DNA methylation signatures in circulating cell-free DNA for the monitoring of at-risk populations progressing to lung cancer

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Alteration of DNA methylation patterns at the 5′ position of cytosines, in the context of CpG dinucleotides, is one of the most promising epigenetic biomarkers for many biomedical applications and has been actively studied in many diseases and developmental questions for the last twenty years (How Kit et al., 2012). In comparison to genetic alterations such as mutations, which are usually distributed throughout the gene, DNA methylation alterations are concentrated in a defined area, often the promoter, facilitating analysis. DNA methylation can be reliably analyzed in freshly frozen and archived clinical specimens, but more importantly, also in biofluids, that were either in direct contact with the disease (e.g. stool for colorectal, urine for genitourinary or sputum, bronchoalveolar lavages or brushes for lung cancer) or in cell-free circulating DNA isolated from serum or plasma of patients (How Kit et al., 2012).

Although it is currently unclear if DNA methylation changes are the cause or the consequence of the disease, it is clear that DNA methylation changes occur early during disease pathogenesis and precede disease detection by histological and/or imaging methods. Several DNA methylation-based biomarkers are already commonly used in the clinic, such as the testing of the DNA methylation status of the MGMT promoter for the prediction of the response to chemotherapy using alkylating agents in glioblastoma or of MLH1 for the diagnosis of Lynch Syndrome. Commercial products such as the Epi proColon test, analyzing methylation in the SEPT9 gene (Church et al., 2014) for the population-wide screening for colorectal cancer and approved by the Chinese FDA in July 2015, and the Epi proLung (SHOX2) test (Ilse et al., 2014) are now available. Panels with multiple genes can further improve the specificity and sensitivity of DNA methylation signatures as recently demonstrated in a stool-based screening test for colorectal cancer ((Imperiale et al., 2014), Cologuard, Exact Sciences), where DNA methylation alterations were combined with mutation detection and which received the first US-FDA approval for a DNA methylation-based diagnostic test in 2014.

Lung cancer is the most common cancer in the world and few effective and cheap methods are available for its early detection and screening, especially in at-risk populations such as heavy smokers or patients with chronic obstructive lung disease (COPD) and fibrotic interstitial lung diseases (ILDs). Although histological and cytological examinations are currently the gold standard in lung cancer diagnosis, patients are often at late stages when diagnosis is confirmed. Therefore, there is an urgent need for new diagnostic methods to increase the early diagnostic rate, enhance the confirmed diagnostic rate, monitor potential disease progression to lung cancer in high-risk populations, and improve the differentiation of lung cancer from other lung diseases to avoid overtreatment of patients.

While epigenetic changes are well characterized in lung cancer (Liloglou et al., 2014), our understanding of epigenetic changes in COPD and ILD is still in its infancy, and DNA methylation changes that might be of potential therapeutic importance have been identified (Kabesch and Adcock, 2012; Hagood, 2014). Furthermore, smoking has shown in several epigenome-wide association studies to yield highly reproducible DNA methylation changes at specific loci such as F2RL3 and ARHHD that could contribute to the health risks associated with smoking (Zeilinger et al., 2013).

In the current issue, Wielchier et al. (2015) develop a four gene DNA methylation signature for the detection of lung cancer and its differentiation from other lung diseases with a high risk of cancer development, such as COPD and ILD, in retrospective cohorts and provide a paradigm for the development of DNA methylation-based diagnostic assays. They translate findings in the tissue to a potential diagnostic test in cell-free circulating DNA that can be easily obtained with minimally invasive procedures. The study distinguishes itself positively from the many so-called “biomarker” studies using DNA methylation alterations by the use of relatively large cohorts and an analysis strategy based on solid and appropriate statistics.

Starting from a genome-wide screen of DNA from 129 lung tissues with the widely used illumina 450K BeadChip, the authors identify a panel of 222 DNA methylation-based markers that distinguish either the different disease groups or any disease from healthy tissue. Using a technology that can potentially be easily transferred into a clinical laboratory (digestion with a methylation-sensitive restriction enzyme followed by multiplexed real-time PCR amplification), a panel of 63 multiplex PCR assays was devised and the DNA methylation patterns were analyzed in 204 sera/plasma yielding a high AUC of 0.91 for lung cancer and slightly less for ILD and COPD. Reducing the signature to a laboratory (digestion with a methylation-sensitive restriction enzyme followed by multiplexed real-time PCR amplification), a panel of 63 multiplex PCR assays was devised and the DNA methylation patterns were analyzed in 204 sera/plasma yielding a high AUC of 0.91 for lung cancer and slightly less for ILD and COPD. Reducing the signature to a
prospective studies, especially in patients with COPD and ILD progressing to lung cancer, will be required to assess the clinical value of the presented signature.

While this study is of high quality and analyzes a larger number of samples than most DNA methylation studies, the large number of histologic subgroups together with their subclassification such as TNM for lung cancer and GOLD for COPD makes the validation in multi-centric international collaborations for larger cohorts necessary, potentially going back to a larger gene panel to optimize sensitivity and specificity. Furthermore, it will be important to combine and compare the signature developed by Wielscher et al. (2015) with DNA methylation markers including SHOX2 (Ilse et al., 2014) and other previously reported markers (Liloglou et al., 2014) as well as signatures that have been developed for the early detection and prognosis of lung cancer (Sandoval et al., 2013). Finally, as for other DNA methylation markers and signatures it will be necessary to assess the specificity of the signature by analyzing the DNA methylation changes in other solid tumors at the loci included in the signature.

The presented work is a first major step potentially allowing the detection of lung cancer at early stages, and more importantly the monitoring of at-risk populations for progression into lung cancer. The workflow presented in the study should be an example for future DNA methylation based biomarker studies. However, extensive validation and replication will be required to assess fully its clinical value and utility.

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