Retaining ALK Rearrangement in Cultured Circulating Tumor Cells Derived from Lung Cancer Patients

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PURPOSE

The isolation and culture of CTCs can be applied as a substitute method for tumor tissue biopsy, and may provide many clinical applications, including genomic analysis of tumor and personalized cancer therapy according to the genomic information. This method could provide better understanding of tumor metastasis and noninvasive monitoring the disease progression.

INTRODUCTION

Circulating tumor cells (CTCs) are present in the blood of cancer patients at low concentrations. It has been proposed that CTCs may be a prospective prognostic marker for cancer progression in several types of cancer (1, 2) and a potential source of the metastatic tumor cells(3, 4). Viable CTCs isolated from cancer patients can be a useful tool for identifying molecular targets and developing new cancer treatments (5).

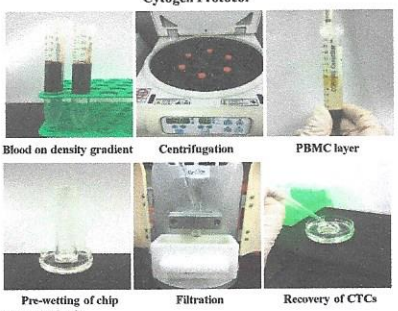
Lung adenocarcinoma is the most common subtype of lung cancer today. Recently, the treatment paradigm for advanced non-small cell lung cancer (NSCLC) has been transformed from conventional chemotherapy to targeted therapy based on molecular aberrations in primary tumor (6). Now, it has been regarded as standard procedure to test lung carcinoma for the presence of *EGFR* mutation and *ALK* rearrangement upon diagnosis, in order to select patients for targeted therapy. However, the detection of such molecular abnormalities is complicated due to the difficulty in obtaining tumor material from repeated tissue biopsies (7). Here, we were able to obtain sufficient amounts of CTCs through CTC cultures, and analyzed cultured CTCs to confirm preexisting ALK rearrangement.

MATERIALS AND METHODS

Blood collection
Blood samples (5-10 ml) from advanced NSCLC cancer patients

Primary culture of CTCs
Cytogen protocol (8)
Culturing in growth medium (RPMI-1640 with 10% FBS, 2% antibiotic-antimycotic) at 37°C, 5% CO_2 for 16-18 days

Cytogen Protocol



Immunofluorescence analysis
EpCAM (Cell Signaling), CD45 (Santa Cruz), DAPI staining

Immunocytochemistry
EpCAM (Cell Signaling)

Quantitative real-time PCR for EML4-ALK fusion detection
AmoyDx EML4-ALK Fusion Gene Diagnostic Kit (Amoy Diagnostics Company Ltd.)

CONCLUSION

In our study, we showed that all **FISH results in primary tumors were corresponding with real-time PCR results in cultured CTCs**. Use of **the cultured CTCs for molecular analysis has a merit of non-invasiveness** and it could be easily repeated at different time points during treatment to guide therapeutic decisions in a patient's treatment course.

For the successful application of this strategy to clinical practice, CTC culture conditions will be further optimized. In addition, further confirmative characterization methods, such as different cell marker staining and molecular profiling, should be developed for precise identification of cultured CTCs.

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RESULTS

CTC cultures

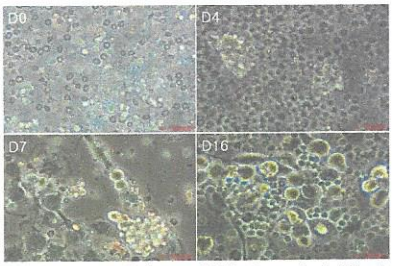


Figure 1. Representative images of CTC culture at day 0, 4, 7, and 16 (X400).

Characterization of CTC features

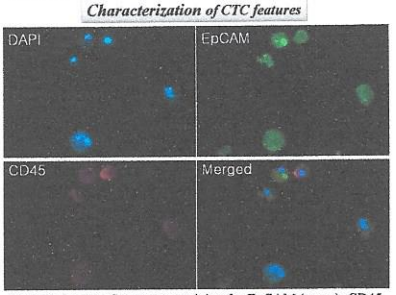


Figure 2. Immunofluorescent staining for EpCAM (green), CD45 (red), and nuclei (blue) (X400).

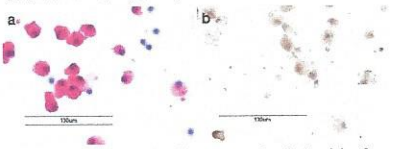


Figure 3. H&E staining (a) and immunocytochemical staining for EpCAM (b) of cultured CTCs.

Identification of preexisting ALK rearrangement in primary tumor tissue

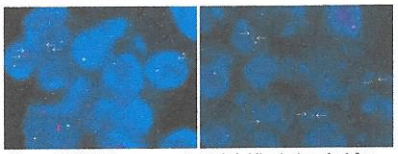
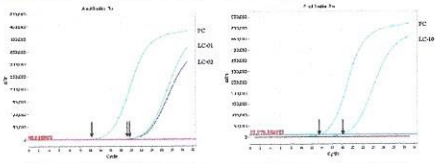


Figure 4. FISH (Fluorescence *in situ* hybridization) method for the detection of *ALK* rearrangement in lung cancer patient's tissue

Identification of preexisting ALK rearrangement in cultured CTCs



	Ct(t)	Ct Threshold
LC01	19.55	2402.4
LC02	19.63	2402.4
NC	ND	1963
PC	12.77	1963
NTC	ND	1963

	Ct(t)	Ct Threshold
LC09	ND	12,079.1
LC10	18.15	12,079.1
NTC	ND	12,079.1
PC	13.79	12,079.1

Figure 5. Real-time PCR analysis of cultured CTCs for ELM4-ALK rearrangement.

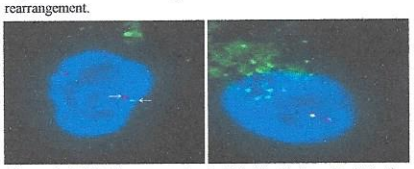


Figure 6. FISH (Fluorescent *in situ* hybridization) method for the detection of *ALK* rearrangement in cultured CTCs from lung cancer patient