Review

Circulating Biomarkers in Non–Small-Cell Lung Cancer: Current Status and Future Challenges

Alexios Matikas,[1] Konstantinos N. Syrigos,[2] Sofia Agelaki[1,3]

Abstract

Despite recent advances, non–small-cell lung cancer remains a devastating disease and carries a grim prognosis. Major contributing factors include difficulties in diagnosing the disease early in its course during the asymptomatic stage and the poor understanding of the biology underlying disease progression. Liquid biopsies, noninvasive blood tests that detect circulating biomarkers such as circulating tumor cells and tumor-derived nucleic acid fragments, are in a rapidly evolving field of research that could provide answers to both of these unmet needs. Herein, we review the relevant data concerning the diagnostic, predictive, and prognostic significance of 3 distinct but potentially complementary circulating biomarkers in non–small-cell lung cancer: circulating tumor cells, cell-free DNA, and microRNAs.

Clinical Lung Cancer, Vol. ■, No. ■, ■-■
Keywords: Cell-free DNA, CTC, Liquid biopsy, miRNA, NSCLC

Introduction

Lung cancer is the leading cause of cancer-related death worldwide.[1] This is partly attributable to the advanced stage of the disease at the time of diagnosis, because more than half of the patients have distant metastases at initial presentation. Multiple efforts have been undertaken toward the detection and screening of early lung cancer, mostly with negative results. Notably, the NLST (National Lung Screening Trial) reported that annual low-dose computed tomography (CT) imaging was effective in reducing lung cancer mortality.[2] Current research concentrates on the refinement of the lung cancer screening process. This includes, among others, efforts toward the discovery of biomarkers suitable for large-scale implementation to improve screening sensitivity by targeting the population at higher risk.

Non–small-cell lung cancer (NSCLC) can now be subdivided into different molecular groups for which specific biomarkers guide treatment selection according to the molecular profile of the tumor.[3] The discovery of epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements revolutionized the treatment of NSCLC by introducing the era of personalized care in this disease. Other actionable genomic alterations include BRAF (B-Raf proto-oncogene, serine/threonine kinase) mutations, MET (MET proto-oncogene, receptor tyrosine kinase) amplification, ROS1 (ROS proto-oncogene 1, receptor tyrosine kinase) and RET (ret proto-oncogene) rearrangements, and HER2 (erb-b2 receptor tyrosine kinase 2) mutations.[4] However, despite the discovery of genetic alterations that drive tumor growth and the development of potent inhibitors that confer high response rates and prolongation of progression-free survival (PFS) in these patients,[5-7] the development of resistance is inevitable. Moreover, most patients are still treated with palliative cytotoxic chemotherapy with dismal results. Thus, it is clear that there are 2 highly unmet needs in lung cancer research: the necessity for early diagnosis, which might lead to prompt therapeutic interventions and higher likelihood of cure, and the optimization of treatment, from a "one size fits all" to a more tailored, personalized approach, adaptive to the consecutive changes of the molecular profile of the tumor.

Indeed, it has now been indisputable that in advanced disease, a repeat biopsy is of paramount importance in an effort to decipher the biology of disease progression after initial response to treatment.[8] Despite its unquestionable value, tissue rebiopsy remains challenging because it is frequently infeasible, it is time consuming, and might lead to biopsy-related complications.[9] Finally, because of the heterogeneity observed within the primary tumor and among the different disease sites, rebiopsy is subjective to sampling bias and might not be representative of the whole molecular profile of the tumor.[10]

Liquid biopsy is an emerging, minimally invasive process that potentially addresses the pressing requirements in early diagnosis and in monitoring of the disease; population-based screening of a

[1]Department of Medical Oncology, University General Hospital of Heraklion, Heraklion, Crete, Greece
[2]Oncology Unit, 3rd Department of Internal Medicine, Sotiria General Hospital, National & Kapodistrian University, Athens School of Medicine, Athens, Greece
[3]Laboratory of Translational Oncology, University of Crete, School of Medicine, Heraklion, Crete, Greece

Submitted: Mar 8, 2016; Revised: May 31, 2016; Accepted: May 31, 2016

Address for correspondence: Sofia Agelaki, MD, PhD, Department of Medical Oncology, University General Hospital of Heraklion, Voutes PO Box 1352, 71110 Heraklion, Crete, Greece
Fax: +30 2810543601; e-mail contact: agelaki@uoc.gr

1525-7304/$ - see frontmatter
http://dx.doi.org/10.1016/j.cllc.2016.05.021

specific biomarker could be easily undertaken with minimal risks. Moreover, in lung cancer patients, liquid biopsy could capture the molecular diversity of the disease, whereas the ease of serial testing facilitates the monitoring of its spatial and temporal genomic evolution. Two categories of biomarkers presenting particular interest in multiple tumors including lung cancer, are circulating tumor cells (CTCs) and circulating cell-free nucleic acids, such as DNA and microRNA (miR). The purpose of this review is to summarize the current available data concerning the contribution of the aforementioned circulating biomarkers in the management of patients with NSCLC.

Circulating Tumor Cells

The blood-borne dissemination of tumor cells and the subsequent development of distant metastases is considered to be one of the hallmarks of cancer.[11] CTCs are shed from the primary tumor into the circulation and are considered to contribute to cancer progression via the process of "self-seeding." CTCs are not isolated in healthy control subjects[12] although circulating epithelial cells have been isolated in patients with nonmalignant diseases.[13] The malignant nature of lung cancer CTCs has been shown in patients with either chemosensitive or chemorefractory small-cell lung cancer (SCLC) because they were shown capable of forming tumors in immune-compromised mice.[14]

Circulating tumor cells were introduced as a clinical research tool 10 years ago, and to date multiple methods have been developed for their isolation, characterization, and enumeration. These techniques present great variability in CTC detection rates, sensitivity, and specificity,[15] reviewed in detail elsewhere.[16] More commonly, CTC assays use an initial enrichment step that uses epithelial markers to deplete blood-derived cell populations and a detection step to identify CTCs. However, the rarity of CTCs, the lack of a consensus definition on biomarker expression in CTCs, and the significant inter- and intrapatient heterogeneity of these cells, represent significant challenges in CTC detection. Although analytical and clinical validity was shown for numerous methods, to date the CellSearch (Veridex) system remains the only US Food and Drug Administration-approved technology for CTC detection and enumeration in metastatic breast, colorectal, or castration-resistant prostate cancer.[17-19] CellSearch (Veridex) uses an epithelial cell adhesion molecule (EpCAM)-based enrichment step and identifies CTCs as intact enucleated cells staining positive for cytokeratins and negative for the leucocyte marker CD45.[12] One major disadvantage of the platform lies in its inability to identify tumor cells that express no/low levels of EpCAM by undergoing epithelial to mesenchymal transition.

The isolation according to size of epithelial tumor cells technology (ISET; RareCell Diagnostics, Paris, France) allows the isolation of CTCs independently of any surface marker.[20] On the basis of this assay, epithelial tumor cells can be isolated using filtration because they are broadly larger in size compared with the peripheral blood leukocytes. ISET (RareCell Diagnostics) permits further phenotypic and molecular characterization of CTCs because selected tumor cells can be recovered using laser microdissection for genetic characterization or can be immunostained for further phenotypic characterization. Several studies have been designed to compare ISET (RareCell Diagnostics) and CellSearch (Veridex) systems regarding their efficacy to detect CTCs in NSCLC patients. The ISET (RareCell Diagnostics) technology permitted higher CTC isolation rates compared with the CellSearch system (Veridex), in early (in 104 patients [50%] vs. 82 patients [39%], respectively) and in advanced NSCLC (in 32 patients [80%] vs. 9 patients [23%], respectively).[21,22] Interestingly, a concordance rate of only 20% between the 2 methods was depicted among patients with early disease. Furthermore, only 39% of the morphologically malignant cells detected using ISET (RareCell Diagnostics) expressed cytokeratins and 11% of them showed vimentin without cytokeratin expression, pointing toward cells bearing an epithelial to mesenchymal transition phenotype. Another promising method of CTC detection is the CTC-chip, which has shown superior detection rates of approximately 100% in metastatic NSCLC.[23] CTC-chip is a microfluidic-based platform capable of separating CTCs from peripheral blood samples on the basis of the interaction of target CTCs with EpCAM-coated microposts in laminar flow conditions, and without requisite prelabeling or processing of samples.

Considering these statements, it is obvious that the lack of a standardized method hinders the ability to properly evaluate the role of CTCs as biomarkers in NSCLC. Also, the CTC identification yield of the current techniques is lower compared with other malignancies including SCLC.[24] However, several efforts with considerable success have been placed concerning the putative diagnostic, predictive, and prognostic role of CTCs in NSCLC.

Diagnosis

Using the CellSearch system (Veridex), Tanaka et al investigated the role of CTC enumeration in the discrimination between primary lung cancer and nonmalignant diseases and in the prediction of distant metastasis. In a cohort of 150 patients clinically suspected to have, or with a diagnosis of primary lung cancer, 38 [30.6%] lung cancer patients and 3 [12.0%] patients with nonmalignant disease had detectable CTCs. Although CTC counts were higher in lung cancer patients, a receiver operating characteristic (ROC) curve analysis showed an insufficient capability of the CTC counts in discriminating between patients with lung cancer and nonmalignant disease (area under ROC [AUC-ROC] curve for CTC count, 0.598 [$P = .122$]). However, the AUC-ROC curve in the prediction of distant metastasis was 0.783 (95% confidence interval, 0.679-0.886; $P < .001$), suggesting that CTC count is a useful surrogate marker of distant metastasis in primary lung cancer.[25] Using the ISET (RareCell Diagnostics) system, CTCs were detected in 5 [3%] chronic obstructive pulmonary disease (COPD) patients and in none of control smoking and nonsmoking healthy individuals. The annual surveillance of the CTC-positive COPD patients using CT-scan screening, detected lung nodules 1 to 4 years after CTC detection, allowing for prompt surgical resection and histopathological diagnosis of early-stage lung cancer. These results suggest that monitoring "sentinel" CTC-positive COPD patients might allow early diagnosis of lung cancer.[26] Further evidence of the diagnostic value of CTC enumeration using the ISET (RareCell Diagnostics) system was provided by Fiorelli et al, who reported that a CTC count of > 25 had high sensitivity and specificity for the differentiation between benign and malignant disease.[27] In another report, CTCs have also been shown capable of differentiating between patients with stage IIIA, IIIB, and IV NSCLC.[28]