ALK rearrangement analysis in circulating tumor cells of lung cancer patients

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ABSTRACT

The evaluation of ALK rearrangement in non-small-cell lung cancer (NSCLC) is a significant tool when considering chemotherapy. It is not always possible to perform a tumor biopsy in patients. We suggest isolation and culturing of circulating tumor cells (CTCs) as an alternative tool to a tumor biopsy for the diagnosis of ALK rearrangement. From 22 patients with NSCLC harboring ALK rearrangement, blood samples were collected and divided into two parts: one for immunofluorescence staining of CTC marker and the other for culturing of CTCs. Both samples were processed by size-based filtration, and Cultured CTCs were analyzed for EML4-ALK translocation by fluorescence in situ hybridization (FISH) using Vysis ALK break apart FISH probe kit. CTC culturing was successful in 18 of 22 cases (81.8%). Among 18 cases of successful CTC cultures, 13 cases showed ALK rearrangement positivity (72.2%). Therefore, we suggest that the CTCs can be used as an alternative method to tissue biopsy for diagnosing ALK rearrangement. In addition, this method may have clinical applications including serial blood sampling for the development of personalized cancer therapy based on individual genomic information.

OBJECTIVES

To evaluate the isolation and culture of circulating tumor cells (CTCs) as an alternative to tumor biopsies for the diagnosis of ALK rearrangement

METHODS

Blood collection and CTC enrichment

Blood samples (15 ml) from NSCLC patient were collected and processed within 4 hrs. Cells enriched from 5 ml of blood were immunostained for identification of CTCs. Remaining cells were used for CTC culturing.

Immunofluorescence analysis

EpCAM, CD45, DAPI

CTC Culturing

Enriched CTCs were collected and cultured in growth medium at 37° C, 5% CO2. After 16-18 days of culturing, cells were fixed in 4% paraformaldehyde for FISH analysis.

ALK FISH (Fluorescent In Situ Hybridization)

Vysis ALK break apart FISH probe kit

RESULTS

Table1. Clinical characteristics of patients.

	No. (%)	
Gender	Male: 8 (36%)	Female: 14 (64%)
Age	32 ~ 82	Average 58.4
Smoking status	Yes: 8 (36%)	No: 14 (64%)
Histologic subtype	21 Adenocarcinomas (21 Mucinous carcinoma, 1 Undifferentiated carcinoma)	
ALK positive rate in biopsy	18 ~ 80 %	Average 41.2%
Metastasis	Yes: 20 (81%)	No: 2 (9%)
Chemo or Radiation Therapy	Yes: 22 (100%)	No: 0 (0%)
Additional Crizotinib Treatment	Yes: 15 (68%)	No: 7 (32%)
Current Disease status	Complete response: 0 (0%)	Stable disease: 8 (36%)
	Partial response: 3 (14%)	Progressive disease: 11 (50%)

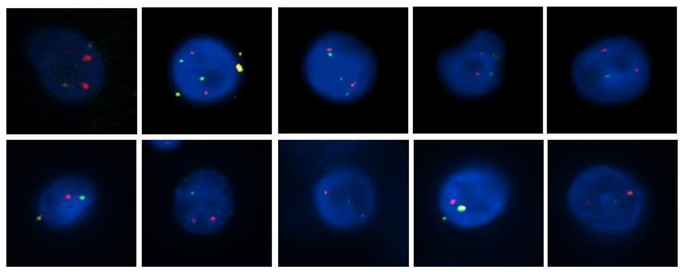

Figure2. Representative FISH images for the detection of ALK rearrangement in CTC from NSCLC patients

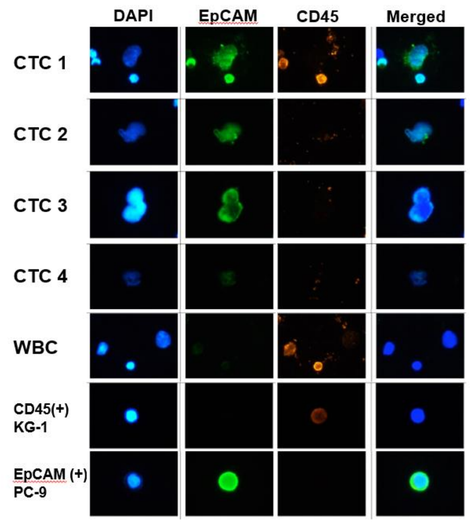

Figure2. Immunofluorescent staining of CTC for EpCAM and CD45.

CONCLUSIONS

we suggest that the CTCs can be used as an alternative method to tissue biopsy for diagnosing ALK rearrangement. In addition, this method may have clinical applications including serial blood sampling for the development of personalized cancer therapy based on individual genomic information.