ORIGINAL ARTICLE

Detection of Lung Cancer through Metabolic Changes Measured in Blood Plasma

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ABSTRACT

Introduction: Low-dose computed tomography, the currently used tool for lung cancer screening, is characterized by a high rate of false-positive results. Accumulating evidence has shown that cancer cell metabolism differs from that of normal cells. Therefore, this study aims to evaluate whether the metabolic phenotype of blood plasma allows detection of lung cancer.

Methods: The proton nuclear magnetic resonance spectrum of plasma is divided into 110 integration regions, representing the metabolic phenotype. These integration regions reflect the relative metabolite concentrations and were used to train a classification model in discriminating between 233 patients with lung cancer and 226 controls. The validity of the model was examined by classifying an independent cohort of 98 patients with lung cancer and 89 controls.

Results: The model makes it possible to correctly classify 78% of patients with lung cancer and 92% of controls, with an area under the curve of 0.88. Important moreover is the fact that the model is convincing, which is demonstrated by validation in the independent cohort with a sensitivity of 71%, a specificity of 81%, and an area under the curve of 0.84. Patients with lung cancer have increased glucose and decreased lactate and phospholipid levels. The limited number of patients in the subgroups and their heterogeneous nature do not (yet) enable differentiation between histological subtypes and tumor stages.

Conclusions: Metabolic phenotyping of plasma allows detection of lung cancer, even in an early stage. Increased glucose and decreased lactate levels are pointing to an increased gluconeogenesis and are in accordance with recently published findings. Furthermore, decreased phospholipid levels confirm the enhanced membrane synthesis.

Keywords: Lung cancer; ^{1}H-NMR spectroscopy; Metabolic phenotype; Blood plasma biomarker; Risk model

Introduction

Lung cancer is the leading cause of cancer death worldwide, with a 5-year survival rate of around 15%.[1,2] A promising screening tool is low-dose computed

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tomography (LDCT), which has been shown to reduce lung cancer mortality by 20% as compared with chest radiography screening.[3] However, LDCT screening has some disadvantages, such as the high cost associated with screening all patients at risk according to current risk models, radiation exposure, and the low positive predictive value (PPV).[4] Because of these limitations, other detection platforms are being evaluated, all with their advantages and shortcomings.[5]

Over the past decade, accumulating evidence has shown that cancer cell metabolism differs from that of normal cells.[6–8] More specifically, it is reprogrammed to promote cell proliferation and survival and is driven by aberrant signaling pathways induced by the activation of oncogenes/inactivation of tumor suppressor genes.[9] One of the main adaptations of cancer cells is that, even in the presence of normal oxygen levels, they rely on anaerobic energy production through glycolysis, a hallmark known as the Warburg effect.[10] As metabolites are the end products of cellular processes, changes in their concentration reflect alterations in the metabolic phenotype.[11] Proton nuclear magnetic resonance (^{1}H-NMR)-based metabolomics allows a fast, noninvasive identification and quantification of complex mixtures of metabolites, as in plasma.[12–14]

This study aims to (1) investigate whether the plasma metabolic phenotype allows discrimination between patients with lung cancer and controls, (2) evaluate the predictive accuracy of the trained classification model in an independent cohort, in addition to being of help in understanding the changed metabolism, and (3) examine whether the metabolic phenotype allows discrimination between histological subtypes and tumor stages.

Materials and Methods

Subjects

Patients with lung cancer (n = 357) were included in the Limburg Positron Emission Tomography Center (n = 273) (Hasselt, Belgium) and at the Department of Respiratory Medicine of University Hospitals Leuven (n = 84) (Leuven, Belgium) from March 2011 to June 2014. The diagnosis was confirmed by a pathological biopsy or by a clinician specialized in interpreting radiological and clinical lung cancer data. Clinical staging was performed according to the seventh edition of the tumor, node, and metastasis classification.[15] The controls (n = 347) were patients with noncancer diseases who were included at Ziekenhuis Oost-Limburg (Genk, Belgium) between March 2012 and June 2014. The absence of malignant disease was confirmed on the basis of the hospital medical files. A double-check of the medical file was accomplished at the time of the statistical analysis for controls who were misclassified. None had malignant disease at the moment of statistical analysis. For both groups, blood sampling and sample preparation was performed according to a fixed protocol and by trained staff.

The exclusion criteria were as follows: (1) not fasted for at least 6 hours, (2) fasting blood glucose concentration of 200 mg/dL or higher, (3) medication intake on the morning of blood sampling, and (4) treatment or history of cancer in the past 5 years. The study was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice and was approved by the involved ethical committees. Study participants provided informed consent.

Both groups were subdivided into a training cohort and a validation cohort (Fig. 1). More specifically, 250 of the 357 patients with lung cancer and 250 of the 347 controls were randomly assigned to the training cohort, leaving a validation cohort of 107 patients with lung cancer and 97 controls. Forty-one statistical outliers in the training cohort (17 patients with lung cancer and 24 controls) and 17 in the validation cohort (9 patients with lung cancer and 8 controls) were excluded. According to their medical files, they showed abnormal concentrations of glucose, lipids, or ketone bodies. Individuals with high glucose levels were determined to have diabetes or an increased fasting glucose, whereas those with high lipid levels suffered from obesity or hyperlipidemia or were taking cholesterol-lowering medication. Most of the individuals with high levels of ketone bodies levels had a low body mass index.

Blood Sampling, Sample Preparation, and NMR Analysis

The protocols used have been described in detail.[16]

Statistical Analysis

Multivariate statistical analysis was performed using SIMCA-P+ (Version 14, Umetrics, Malmö, Sweden). After mean centering and Pareto scaling of the variables, unsupervised principal component analysis (PCA) was performed to identify outliers by a Hotelling's T2 range test and a distance to model plot. After outliers had been removed, orthogonal partial least squares discriminant analysis (OPLS-DA) was used to train a classification model. The validity of the model was confirmed by (1) permutation testing; (2) classification of an independent cohort with a classification cutoff value of 0.5; (3) receiver operating characteristic curve explorer and tester; and (4) last but not least, comparison with the outcome of an independent model constructed by partial least squares discriminant analysis (PLS-DA) (R Version 3.1.2 [R Foundation for Statistical Computing, Vienna, Austria]). An S-plot was used to identify the most