INTRODUCTION

- Presence of circulating tumor cells (CTCs) has been correlated with progression and metastasis in various malignancies.
- Isolation and characterization of CTCs at the protein or nucleic acid level have potential for the development of minimally invasive methods to detect molecular alterations in patients with advanced cancers.
- To address the lack of an efficient CTC isolation technology that can successfully isolate viable CTCs from a wide variety of cancer types including different subsets of CTCs such as cells in the epithelial mesenchymal transition, ApoCell has developed a multiplex assay using ApoStream® and to detect genetic abnormalities including EGFR mutations and ALK gene rearrangement in the isolated CTCs.

METHODS

- An immunofluorescent multiplex panel for CTC detection was developed using a sequential protocol involving a cocktail of antibodies targeting epithelial and mesenchymal phenotypes.
- Hematopoietic (CD45, CD15), epithelial (EpCAM, CK, E-Cadherin), and mesenchymal (N-cadherin, vimentin, β-catenin, Twist-1) markers were used.
- Nuclear marker DAPI was also included.
- specificity of the multiplex CTC enumeration assay was verified using control cell lines and PBMCs.
- Blood from 34 advanced NSCLC patients and 11 normal donors was collected.
- Isolation and characterization of CTCs at the protein or nucleic acid level were performed.

RESULTS

- Enumeration of CTC Phenotypes
- 25 of 34 patients (74%) had detectable levels of CTCs (epithelial+ or mesenchymal+), with a mean of 86 CTCs/8 mL of blood (range 19 - >500 CTCs per 8 mL blood), above the cut-off value (18 CTCs per 8 mL) established from normal donors.
- CTCs from all 25 patient samples exhibited mesenchymal phenotype while 12 (70%) patients also had CTCs of epithelial lineage.
- EGFR positive CTCs were not detected in a majority of patient samples.
- EGFR mutations (T790M, L858R) were detected in CTCs of 42% of patients.
- CTCs from epithelial and/or mesenchymal lineages were detected in 25 of 34 (74%) advanced NSCLC patients.
- From all patients, CTCs were isolated from peripheral blood of patients with NSCLC patients. ALK rearrangement was detected in single CTCs isolated from patient blood.
- CTCs from all 25 patients were isolated and examined for the presence of ALK rearrangement.
- ALK rearrangement positive CTCs were detected in 10 out of 24 patients (42%).
- ALK gene rearrangement was detected by FISH in single CTCs isolated from peripheral blood of patients with EML4-ALK fusion in their tissue biopsy.
- The results from this study successfully demonstrated the ability to use ApoStreamTM to enrich a phenotypically heterogeneous population of CTCs from peripheral blood of NSCLC patients and these cells were confirmed to carry genetic abnormalities through molecular assays.

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REFERENCES