

Circulating Aneuploid Cells Detected in the Blood of Patients with Infectious Lung Diseases

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The identification of circulating tumor cells (CTCs) is clinically important for diagnosing cancer. We have previously developed a size-based filtration platform followed by epithelial cell adhesion molecule immunofluorescence staining for detecting CTCs. To characterize CTCs independently of cell surface protein expression, we incorporated a chromosomal fluorescence *in situ* hybridization (FISH) assay to detect abnormal copy numbers of chromosomes in cells collected from peripheral blood samples by the size-based filtration platform. Aneuploid cells were detected in the peripheral blood of patients with lung cancer. Unexpectedly, aneuploid cells were also detected in the control group, which consisted of peripheral blood samples from patients with benign lung diseases, such as empyema necessitatis and non-tuberculous mycobacterial lung disease. These findings suggest that chromosomal abnormalities are observed not only in tumor cells, but also in benign infectious diseases. Thus, our findings present new considerations and bring into light the possibility of false positives when using FISH for cancer diagnosis.

Key words: 1. Circulating neoplastic cells
2. Fluorescent *in situ* hybridization
3. Lung neoplasms

Case report

The identification and characterization of circulating tumor cells (CTCs) in the blood of cancer patients can help to diagnose cancer more accurately. Epithelial cell adhesion molecule (EpCAM)-based platforms are usually used to capture and identify CTCs [1-3]. CTCs can be successfully characterized by fluorescence *in situ* hybridization (FISH) following enrichment using the EpCAM-positive selection platforms [4]. However, as CTCs undergoing epithelial-mesenchymal transition frequently lose the expression of EpCAM, technologies targeting only EpCAM-

positive cells may underestimate the actual number of CTCs [5,6]. Once CTCs are enumerated, they have to be properly characterized to ensure the sensitivity and reliability of the CTC enumeration platform used. We have previously developed a highly sensitive method for detecting CTCs that employs size-based filtration and EpCAM immunofluorescence staining [7]. CTCs of epithelial origin were distinguished from blood cells as all 4',6-diamidino-2-phenylindole (DAPI)-positive, leukocyte common antigen (CD45)-negative, and EpCAM-positive cells. To characterize CTCs independently of cell surface protein expression, we incorporated a chromosomal FISH assay to detect ab-

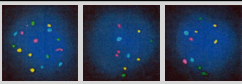
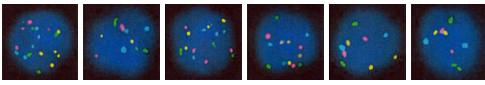
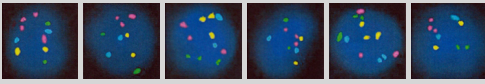
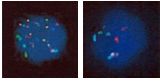
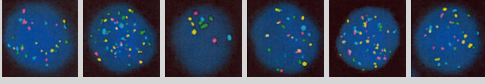
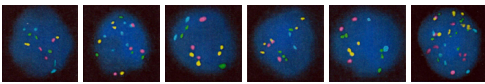
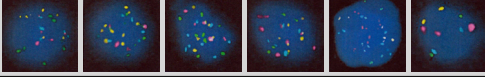
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Table 1. Detection of aneuploid cells in the blood of patients with lung diseases

Patient code	EpCAM+/CD45-/DAPI+cell count ^{a)}	Aneuploid cell count	Chromosomal FISH ^{b)} : oligo probe: chromosome #1, aqua; #7, green; #8, gold; #20, red	Clinical description
LGC2013036	13	3		Primary tumor location: lung cancer Metastasis: lung-to-lung, pleural seeding Pathologic stage: 4 Primary tumor cell type: ADC Tumor size (CT, cm): not measurable
LGC2013042	3	6		Primary tumor location: r/o lung cancer Metastasis: no Pathologic stage: 4 Primary tumor cell type: ADC Tumor size (CT, cm): 2
LGC2013050	0	6		Primary tumor location: r/o lung cancer Metastasis: no; lymphatic invasion: yes Pathologic stage: 3 Primary tumor cell type: SCC Tumor size (measured size, cm): 5.5×4×3.5
LGC2013051	1	2		Primary tumor location: lung cancer Metastasis lung, brain Pathologic stage: 4 Primary tumor cell type: ADC Tumor size (CT, cm): 5
LGC2013054	3	6		Primary tumor location: lung cancer Metastasis: no; lymphatic invasion: yes; vascular invasion: yes Pathologic stage: 3 Primary tumor cell type: SCC Tumor size (measured size, cm): 7.5×5
LGC2013043	2	6		Emphyema necessitates
LGC2013049	5	6		NTM lung disease (<i>M. Abscessus</i>)

ADC, adenocarcinoma; CT, computed tomography; SCC, squamous cell carcinoma; NTM, non-tuberculous mycobacteria; CD45, leukocyte common antigen; mAb, monoclonal antibody; EpCAM, epithelial cell adhesion molecule; DAPI, 4',6-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization.

^{a)}The collected cells were underwent immunofluorescence staining with anti-human-CD45 mAb, anti-human EpCAM mAb, and DAPI and then counted under a fluorescence microscope. ^{b)}Chromosomal FISH image magnification, ×1,000.

normal copy numbers of chromosomes in cells collected from peripheral blood samples using the size-based filtration platform. Abnormal copy numbers of chromosomes were detected not only in tumor cells, but also in certain benign cells, thus highlighting the limitations of the chromosomal FISH assay for cancer diagnosis.

Five patients with non-small cell lung cancer and 2 patients with benign lung diseases were enrolled in this study, and no patient was excluded. This study was approved by the institutional review board (No.

2011-12-024). A 10-mL blood draw was collected from the patients and processed on a size-based filtration platform. The collected cells were divided and loaded onto 2 microscopic slides. On 1 slide, half of the collected cells were immunofluorescently stained with anti-human CD45 monoclonal antibody (mAb), anti-human EpCAM mAb, and DAPI to check for EpCAM+/CD45-/DAPI+ cells. The remaining cells on the other slide were stained with 4-color fluorescent-conjugated oligo-FISH probes specific to chromosomes 1 (aqua), 7 (green), 8 (gold), and 20 (red). The patient

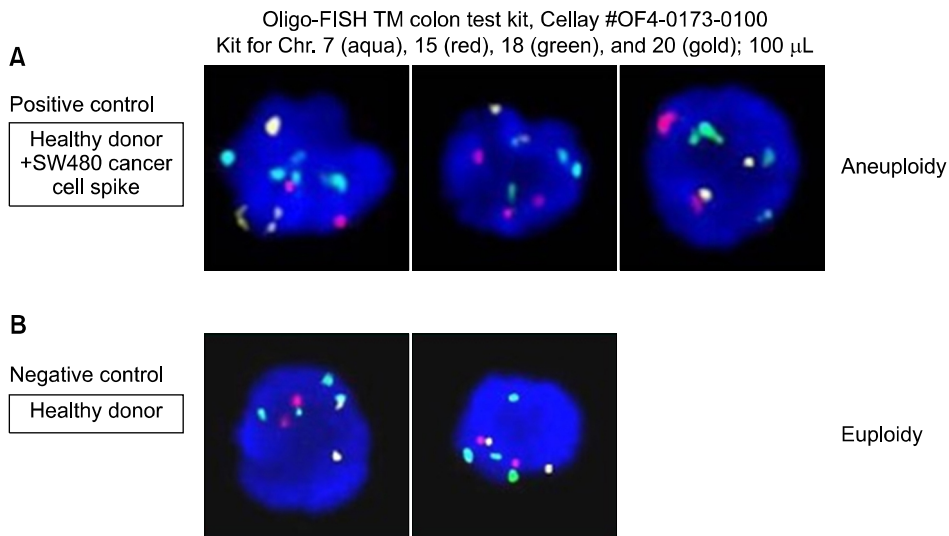


Fig. 1. The positive and negative controls for chromosomal fluorescence *in situ* hybridization assay. (A) Positive control. The cells from the SW380 cell line (the colon adenocarcinoma cell line) were spiked into human blood from a healthy donor, and then filtrated. In the chromosomal FISH assay, aneuploidy was observed. (B) Negative control. The blood sample from the healthy donor was filtrated, and euploidy was observed in the FISH assay. FISH, fluorescence *in situ* hybridization.

characteristics, EpCAM+/CD45-/DAPI+ cell counts, and aneuploid cell counts of the collected cells are summarized in Table 1. Aneuploid cells were detected in the peripheral blood of patients with lung cancer. Unexpectedly, aneuploid cells were also detected in the peripheral blood of patients with benign lung diseases, such as empyema necessitatis and non-tuberculous mycobacterial lung disease, which served as negative controls.

Discussion

For better identification of CTCs in patients with lung cancer, we performed both immunofluorescent staining of EpCAM+/CD45-/DAPI+ cells and chromosomal FISH assays on the cells collected from the size-based filtration platform. The collected cells in the peripheral blood of patients with lung cancer showed aneuploidy. Unexpectedly, aneuploid cells were also detected in the peripheral blood of patients with benign lung diseases, such as empyema necessitatis and non-tuberculous mycobacterial lung disease. Furthermore, the enumeration of aneuploid cells did not correlate with the immunofluorescence staining results. Pantel et al. [8] have shown that circulating epithelial cells in patients with benign colon diseases were detected as false positives using both CellSearch and epithelial immunospot assays. Positive events were detected most frequently in patients with benign colon diseases, such as diverticulosis and Crohn disease.

FISH is a highly reliable method for detecting aneuploidy in chromosomal assays. It has high sensitivity (>90%) and specificity (100%), and it also has a low false-negative rate (<10%) and false-positive rate (0%) [9]. Thus, FISH assays pose little potential for false positives in patients with benign lung disease.

The link between aneuploidy and inflammatory diseases has been highlighted by numerous studies, indicating that aneuploidy is not a unique feature of cancer [10,11]. According to Tsai et al. [10], the copy numbers of chromosomes in mouse embryo cells infected with mycoplasma were observed to change with each passage, indicating that mycoplasma infection can induce aneuploidy in infected cells. In addition, Lay et al. [11] have shown that a high number of nuclei were cells with aneuploidy induced by *Mycobacterium tuberculosis* infection. Furthermore, granulomas in inflamed tissues have been observed to form giant cells that block mitotic cell division that would inhibit the inflammatory response.

We report, for the first time, evidence that aneuploid cells can also be detected in the blood samples of patients with benign disease. However, the source of these cells is not clear. In future studies, comparisons should be performed on peripheral blood and tumor tissue samples from patients with lung cancer as well as patients with infectious diseases to determine the actual origin of the aneuploid cells.

The specificity and sensitivity of CTC identification are important for cancer diagnosis. Our platform can detect CTCs, but the collected cells have to be char-

acterized using other techniques to increase the accuracy of cancer detection. Flaig et al. [12] have reported that FISH is a good prognostic marker for detecting CTCs, comparable to EpCAM-positive selection platforms in urothelial cancer patients. Counts of CTCs collected by either density gradient centrifugation or filtration from the blood of prostate, ovarian, and colorectal cancer patients are correlated with the aneuploid cell counts from FISH in the same patients [13]. In our study, positive chromosomal abnormalities were not detected in healthy donor samples (Fig. 1), but they were detected in both patients with cancer and patients with benign infectious diseases. Our report thus suggests the risk of false positives and raises concerns regarding the reliability of the FISH assay for cancer diagnosis.

In conclusion, we evaluated EpCAM+/CD45−/DAPI+ cells and aneuploid cells from the cells collected by a size-based filtration platform. Aneuploid cells were detected in the peripheral blood of patients with lung cancer and, unexpectedly, in the peripheral blood samples of patients with benign lung diseases, such as empyema necessitatis and non-tuberculous mycobacterial lung disease, which served as the negative control group. We conclude that chromosomal abnormalities can also be detected in patients with benign infectious diseases of the lung, thus indicating that they are not a unique property of tumor cells.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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