Editorial

Looking for sputum biomarkers in lung cancer secondary prevention: where are we now?

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Secondary prevention (i.e., early detection) is fundamental to enlarge the window of opportunity for potentially curative treatments in lung cancer patients. The National Lung Screening Trial (NLST) demonstrated that low-dose computed tomography (LDCT)-based screening of high-risk individuals is an effective strategy, leading to 20% relative reduction in lung cancer-specific mortality (1). Despite this clinical benefit, the widespread employment of this screening approach has one major limitation: only less than 4% of LDCT-detected lung nodules are malignant (1). The high false-positive rate would result in anxiety and fear for the patients and their family, and unnecessary invasive procedures with subsequent unbearable medical expenses. Thus, it is imperative to develop additional interventions that might be used alone or, more realistically, to enhance the cost-effectiveness of LDCT-based identification of early-stage lung cancers.

With the dawning of molecular pathology technologies in the clinic, there have been extensive efforts to integrate lung cancer imaging-based screening with cutting-edge molecular tests on biological fluids, such as blood, sputum, urine, or saliva (2-13). The interrogation of molecular biomarkers in these noninvasively and easily accessible biosources has the potential to restrict the false positive rate of LDCT, with the identification of tumor-related molecular aberrations (14). To date, a multitude of analytic approaches has been proposed for the early detection of lung cancer based on different sample types, biomarkers, and available devices.

Several sputum biomarkers have been developed during the past few years for lung cancer early identification, including DNA mutations, loss of heterozygosity (LOH), micro RNAs (miRNAs), messenger RNA (mRNA), free DNA, and DNA hypermethylation (14). Lately, chromosomal aneusomy assessed by fluorescence *in situ* hybridization (15) and even the sputum microbiome has been also investigated (16). Not surprisingly, each of these tools has specific limitations that should be considered for their implementation in the everyday clinical practice.

Searching for DNA mutations, for instance, is a very sensitive, low-cost, rapid, and simple method that can be easily carried out on sputum samples in most pathology laboratories. Unfortunately, lung cancers, and particularly non-small cell lung cancers, have only a few highly recurrently mutated genes (e.g., *TP53*, *KRAS*, *EGFR*) and hotspot mutations are relatively rare (17,18). Likewise, the LOH analysis in the sputum has relevant disadvantages, given that the percentage of tumor cells in the sputum is extremely low and allelic unbalances are present at very low frequency in the lung (9). For both mutational and LOH analyses in sputum, novel and efficient tumor cell enrichment techniques coupled with ultra-sensitive detection methods are expected. Another family of important biomarkers in lung cancer screening is represented by miRNAs. These small non-coding RNA molecules are stably present in the sputum and are currently

viewed with optimism in lung cancer screening (19). Various miRNAs have been identified as potential sputum biomarkers, such as miRNA-210, 21, 31, 143, and 155 (20). A tailored multi-miRNA panel could lead to high sensitivity and specificity levels while discriminating between healthy individuals and lung cancer patients.

DNA methylation is one of the earliest epigenetic events in lung cancer and occurs in most of the neoplastic cells. The fact that silencing genes through hypermethylation or activating genes through hypomethylation play an important role in the initiation and progression of lung cancer, has stimulated the development of screening approaches to identify additional genes and pathways that are disrupted within the epigenome. The advance of high-fidelity sequencing technologies has made the methylation detection a stable and cheap test that could provide the necessary conditions for clinical utility in sputum analysis. In this scenario, methylation-specific PCR (MSP) in sputum samples is emerging as a powerful method for lung cancer secondary prevention. To date, panels of cancer-associated methylated genes, rather than a single gene, are preferred in lung cancer risk stratification. There is a growing body of literature on this topic, confirming the high expectations in methylation-based secondary prevention programs (14). However, none of these biomarkers have been universally implemented in the clinical screening workup.

In a recent study, Leng and collaborators employed an 8-gene panel for the study of DNA methylation in the sputum as a classifier for lung cancer risk in clinically CT-screen eligible smokers (21). The eight genes included *CDKN2A*, *MGMT*, *DAPK*, *RASSF1A*, *GATA4*, *GATA5*, *PAX5A*, and *PAX5B*. The performance of this panel was previously assessed in a nested case-control study (22). In the current study, the authors explored the accuracy of these sputum biomarkers as an aid in improving lung cancer risk stratification to define high-risk individuals eligible for CT-screening. They analyzed three large cohorts, including stage I surgically resected lung cancer patients (from ECOG-ACRIN trial n=487), and current and former smokers at high risk (from Lovelace n=1,380 and PLuSS trials n=718). The prevalence of gene methylation events was significantly increased in lung cancer smokers compared to cancer-free smokers. Joining molecular analysis and clinical variables for CT screen eligibility, increased prediction accuracy from approximately 75% to 90% and specificity from 25% to 54%, at 95% of sensitivity. So far, the addition of these methylation biomarkers to the clinical data may be able to reduce the false positive rates of LDCT and increase lung cancer detection rates.

The assessment of the clinical utility of these lung cancer screening biomarkers requires multi-institutional prospective clinical trials. However, it is not trivial as it requires very large populations of high-risk individuals to obtain statistically powerful numbers of cases (i.e., patients who develop lung cancer) and thus, acceptable results for clinical approval. Moreover, the relatively low specificity shown in the study by Leng and collaborators might be overcome by the combination of different biomarkers from the same or different biosources. DNA methylation and miRNA are both modulators of gene expression and play critical roles in various cellular and cancer processes. Furthermore, DNA methylation is associated also with the regulation of miRNAs (23), suggesting that MSP and miRNAs profiling could be synergic in lung cancer screening. Ad hoc meta-analysis and clinical trials are required to establish the best combination strategy. Finally, the right time of the molecular evaluation (e.g., before or after imaging-based screening), as well as the population eligible for molecular screening, should be determined. Indeed, lung cancers are vastly heterogeneous, encompassing a wide spectrum of tumors with genetic, epigenetic, transcriptomic, histologic, and clinical differences. These characteristics show impressive levels of diversity in specific subpopulations, based on ethnicity, world areas, economic factors, professional and personal behavior, and individual susceptibility. In this era of precision medicine, the definition of high-risk populations based solely on the age and smoking status appears to be suboptimal, as different populations may be at risk for different diseases, and that different diseases may need to be detected using different methods. Additional criteria to stratify high-risk individuals into clinically meaningful subgroups should be implemented to allow for tailored molecularly-driven secondary prevention programs. So, where are we now? The combination of molecular testing and imaging screening remains the highway to be journeyed for lung cancer secondary prevention, providing its clinically utility and cost-effectiveness. To this end, sputum methylation analysis might represent one of the valuable tools in this combined screening strategy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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