0.471	1	0.652	0	0.424	0	0.277
> 60	4.5		1.5		1		0.5	
Gender								
Male	3.5	0.412	1	0.235	1	0.957	0	0.731
Female	6		4		0		0	
Histologic subtype								
Squamous cell carcinoma	2	0.592	2	0.277	0	0.051	0	
Adenocarcinoma	4		1		1		0	
Other	2		2		0		0	
Smoking status								
Never-smoker	4.5	0.913	2	0.133	0.5	0.486	0	0.227
Current/Former smoker	4		0		1		1	
Tumor Size								
≤ 3 cm	2	0.117	1	0.083	0	0.451	0	
> 3 cm	5		2		1		0	
Lymph nodes								
Negative	3	0.176	1	0.026	1	0.134	0	0.254
Positive	6		5		0		1	
TNM stage								
IA	1	0.026	0.5	0.037	0	0.148	0	0.276
IB	3.5		1.5		1		0	
IIIA	6		5		0		1	
Pleura invasion								
No	2	0.053	1.5	0.077	0.5	0.507	0	0.270
Yes	6		3		1		1	
EGFR mutations								
Negative	3	0.116	1	0.602	0	0.267	0	0.318
Positive	6		2		1		0	
CEA								
< 5 ng/ml	4	0.349	1	0.338	1	0.626	0	0.060
≥ 5 ng/ml	5		3		0		2	
Cyfra 21-1								
< 3.3 ng/ml	3	0.204	1	0.204	1	0.406	0	0.490
≥ 3.3 ng/ml	5.5		2		0		0.5	
NSE								
< 13 ug/ml	2	0.142	1	0.023	0	0.562	0	
≥ 13 ug/ml	5		3		1		0	
SCC								
< 5 ug/L	4	0.406	1	0.270	1	0.538	0	0.624
≥ 5 ug/L	7		4		2		1	

Discussion

It is considered that CTCs from solid tumors are associated with metastasis [11]. Monitoring CTCs in the blood will provide a powerful and noninvasive approach for the detection of disease [12]. The change from epithelial to mesenchymal or biphenotypic CTCs makes CTCs more

Table 3. Association of TAZ expression with clinical characteristics

Characteristic	Total CTC	*P*	Epithelial CTC	*P*	Biphenotypic CTC	*P*	Mesenchymal CTC	*P*
Age, year								
≤ 60	60.5	0.135	68.3	0.346	60.7	0.471	33.3	1.000
> 60	49.5		51.8		51.5		38.9	
Gender								
Male	54.5	0.964	62.7	0.402	57.1	0.743	28.6	0.321
Female	54.1		54.3		52.6		55.6	
Smoking status								
Never-smoker	60.0	0.06	59.4	0.184	65.4	0.191	53.3	0.130
Current/Former smoker	46.2		57.1		48.6		20.0	
Tumor Size								
≤ 3 cm	53.5	0.887	73.3	0.336	47.8	0.523	28.6	0.952
> 3 cm	54.5		56.1		60.5		39.1	
Lymph nodes								
Negative	56.4	0.361	65.5	0.099	54.4	0.778	33.3	0.643
Positive	49.1		48.7		75.0		41.7	
TNM stage								
IA	60.0	0.579	100.0	0.579	47.6	0.546	25.0	0.831
IB	55.2		59.6		57.1		35.7	
IIIA	49.1		48.7		75.0		41.7	
Pleura invasion								
No	54.0	0.361	58.5	0.969	59.4	0.548	28.6	0.631
Yes	54.5		58.9		51.7		43.8	
EGFR mutations								
Negative	60.0	0.016	64.6	0.249	77.8	0.05	46.2	0.346
Positive	46.2		53.1		46.5		29.4	
CEA								
< 5 ng/ml	54.5	0.648	60.6	0.525	57.4	0.642	26.3	0.180
≥ 5 ng/ml	60.0		50.0		100		62.5	
Cyfra 21-1								
< 3.3 ng/ml	59.4	0.182	61.5	0.672	62.5	0.535	47.1	0.321
≥ 3.3 ng/ml	48.2		56.8		44.4		20.0	
NSE								
< 13 ug/ml	45.8	0.027	60.7	0.839	41.9	0.003	23.1	0.294
≥ 13 ug/ml	63.8		58.3		88.9		50.0	
SCC								
< 5 ug/L	55.3	0.980	61.9	0.293	55.6	0.083	37.5	1.000
≥ 5 ug/L	55.0		46.2		100		33.3	

aggressive and more likely to form distant metastases [10, 13]. TAZ has been shown as a novel oncogene in NSCLC by promoting EMT [14, 15]. To our knowledge, our study is the first to detect TAZ expression in CTCs of NSCLC patients and analyze the correlation of TAZ expression with clinicopathological features of NSCLC patients.

Because of the rarity of CTCs, different methods have been developed to detect them. The *CellSearch system* is considered the most reliable FDA-approved technique for CTC enumeration [16]. Although the *CellSearch system* is a powerful method, it is unable to detect mesenchymal CTCs, whose main biomarker is vimentin [5]. Compared with other methods, the

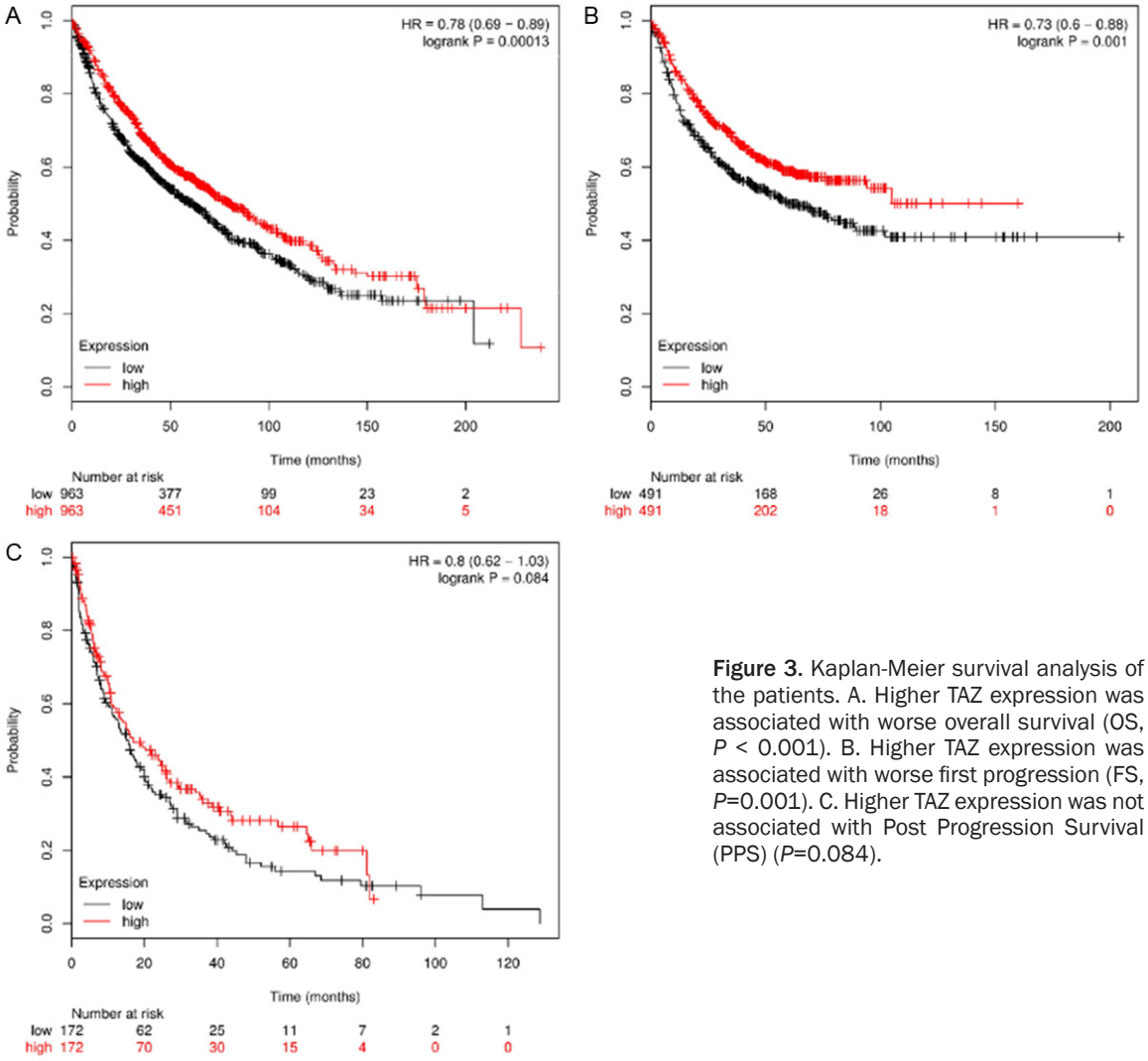

Figure 3. Kaplan-Meier survival analysis of the patients. A. Higher TAZ expression was associated with worse overall survival (OS, $P < 0.001$). B. Higher TAZ expression was associated with worse first progression (FS, $P=0.001$). C. Higher TAZ expression was not associated with Post Progression Survival (PPS) ($P=0.084$).

CanPatrol CTC enrichment technique can capture mesenchymal and biphenotypic CTCs. Moreover, the captured CTCs are still alive. Therefore, we can observe EMT of CTCs and monitor their changes in each patient to evaluate the treatment effect.

In previous studies, different CTC phenotypes have been correlated with the prognosis in several cancer types [17, 18]. Lower TNM stages correspond to lower numbers of all CTCs and epithelial-type CTCs in the blood, and increased numbers of CTCs correspond to a poorer prognosis [19, 20]. The number of CTC was closely correlated with the TNM staging. In this study, we found significantly more epithelial CTCs when the TNM stage was higher. Therefore, we suggest that the detection of epithelial CTCs can be applied in any stage of NSCLC patients to indicate the severity of illness. In recent years, NSE was proposed as a diagnostic marker for non-small cell lung cancer [21-23]. Individuals with abnormal NSE levels had significantly more epithelial CTCs. NSE was shown as an independent predictor of survival in NSCLC patients [24]. Thus we speculate that CTCs and NSE could be combined to predict the prognosis of NSCLC patients.

Interestingly, we found that TAZ-positive rate in all CTCs and biphenotypic CTCs from EGFR mutation-negative patients was higher than in EGFR mutation-positive patients. Thus treatment with tyrosine kinase inhibitor (TKI) drugs will lead to a better prognosis for individuals with EGFR mutation than those without EGFR mutation [25]. In addition, TAZ-positive rate in all and biphenotypic CTCs with normal NSE lev-

els was significantly lower than in patients with abnormal NSE levels. Since mesenchymal CTCs or biphenotypic CTCs are more invasive than epithelial CTCs. This may explain why patients with abnormal NSE level have worse prognosis.

In a study of 56 patients who had undergone radical surgery for previously untreated NSCLC, CTC-positive rate was significantly lower one month after surgery (32.1% vs. 51.8% before surgery, P=0.034) [26]. In our study, some patients presented this kind of trend, but there were no statistical significances. However, the number of CTCs in some patients increased. This could be due to systematic mediastinal lymphadenectomy or disturbance of the solid tumor during the lung operation. Such an operation could lead to CTCs spreading into the blood. Many postoperative CTCs can undergo apoptosis. It is noteworthy that the number of mesenchymal CTCs in patients who smoked and patients with visceral pleural tumor invasion or with high CEA level decreased significantly after surgery. Additionally, TAZ expression in all CTCs decreased after surgery in patients with positive mediastinal lymph nodes, high TNM stage, and high CEA and NSE levels. These results demonstrate therapeutic effects of surgery.

In summary, this is the first report on TAZ expression in three distinct CTCs in NSCLC patients. Kaplan-Meier survival analysis showed that higher TAZ expression in CTCs was associated with worse overall survival and worse first progression of NSCLC patients. Our study suggests that TAZ expression in CTCs could be used as a potential predictor of survival of NSCLC patients.

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Disclosure of conflict of interest

None.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[2] Wittekind C, Neid M. Cancer invasion and metastasis. Oncology 2005; 69 Suppl 1: 14-16.

[3] Paterlini-Brechot P, Benali NL. Circulating tumor cells (CTC) detection: clinical impact and future directions. Cancer Lett 2007; 253: 180-204.

[4] Pierga JY, Bidard FC, Mathiot C, Brain E, Delaloge S, Giachetti S, de Cremoux P, Salmon R, Vincent-Salomon A, Marty M. Circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer in a phase II randomized trial. Clin Cancer Res 2008; 14: 7004-7010.

[5] Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. Nat Rev Clin Oncol 2014; 11: 129-144.

[6] Bonnomet A, Brysse A, Tachsidis A, Waltham M, Thompson EW, Polette M, Gilles C. Epithelial-to-mesenchymal transitions and circulating tumor cells. J Mammary Gland Biol Neoplasia 2010; 15: 261-273.

[7] Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist LV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DA, Maheswaran S. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 2013; 339: 580-584.

[8] Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. Nat Rev Cancer 2013; 13: 246-257.

[9] Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, Pei XH, Zhao S, Xiong Y, Guan KL. TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. Mol Cell Biol 2008; 28: 2426-2436.

[10] Wu S, Liu S, Liu Z, Huang J, Pu X, Li J, Yang D, Deng H, Yang N, Xu J. Classification of circulating tumor cells by epithelial-mesenchymal transition markers. PLoS One 2015; 10: e0123976.

[11] Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, Doyle GV, Matera J, Allard WJ, Miller MC, Fritsche HA, Hortobagyi GN, Terstappen LW. Circulating tumor cells: a novel

prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005; 23: 1420-1430.
[12] Maheswaran S, Haber DA. Circulating tumor cells: a window into cancer biology and metastasis. Curr Opin Genet Dev 2010; 20: 96-99.
[13] Bulfoni M, Gerratana L, Del Ben F, Marzinotto S, Sorrentino M, Turetta M, Scoles G, Toffoletto B, Isola M, Beltrami CA, Di Loreto C, Beltrami AP, Puglisi F, Cesselli D. In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis. Breast Cancer Res 2016; 18: 30.
[14] Chan SW, Lim CJ, Guo K, Ng CP, Lee I, Hunziker W, Zeng Q, Hong W. A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer Res 2008; 68: 2592-2598.
[15] Zhou Z, Hao Y, Liu N, Raptis L, Tsao MS, Yang X. TAZ is a novel oncogene in non-small cell lung cancer. Oncogene 2011; 30: 2181-2186.
[16] Tibbe AG, Miller MC, Terstappen LW. Statistical considerations for enumeration of circulating tumor cells. Cytometry A 2007; 71: 154-162.
[17] Satelli A, Brownlee Z, Mitra A, Meng QH, Li S. Circulating tumor cell enumeration with a combination of epithelial cell adhesion molecule-and cell-surface vimentin-based methods for monitoring breast cancer therapeutic response. Clin Chem 2015; 61: 259-266.
[18] Scher HI, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D, Heller G. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. Lancet Oncol 2009; 10: 233-239.
[19] Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26: 3213-3221.
[20] Molina R, Filella X, Auge JM, Fuentes R, Bover I, Rifa J, Moreno V, Canals E, Viñolas N, Marquez A, Barreiro E, Borras J, Viladiu P. Tumor markers (CEA, CA 125, CYFRA 21-1, SCC and NSE) in patients with non-small cell lung cancer as an aid in histological diagnosis and prognosis. Comparison with the main clinical and pathological prognostic factors. Tumour Biol 2003; 24: 209-218.
[21] Foa P, Fornier M, Miceli R, Seregni E, Santambrogio L, Nosotti M, Cataldo I, Sala M, Caldiera S, Bombardieri E. Tumour markers CEA, NSE, SCC, TPA and CYFRA 21.1 in resectable non-small cell lung cancer. Anticancer Res 1999; 19: 3613-3618.
[22] Shibayama T, Ueoka H, Nishii K, Kiura K, Tabata M, Miyatake K, Kitajima T, Harada M. Complementary roles of pro-gastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) in diagnosis and prognosis of small-cell lung cancer (SCLC). Lung Cancer 2001; 32: 61-69.
[23] Ferrigno D, Buccheri G, Giordano C. Neuron-specific enolase is an effective tumour marker in non-small cell lung cancer (NSCLC). Lung Cancer 2003; 41: 311-320.
[24] Izar B, Sequist L, Lee M, Muzikansky A, Heist R, Iafrate J, Dias-Santagata D, Mathisen D, Lanuti M. The impact of EGFR mutation status on outcomes in patients with resected stage I non-small cell lung cancers. Ann Thorac Surg 2013; 96: 962-968.
[25] Cai KC, Liu DG, Wang YY, Wu H, Huang ZY, Cai RJ, Wang HF, Xiong G, Zhang ZL. Gefitinib maintenance therapy in Chinese advanced-stage lung adenocarcinoma patients with EGFR mutations treated with prior chemotherapy. Neoplasma 2015; 62: 302-307.
[26] Bayarri-Lara C, Ortega FG, Cueto Ladron de Guevara A, Puche JL, Ruiz Zafra J, de Miguel-Perez D, Ramos AS, Giraldo-Ospina CF, Navajas Gómez JA, Delgado-Rodriguez M, Lorente JA, Serrano MJ. Circulating tumor cells identify early recurrence in patients with non-small cell lung cancer undergoing radical resection. PLoS One 2016; 11: e0148659.