Preliminary Investigation of the Clinical Significance of Detecting Circulating Tumor Cells Enriched from Lung Cancer Patients

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Background: Enumeration of circulating tumor cells (CTCs) may be valuable for lung cancer treatment and monitoring cancer patient relapse. In the present study we report clinical significance of lung cancer CTC.

Methods: CTCs were enriched from peripheral blood of 47 lung cancer patients by means of a modified enrichment strategy, followed by identification with immunofluorescence staining using anticytokeratins 18 and 19 monoclonal antibodies.

Results: A control group consisted of 18 healthy donors and 13 nonmalignant pulmonary tuberculosis patients had no positive subject detected. Among 41 newly diagnosed and 6 recurrent lung cancer patients (3 stage I–II, 22 stage III and 22 stage IV) including 27 adenocarcinoma (ADC), 7 squamous cell carcinoma and 13 small cell lung cancer (SCLC), positive detection rate of newly diagnosed patients with CTC ≥2/7.5 ml blood was 78% (ADC stage III), 75% (squamous cell carcinoma stage III) and 60% (SCLC stage III), respectively. Whereas 46% (ADC IV) and 71% (SCLC IV) were observed for stage IV patients. Recurrent patients showed highest detection sensitivity of 83%. A small scale follow-up study was performed on 12 patients following 2 courses of first line chemotherapy. This demonstrated a good correlation of CTC enumeration with radiographic response.

Conclusions: Results of the present study suggest potential clinical utilities of CTC enumeration on lung cancer patients in terms of rapid evaluation of chemotherapy effect in real time and monitoring lung cancer recurrence. A large scale of study which is necessary for further validation of the significance of lung cancer CTC is being performed.

Key Words: Circulating tumor cell (CTC), Lung cancer, Enrichment, CT, Chemotherapy.

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Lung cancer is the leading cancer of all cancer-related death in most countries. Roughly 20% are from small cell lung cancer (SCLC), and 80% are non-small cell lung cancer (NSCLC) which includes adenocarcinoma (ADC) and squamous cell carcinoma (SCC).[1] Worldwide, 5-year survival rate of lung cancer patient is less than 5 to 15%[2] Nearly 50% of early-stage lung cancer patients will relapse or develop metastases within 5 years after surgical removal of tumor mass, indicating the existence of occult metastatic cells which can not be effectively detected by current methods.

Circulating tumor cells (CTCs) are tumor cells shed from the primary tumor into blood circulation. Though some people debate the biologic significance of CTC due to tumor genomic instability and potential metastatic inefficiency,[3] the implication of CTC in metastasis,[4,5] prognosis and predicting progression-free and overall survival[1,6–8] etc. from many studies have been described elsewhere. In addition, clinical significance of molecular characterization of the mutated epidermal growth factor receptor gene on CTC in NSCLC patients with respect to predicting chemotherapy response has been recently reported as well.[9] In view of its obvious clinical relevance, CTC has been recently recommended by the American Society of Clinical Oncology as an acceptable breast cancer (BCA) marker.[10]

Although various methods,[11,12] including chip technology,[13] filtration, immunoparticle isolation, flow cytometry,[14] reverse transcription-polymerase chain reaction,[15,16] and fluorescence in situ hybridization [17] have been developed and employed in an attempt to detect CTC in peripheral blood, the

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approach for detecting lung cancer CTC still needs to be improved in terms of sensitivity and specificity.[13,18]

The purpose of the present study was to validate a new CTC enrichment strategy on the peripheral blood from lung cancer patients and to perform a preliminary study to investigate clinical significance of lung cancer CTC. The approach applied in this study for lung cancer CTC detection showed both high sensitivity and specificity. In addition, a good correlation of CTC with radiographic response to chemotherapy was demonstrated in a follow-up study.

PATIENTS AND METHODS

Enrichment and Identification of CTC from Peripheral Blood

Enrichment of CTC

The Cytelligen method is essentially similar to that previously published by others with some modifications.[19] Seven and half ml of peripheral blood collected in BD Vacutainer tube (Becton, Dickinson and a Company, Franklin, NJ) were washed with phosphate buffered saline (PBS) once, followed by lysis of red blood cell (RBC). The reaction mixture was spun down at 300*g* for 5 minutes. Resulting cell pellet was resuspended in PBS and subsequently incubated with 0.5 ml of antileukocyte surface marker CD45 monoclonal antibody coated magnetic beads for 30 minutes, followed by separation of magnetic beads using a magnetic stand (Promega, Madison, WI). Supernatants were transferred into a centrifuge tube followed by spinning at 500*g* for 3 minutes. Cell pellet was resuspended, and subsequently subjected to immunofluorescence analysis.

Immunostaining and identification of enriched CTC

Identification of CTC by means of Cytelligen immunostaining approach was performed essentially according to the protocol previously published with some modifications.[20–22] Briefly, cells fixed by 2% paraformaldehyde on slides were permeabilized with 0.1% Triton X-100, followed by incubation with monoclonal antibodies anticytokeratin 18 conjugated to Alexa Flora 594 (Invitrogen, Carlsbad, CA) and anticytokeratin 19 conjugated to Alexa Flora 488 for 1 hour. Sample was washed three times with PBS, followed by mounting with 4′-6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA) containing mounting media, and subsequently subjected to image analysis using a three-color fluorescence microscope. The leukocytes marker CD45 was stained with immunohistochemical staining. Each positive CTC has to meet following criteria: cell size >4 μm, cells are intact with round to oval morphology with visible DAPI stained nucleus, and positive for both anticytokeratin 18 and 19 staining.

Spiking Study

A validation study was performed to establish the accuracy of the method to detect cancer cells in blood. Live human lung cancer cells A549 were labeled with DAPI for 1 hour. Cells were counted with a microscope or were serially diluted to provide, 5, 10. 20, 50, and 100 cells respectively and each sample were placed into 7.5 ml blood. This was followed by the enrichment isolation procedure. Recovered cancer cells were enumerated using a fluorescent microscope by a blinded observer. The experiment was repeated in triplicate for each number of cancer cells, and the total spiking study was repeated four times.

Patients and Specimens

Consent forms signed by all human subjects including lung cancer and nonmalignant pulmonary tuberculosis patients recruited in this study were approved by the Ethics Review Committee of Peking Union Medical College Hospital. Healthy donors were selected from the Clinical Trial Center in Peking Union Medical College Hospital. Staging of lung cancer patients was performed according to the National Comprehensive Cancer Network guidelines upon chest radiography, bronchoscopy, brain, and thoracic computed tomography (CT), positron emission tomography and bone scintigraphy. Clinical responses of first-line chemotherapy in follow-up study were classified by CT scanning according to the Response Evaluation Criteria in Solid Tumors.

Forty-seven lung cancer patients including 3 stage I/II, 22 stage III and 22 stage IV of 27 ADC, 7 SCC, and 13 SCLC patients, plus 18 health donors (HD) and 13 nonmalignant tuberculosis patients (TB) with multiple nodules in lung were enrolled in this blinded study. Among the 47 lung cancer patients, 41 were newly diagnosed, and another 6 were recurrent.

Before each collection of blood samples, the first 2 ml of blood were discarded to avoid potential epithelial cell contamination, 7.5 ml peripheral blood was subsequently drawn from the median cubital vein into a BD Vacutainer tube. All blood samples were processed within 24 hours after collection.

To avoid bias, all blood samples (including control and patient) collection, encoding, enrichment, and result reading were blindly performed by different personnel. Decoding and evaluation of correlation between CTC counting and patient clinical status were coperformed by cross blinded physicians and research scientists.

Statistical Analysis

One way-analysis of variance and *t* test biostatic analysis of the results obtained from all different categories of lung cancer patients were performed and plotted by means of the GraphPad Prism, Version 4.0. San Diego, CA.

RESULTS

Validation of the New Enrichment Strategy with Spiking Study

To validate the ability of our method of detecting lung cancer cells in blood a series of blinded spiking studies were performed using human lung cancer cell line A549. Results show that almost all the spiked cells at different range of 5, 10, 20, 50, and 100 spiked cells can be recovered, (Figure 1) and white blood cell (WBC) depletion is constantly maintained at 3–4 logs (data not shown), indicating that the

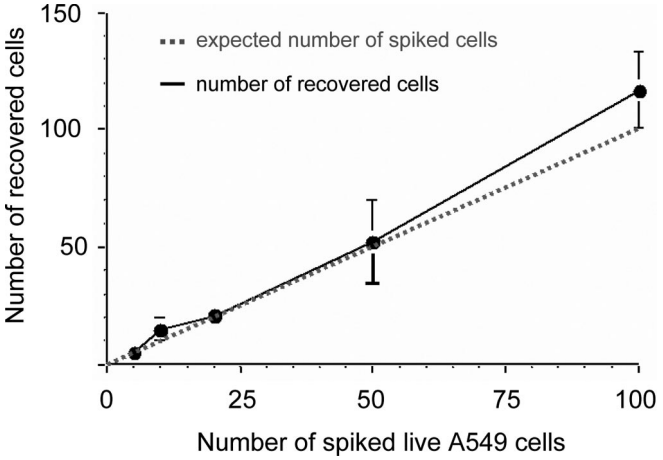

FIGURE 1. Blinded validation of the new enrichment strategy by spiking study. Live human lung cancer cells A549 were labeled with 4′-6-diamidino-2-phenylindole (DAPI), followed by trypsinization and washed. The desired number of cells ranging from 5, 10, 20, 50, to 100 (determined under microscope or from series dilution) were spiked into 7.5 ml blood, followed by enrichment procedure. Obtained cells were enumerated under microscope. The plot was obtained from an average of four separate triplicate experiments. The doted line represents initial spiked cell number, and solid line represents the recovered number of cells. Result shows the number of recovered cells is almost identical to cells spiked in the blood.

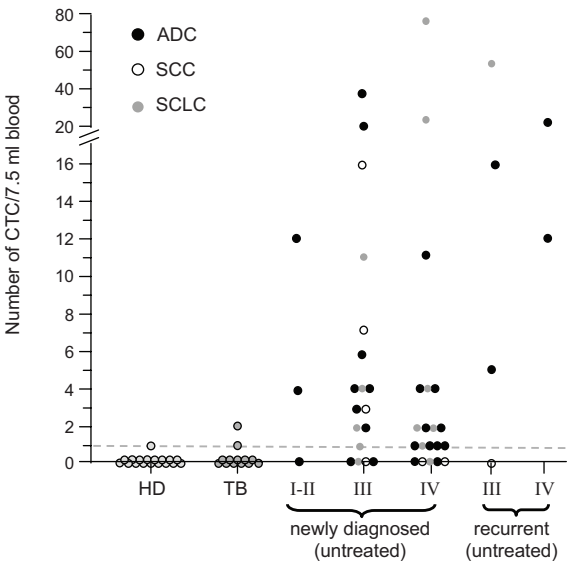

FIGURE 2. Distribution of circulating tumor cell (CTC) enriched from nontreated lung cancer patients. The new enrichment strategy was applied to isolate CTC from 47 lung cancer patients, including 41 newly diagnosed and 6 recurrent patients before subjected to chemotherapy. The gray dash line indicates 1 cell per 7.5 ml blood as the cutoff point. A cutoff of ≥2 cancer cells per 7.5 ml blood was classified as positive. In 18 healthy donors (HD), only 1 had a positive cell. In 13 of tuberculosis patients (TB), 1 patient had 1 and another had 2 positive cells. In the group of 41 newly diagnosed patients 2 of 3 patients with stage II/II were positive for CTC. Among 18 stage III patients, 13 subjects (including 7 adenocarcinoma [ADC], 3 squamous cell carcinoma [SCC] and 3 small cell lung cancer [SCLC]) were found to be positive, All of the 20 stage IV patients were positive. In the group of 6 recurrent patients, 2 stage III plus 2 stage IV ADC as well as 1 of SCLC (stage III) were positive for CTC detection.

current protocol is able to efficiently remove WBC and enrich spiked lung cancer cells from peripheral blood, with a high degree of accuracy.

Enrichment of CTC from Lung Cancer Patients

Eighteen HD, 13 nonmalignant TB patients, and 47 lung cancer patients including 3 stage I/II, 22 stage III and 22 stage IV of 27 ADC, 7 SCC, and 13 SCLC patients were enrolled in this blinded study. Blood samples were collected before chemotherapy therapy unless indicated, and subsequently subjected to enrichment and CTC counting. A positive patient was defined as one whose CTC count in 7.5 ml blood was ≥2. Distribution of positive cells among all control groups and patients is demonstrated in Figure 2.

CTC Detection Sensitivity on Newly Diagnosed and Recurrent Lung Cancer Patients

As shown in Figure 2 and summarized in Table 1, no HD was positive. One nonmalignant TB patient had 2 cells in 7.5 ml blood. 67% of ADC stage I–II (2of 3), 78% ADC stage III (7 of 9) as well as 46% ADC stage IV (5 of 11) were found to have ≥2 CTC in 7.5 ml blood. For SCC patients, 75% SCC stage III (3 of 4) were positive for CTC ≥2. There were no detectable CTC in 2 of SCC stage IV patients. With respect to SCLC, 60% SCLC III (3 of 5) and 71% SCLC IV (5 of 7) were positive. For those six nontreated yet recurrent patients, all but one (SCC stage III) were positive for CTC detection (5 of 6 patients, 83%). The average detection sensitivity for all histologic cancers according to stage I–II, III and IV were 67, 72, and 50%, respectively. The recurrent patients showed the highest detection sensitivity of 83%.

Analysis of CTC Detection on Patients Classified by Different Pathologic Types and Stages

To evaluate the efficiency of the approach applied in this study on the enrichment of lung cancer CTC, all of nontreated patients including 41 newly diagnosed and 6 recurrent patients were pooled and classified into the different pathologic types and stages.

As revealed in Figure 3, in the category of 27 ADC patients, 67% stage I–II (2 of 3), 82% of stage III (9 of 11) and 54% of IV (7 of 13) were found to have positive CTC (CTC ≥2 cells/7.5 ml blood). Among the 2 of CTC ≥2 stage I–II patients, 1 patient had CTC ≥5. In the total of 9 ADC stage III CTC ≥2 patients, 5 patients have CTC higher than 5 cells/7.5 ml blood, which is about 56% (5 of 9). However, in 5 of ADC IV CTC ≥2 patients, 43% (3 of 7) have CTC ≥5. In the group of 7 SCC patients, 60% (3 of 5) of SCC stage III were found to be CTC positive, and among those

TABLE 1. Analysis of CTC Detection Sensitivity

		CTC ≥2	
Pathological Types	*n*	**Positive Patient Number**	**%**
Health donors	18	0	
Tuberculosis	13	0	
I–II			
ADC	3	2	67
III			
ADC	9	7	78
SCC	4	3	75
SCLC	5	3	60
Sensitivity	18	13	72
IV			
ADC	11	5	46
SCC	2	0	0
SCLC	7	5	71
Sensitivity	20	10	50
Recurrence			
Sensitivity	6	5	83

CTC, circulating tumor cell; ADC, adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small cell lung cancer, *n*, number.

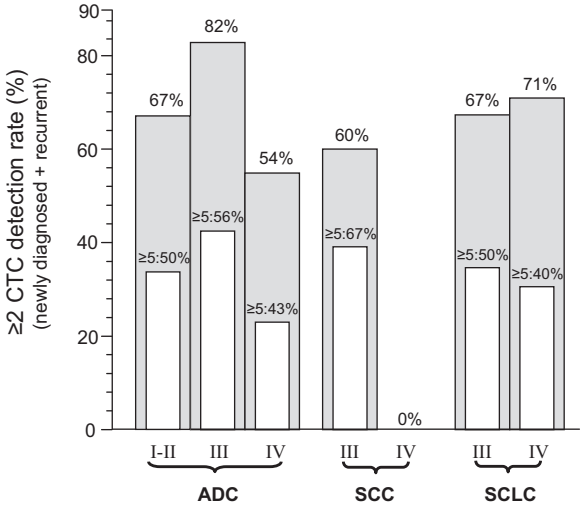

FIGURE 3. Circulating tumor cell (CTC) detection rate of pooled nontreated lung cancer patients. All of nontreated patients including both newly diagnosed and recurrent are classified into stage I–II, III, and IV on each adenocarcinoma (ADC), squamous cell carcinoma (SCC) and small cell lung cancer (SCLC) pathologic types. Sixty-seven percent of ADC I–II (2 of 3), 82% ADC III (9 of 11) and 54% of ADC IV (7 of 13) were positive for CTC (CTC ≥2 cells/7.5 ml blood). The percentage of CTC ≥5 among those CTC ≥2 patients are 50, 56, and 43%, respectively. Among SCC patients, 60% of stage III (3 of 5) were CTC positive with 67% (2 of 3 positive patients) of CTC ≥5. No CTC was detected in 2 of SCC IV patients. In the category of SCLC, 67% (4 of 6) and 71% (5 of 7) patients were CTC positive for stage III and IV, and their CTC ≥5 rate is 50 (2 of 4) and 40% (2 of 5), respectively.

subjects, 67% (2 of 3) had CTC ≥5. In two of SCC IV patients, no CTC was detected.

In the category of 13 SCLC, 67% (4 of 6) SCLC stage III and 71% (5 of 7) SCLC stage IV were noted to have positive CTC (CTC ≥2). Among those, 50% of stage III and 40% of stage IV CTC positive patients were CTC ≥5, respectively.

Results in Figure 3 indicate that ADC stage III had the highest CTC ≥2 positive rate of 82%, followed by ADC stage I–II (67%) and ADC stage IV (54%). In view of the important clinical significance of CTC ≥5 reported on other cancers such as BCA,[7,8] we analyzed percentage of lung cancer patients with CTC ≥5 among the positive patients of CTC ≥2. The percentage of CTC ≥5 among those CTC ≥2 patients were 56% (III), 50% (I–II) and 43% (IV), respectively. SCC III had the highest CTC ≥5 rate of 67% in positive patients though its CTC ≥2 detection is about 60%. In the case of SCLC, both stage III and IV patients had similar CTC ≥2 detection rate of 67 to 71%, with CTC ≥5 of 50% (III) and 40% (IV), respectively.

Statistical Analysis of CTC*ave*

In view of the published important clinical significance of the number of detected CTC on breast cancer patients, further statistical analysis was performed on both newly diagnosed and recurrent lung cancer patients in this study to analyze the average number of counted CTC (CTC*ave*). As shown in Figure 4*A*, ADC stages I–II, III, and IV had CTC*ave* value of 5, 8.5, and 2.5, respectively. SCC III had a value of 6.5. The CTC*ave* value of SCLC III and IV are 3.5 and 10.5, respectively. However, recurrent patients have the highest value of 11. Similar analysis was performed on pooled newly diagnosed patients based on different stages. Result in Figure 4*B* indicates that among a total of 3 stage 1-II, 18 stage III and 20 stage IV patients, the obtained CTC*ave* value is 5, 7, and 7, respectively.

Correlation of CTC Enumeration and CT Evaluation in a Follow-Up Study

A small scale of follow-up study was conducted to evaluate a correlation of radiographic appearance (CT scanning) and CTC counts in this study. As shown in Figure 5, 12 patients including 7 ADC, 2 SCC, and 3 SCLC were subjected to CTC counting before and after 2 courses of first line chemotherapy, followed by CT examination. Eight of 12 patients (patient 1–8) including 5 ADC, 2 SCC, and 1 SCLC were classified by CT as stable disease. Among those 8 patients, 5 patients (patient 3, 4, 5, 6, and 8) dropped their CTC count to 0, patient 4 decreased from 21 to 3, patient 2 and 7 CTC remained 0 after 2 courses of treatment. Patient 1 was noted to have an increase from 2 to 3 following therapy. For partial response patients including 2 SCLC and 1 ADC (patient 9–11), patient 9 CTC drops from 52 to 0, and another 2 patients had a CTC count remaining below 2. The only patient whose CTC dramatically increased from 2 to 12 after 2 cycles of therapy was patient 12, and this patient was thereafter confirmed to have progression of disease (PD) by CT scanning. Clinical responses in this study were classified

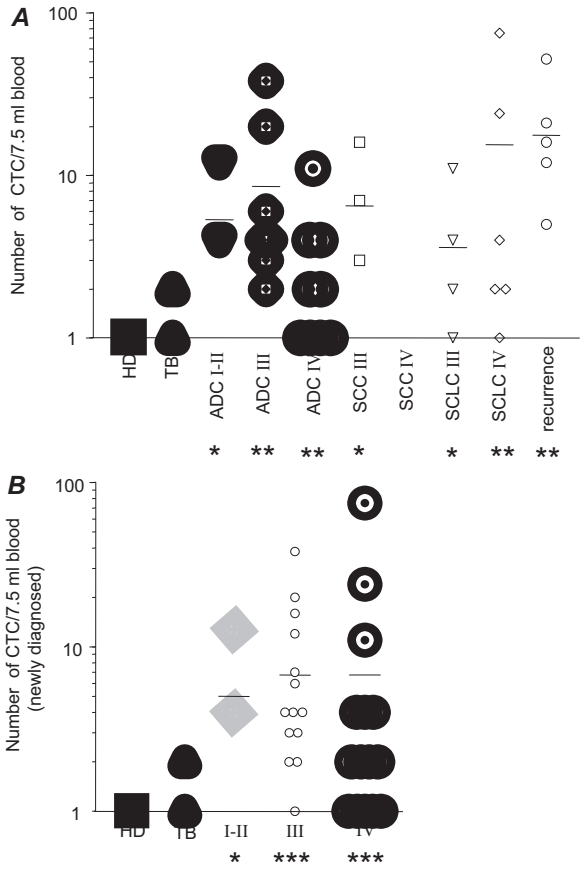

FIGURE 4. Statistical analysis of circulating tumor cell (CTC) counting. *A,* Newly diagnosed and recurrent patients (all pathologic types): statistical analysis indicates the variation of the number of CTC enriched from different pathologic types at different stages. ADC stages I–II, III and IV have an average counting (Mean, CTC*ave*) of 5, 8.5, and 2.5 cells in 7.5 ml blood, respectively. Squamous cell carcinoma (SCC) III has an CTC*ave* value of 6.5. No detectable CTC is observed in 2 of SCC IV patients. Regarding small cell lung cancer (SCLC) patients, CTC*ave* 3.5 and 10.5 are found on SCLC III and IV, respectively. The recurrent group of patients has highest CTC*ave* value of 11. The significance of difference between health donors (HD) and each of pathologic types is denoted by (*$p < 0.05$) and (**$p < 0.01$). *B,* Pooled newly diagnosed patients: statistical analysis on stage I–II, III and IV patients from pooled various pathologic types of newly diagnosed patients shows that CTC*ave* of stage I–II ($n = 2$), stage III ($n = 13$) and stage IV ($n = 10$) is 5 (*$p < 0.05$), 7 (***$p < 0.0001$), and 7 (***$p = 0.0005$), respectively.

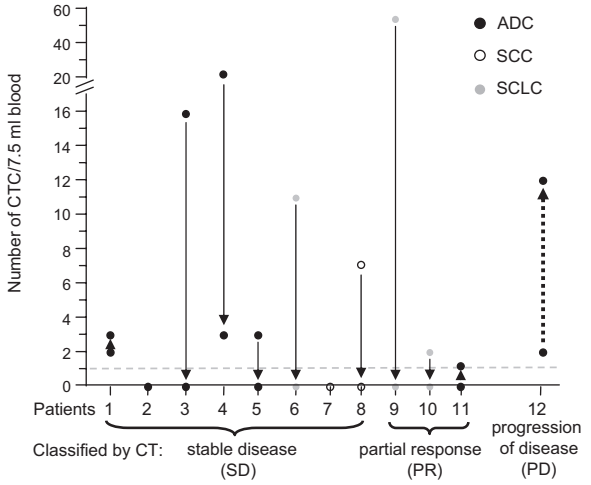

FIGURE 5. Correlation of circulating tumor cell (CTC) counting and computed tomography (CT) scanning with a follow-up study. Correlation of CT scanning and CTC counting was evaluated on a small scale of follow-up study. CTC was enumerated from each patient before chemotherapy started. After two courses of first line chemotherapy, patient CTC was measured, followed by CT examination. Eight of 12 patients (patient 1–8) including 5 adenocarcinoma (ADC), 2 squamous cell carcinoma (SCC) and 1 small cell lung cancer (SCLC) were classified by CT as stable disease. Among those 8 patients, 5 patients dropped their CTC to 0, 1 patient dropped from 21 to 3, 2 patients remained 0, and in 1 patient the count increased from 2 to 3. Three patients had a partial radiologic response: For partial response (PR) patients (patients 9–11), one was noted to have a CTC drop from 52 to 0, while the CTC in the other 2 patients remained below 2. Only one patient was noted to have an increasing CTC, from 2 to 12 after treatment, and this patient was confirmed to have progressive disease (PD). Clinical responses are classified by CT scanning according to the Response Evaluation Criteria in Solid Tumors (RECIST).

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DISCUSSION

The clinical significance of CTCs is described elsewhere.[4,11] The method of capturing CTC from BCA patients which is based on the expression of adhesion molecule EpCAM on the tumor cell surface has been well established.[7,18] However, due to the heterogenic expression of EpCAM among different pathologic types of cancer,[18,23] the ability of this tumor cell surface molecule based detection in other cancers is unclear. In this study, instead of attempting to directly capture CTC, we designed a strategy to enrich CTC via depleting RBC, WBC, and serum proteins, in essence a negative enrichment concept. Obtained lung cancer CTC shows good morphology as shown in Figure 6.

A series of blinded validation studies performed by spiking cultured A549 cells into blood show that this strategy is accurate in detecting the number of cancer cells from the blood specimens. In the validation study (Figure 1), an almost identical number of cells could be recovered from the blood samples. With respect to the recovery on samples where 50 and 100 cells were spiked, variation might be due to imprecise number of spiked cells from series dilution.

We subsequently performed further clinical validation study on 34 NSCLC and 13 SCLC patients plus 18 HD and 13 of nonmalignant tuberculosis (TB) patients. To avoid interference of chemotherapy on CTC enumeration, blood was drawn from patients before they were subjected to

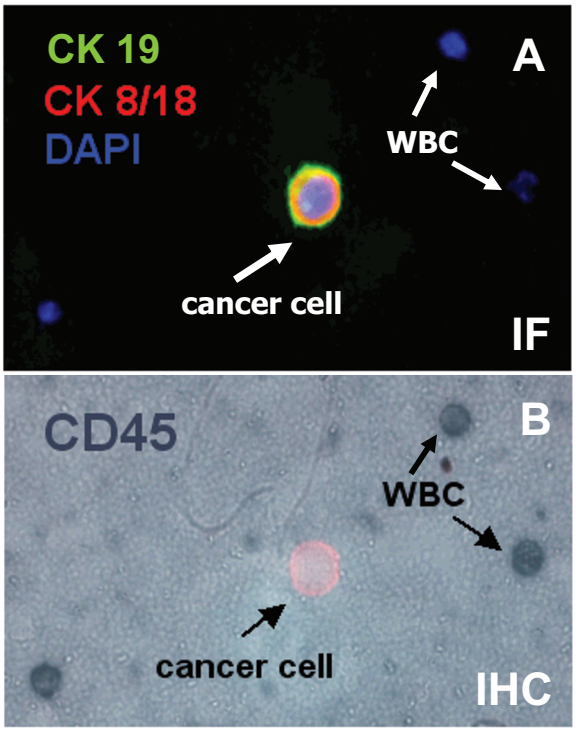

FIGURE 6. Visualization of lung cancer circulating tumor cell (CTC). CTC enriched from lung cancer patient was subjected to immunofluorescent (IF) staining (A) for identification of CTC and immunohistochemical (IHC) staining (B) for counter staining. *A,* Showed that CTC was specifically immunostained with both anti-Cytokeratin (CK) 8/18 and anti-CK 19, but not with anti-CD45 IHC staining as revealed in (*B*). Nucleus of cells were stained with 4′-6-diamidino-2-phenylindole (DAPI) (A). Only white blood cells (WBCs) were found CD45 positive but CK negative as demonstrated in *B*.

chemotherapy. Among 47 lung cancer patients, there were 3 stage I–II, 22 stage III, and 22 stage IV of 27 ADC, 7 SCC, and 13 SCLC patients. In the HD group, none of donors was found to have CTC more than 2 in 7.5 ml blood, which is in consistent with that previously reported by others.[18] Taking CTC ≥ 2/7.5 ml blood as a cutoff point, as shown in Table 1, newly diagnosed patients of ADC stage III and SCC III have highest detection rate of 75 to 78%, and average detection sensitivity of stage III including ADC, SCC, and SCLC is about 72%. Stage I–II patients have a comparable similar detection rate of 67% though there are only three cases examined in this study. About 50% of the average detection sensitivity is found on stage IV patients. However, recurrent patients are found to have highest detection sensitivity of 83%. Interestingly, it has been realized that ADC stage IV patients have lower CTC detection sensitivity compared with others demonstrated in the Table 1. Similar observation is obtained when analysis is performed on the patients pooled from both newly diagnosed and recurrent as shown in Figure 3. The reason stage IV patients did not have a higher prevalence of positive CTC counts is unknown.

Important clinical implication of the number of counted CTC on BCA from various studies has been reported.[4,6,11,24,25] Five BCA CTC/7.5 ml blood is of particular clinical significance and have been taken as the clinical cutoff point for BCA.[7,8] Despite unavailable cell number as a clinical cutoff point for lung cancer, we statistically analyzed the number of counted lung cancer CTC by taking BCA as an example. Results from the analysis performed on pooled nontreated patients of newly diagnosed and recurrent as shown in Figure 3 indicate that among those patients with CTC ≥2, about 50 ± 6% of ADC positive patients have CTC ≥5; 45 ± 5% of SCLC are CTC ≥5, whereas SCC III have the percentage of 67%. Further analysis of the mean of counted CTC (CTC*ave*) on separate newly diagnosed and recurrent patient in Figure 4*A* indicates that SCLC IV has CTC*ave* value of 10.5 in newly diagnosed patients, whereas recurrent patients show the highest CTC*ave* value around 11. Of particular interest is the observation that recurrent lung cancer patients have both highest detection sensitivity (83% in Table 1) and CTC*ave*. It has been recently reported that CTC detected by reverse transcription polymerase chain reaction correlates to recurrence of colorectal cancer.[26] Results from our study suggest the potential significant clinical utility in terms of detecting elevated CTC with respect to monitoring lung cancer relapse. Additional statistical analysis on pooled all newly diagnosed patients classified upon stage as shown in Figure 4*B* reveals the CTC*ave* value for each stage is 5, 7, and 7, respectively, indicating that clinically, CTC enumeration on newly diagnosed patients with different stages may be significant.

Clinical impact of counting CTC during therapy for metastatic BCA in a follow-up study were previously published by others.[6–8] In those studies, correlation between CTC and response to chemotherapy on BCA patients and correlation of CTC counts with local PD were observed, indicating that CTC relates to disease status and tumor load. To observe correlation between lung cancer CTC enumeration and CT scanning, a small scale follow-up study was conducted by us on 12 lung cancer patients. Result of the blind study demonstrated in Figure 5 showed that after 2 courses of first line chemotherapy 7 patients were found to have a decrease in CTC counts, and another 4 patients remained stable. There was only 1 patient whose CTC increased from 2 to 12, and this patient was subsequently classified by CT scanning as PD. In the present small scale study, our observation of the correlation between CTC enumeration and CT scanning suggests that CTC has the potential to correlate to disease status in lung cancer.

Taken together, this report demonstrates that lung cancer CTC can be efficiently enriched and detected by the approach applied in this study. The potential significant clinical utility of detecting lung cancer CTC with respect to monitoring recurrence and rapid evaluation of therapeutic effects in real time are revealed in this preliminary study. Certainly a large scale study would be necessary in the future to further shed light on illustrating clinical utility of lung cancer CTC.

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