



# Early detection of lung cancer based on DNA methylation analysis in sputum and plasma

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Lung cancer kills millions of people each year in the world and is the main cause of cancer death in men and the second cause in women (1). Surprisingly, a decade ago, lung cancer was not so frequently diagnosed compared to breast and prostate cancer, explained by the fact that these cancers can be detected at a very early stage. For lung cancer, the five-year survival rate was as low as 15%. However, when lung cancer can be detected at an early stage, the survival rate increases dramatically (2). Using low-dose computed tomography (CT) screening, a 20% reduction in lung cancer mortality has been more recently reported (3). Thus, during this last decade, many efforts have been made to develop innovative technologies to detect lung cancer very early, with a special focus on molecular markers as an alternative approach to different imaging and cytology-based strategies.

The discovery of highly sensitive and specific biomarkers highlighting pathological changes early enough to allow clinical intervention is of great importance for the early detection of lung cancer. During the last decade, epigenetics and particularly research on DNA methylation have provided important information towards a better understanding of lung cancer pathogenesis. Novel and promising molecular biomarkers for diagnosis and prognosis of lung cancer are continuously emerging in this area, requiring further evaluation. This process includes extensive validation in prospective clinical trials before they can be routinely used in a clinical setting. Especially

epigenetic biomarkers for lung cancer, focusing on DNA methylation are highly promising for early detection (4).

In this context, the work of Hulbert *et al.* (5) reported very interesting data on improving the diagnostic accuracy of non-small cell lung cancer (NSCLC) screening by using ultrasensitive methods for the detection of DNA promoter methylation of a lung cancer specific gene panel in sputum and plasma.

In the present study, Hulbert *et al.* combined a previously developed highly sensitive and specific methylation-on-beads (MOB) assay (6) with real-time quantitative methylation-specific PCR (qMSP) to detect the promoter methylation of six genes frequently methylated in lung cancer namely: *SOX17*, *TAC1*, *HOXA7*, *CDO1*, *HOXA9*, and *ZFP42*. Patients with suspicious nodules on CT imaging have been enrolled and plasma plus sputum were collected pre-operatively; 150 patients had node negative (stage I and IIA) NSCLC. In addition, 60 subjects with non-cancerous lung lesions have been included as controls. Interestingly, the proportion of smokers, former smokers and never smokers was not different between cancer patients and controls; however, cancer patients smoked more. DNA methylation analysis for six cancer specific gene promoters was performed using real-time qMSP and methylation on beads.

According to the results presented, high diagnostic accuracy for early stage lung cancer can be obtained using a combination of a panel of six genes through their promoter methylation detection in sputum or plasma. The authors have shown that

in patients with early stage NSCLC 5 out of 6 genes studied were significantly higher methylated when compared to the control group ( $P < 0.001$ ). Both sensitivity and specificity for lung cancer diagnosis were high in sputum as well as in plasma. A three-gene combination of the best individual genes had a remarkable sensitivity and specificity (98% and 71% using sputum and 93% and 62% using plasma) and this was verified by receiver operating curve (ROC) analysis as well. The most striking finding is that when combining gene promoter DNA methylation analysis in sputum and plasma with clinical data, the authors could correctly diagnose the presence of early lung cancer in 91% and 85% of cases, respectively.

In this study, it has been clearly shown that by using a non-invasive and relatively simple DNA methylation analysis approach in plasma or sputum for a selected panel of methylated promoter genes a high diagnostic accuracy of early stage NSCLC can be obtained. It is important to note that the promoter methylation levels of these genes were found to be associated with a high NSCLC cancer risk independently of age, pack-year and nodule size. This panel could be used to identify patients at high risk for lung cancer, reducing false positive results, unnecessary tests, and improving the diagnosis of lung cancer at an earlier stage. From this point of view the results of this study are of significant clinical importance.

It has already been previously shown that the promoters of five out of these six genes were highly methylated in early stages of lung cancer. More specifically, the same group has previously shown that DNA methylation of *CDO1*, *HOXA9*, and *TAC1* promoters is cancer specific and can be used as a three-gene panel, for the diagnosis of lung cancer (7). Rauch *et al.* have already shown in 2007 that all four HOX gene clusters on chromosomes 2, 7, 12, and 17 are preferential targets for DNA methylation in cancer cell lines and in early-stage lung cancer (8). They reported that methylation analysis of *HOXA* genes in primary squamous cell carcinomas of the lung led to the identification of the *HOXA7*- and *HOXA9*-associated CpG islands as frequent methylation targets in stage 1 tumors. Moreover, it has already previously been shown that *SOX17* promoter is highly methylated in primary tumors and in corresponding plasma samples both in operable and advanced NSCLC (9). Especially in the advanced setting, *SOX17* promoter methylation in plasma ctDNA has a statistical significant influence on NSCLC patient's survival time, while the detection of *SOX17* promoter methylation in plasma provides prognostic information and merits to be further evaluated as a circulating tumor biomarker in patients with operable and advanced NSCLC (9).

Nowadays, the importance of liquid biopsy, an approach that enables following tumor evolution in real time through the analysis of molecular markers in peripheral blood, is

becoming clearer and clearer (10). In many types of solid cancers, but particularly in NSCLC, obtaining a biopsy of solid tumors requires invasive procedures that strongly limit patient compliance. In contrast, a blood extraction is safe, can be performed at many time points during the course of the disease and encourages appropriate therapy modifications, potentially improving the patient's clinical outcome and quality of life. In lung cancer, numerous molecular alterations such as fusion of the tyrosine kinase genes anaplastic lymphoma kinase (ALK), C-ROS oncogene 1 (ROS 1), rearranged during transfection (RET) and neurotrophic tyrosine kinase 1 (NTRK1) constitute therapeutic targets for tyrosine kinase inhibitors (11) and can potentially be detected in plasma. Especially in lung cancer, liquid biopsy is based on the detection and analysis in the peripheral blood of cancer patients of circulating tumor cells (CTCs) (12), circulating tumor DNA (ctDNA) (13) at the gene mutation level (14), DNA methylation level (15), as well as on the analysis of circulating miRNAs (16) and exosomes (17).

It is now established though many studies that DNA methylation of tumor suppressor genes is a very early step in oncogenesis (18). Recent studies in breast cancer have compared the methylation status of tumor suppressors and metastasis suppressor genes in CTCs and ctDNA (19). *SOX17* promoter was found to be highly methylated in primary breast tumors, in CTCs isolated from patients with breast cancer as well as in corresponding cfDNA samples (19). These findings indicate a direct connection at the DNA methylation level between the presence of CTCs and cfDNA in cancer patients even after surgical removal of the primary tumor. Analysis of cfDNA has revolutionized the clinical care of advanced lung cancer patients undergoing targeted therapies. However, the low concentration of cfDNA in the blood of early-stage NSCLC patients has hampered its use for management of early disease (20). The development of highly specific and sensitive methodologies for the detection and analysis of cancer specific DNA alterations at the DNA methylation level in plasma and sputum are very promising for early stage detection and even screening purposes in NSCLC.

From this point of view, this study is very promising for NSCLC screening at very early stages. The findings of this study should be prospectively validated in clinical studies, encompassing a large number of patients.

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## References

1. Anglim PP, Alonzo TA, Laird-Offringa IA. DNA methylation-based biomarkers for early detection of non-small cell lung cancer: an update. *Mol Cancer* 2008;7:81.
2. International Early Lung Cancer Action Program Investigators, Henschke CI, Yankelevitz DF, et al. Survival of patients with stage I lung cancer detected on CT screening. *N Engl J Med* 2006;355:1763-71.
3. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med* 2011;365:395-409.
4. Balgkouranidou I, Liloglou T, Lianidou ES. Lung cancer epigenetics: emerging biomarkers. *Biomark Med* 2013;7:49-58.
5. Hulbert A, Jusue Torres I, Stark A, et al. Early Detection of Lung Cancer using DNA Promoter Hypermethylation in Plasma and Sputum. *Clin Cancer Res* 2016. [Epub ahead of print].
6. Bailey VJ, Zhang Y, Keeley BP, et al. Single-tube analysis of DNA methylation with silica superparamagnetic beads. *Clin Chem* 2010;56:1022-5.
7. Wrangle J, Machida EO, Danilova L, et al. Functional identification of cancer-specific methylation of CDO1, HOXA9, and TAC1 for the diagnosis of lung cancer. *Clin Cancer Res* 2014;20:1856-64.
8. Rauch T, Wang Z, Zhang X, et al. Homeobox gene methylation in lung cancer studied by genome-wide analysis with a microarray-based methylated CpG island recovery assay. *Proc Natl Acad Sci U S A* 2007;104:5527-32.
9. Balgkouranidou I, Chimonidou M, Milaki G, et al. SOX17 promoter methylation in plasma circulating tumor DNA of patients with non-small cell lung cancer. *Clin Chem Lab Med* 2016;54:1385-93.
10. Alix-Panabières C, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. *Cancer Discov* 2016;6:479-91.
11. Aguado C, Giménez-Capitán A, Karachaliou N, et al. Fusion gene and splice variant analyses in liquid biopsies of lung cancer patients. *Transl Lung Cancer Res* 2016;5:525-31.
12. Carter L, Rothwell DG, Mesquita B, et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. *Nat Med* 2017;23:114-9.
13. Pérez-Callejo D, Romero A, Provencio M, et al. Liquid biopsy based biomarkers in non-small cell lung cancer for diagnosis and treatment monitoring. *Transl Lung Cancer Res* 2016;5:455-65.
14. Normanno N, Denis MG, Thress KS, et al. Guide to detecting epidermal growth factor receptor (EGFR) mutations in ctDNA of patients with advanced non-small-cell lung cancer. *Oncotarget* 2016. [Epub ahead of print].
15. Di Paolo A, Del Re M, Petrini I, et al. Recent advances in epigenomics in NSCLC: real-time detection and therapeutic implications. *Epigenomics* 2016;8:1151-67.
16. Markou A, Sourvinou I, Vorkas PA, et al. Clinical evaluation of microRNA expression profiling in non small cell lung cancer. *Lung Cancer* 2013;81:388-96.
17. Reclusa P, Sirera R, Araujo A, et al. Exosomes genetic cargo in lung cancer: a truly Pandora's box. *Transl Lung Cancer Res* 2016;5:483-91.
18. Esteller M, Sanchez-Céspedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res* 1999;59:67-70.
19. Chimonidou M, Strati A, Malamos N, et al. SOX17 promoter methylation in circulating tumor cells and matched cell-free DNA isolated from plasma of patients with breast cancer. *Clin Chem* 2013;59:270-9.
20. Pérez-Ramírez C, Cañadas-Garre M, Robles AI, et al. Liquid biopsy in early stage lung cancer. *Transl Lung Cancer Res* 2016;5:517-24.

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