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Research paper

Circulating metabolite profiles to predict overall survival in advanced non-small cell lung cancer patients receiving first-line chemotherapy

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ABSTRACT

Objectives: The prognosis for advanced-stage non-small cell lung cancer (NSCLC) is usually poor. However, survival may be variable and difficult to predict. In the current study, we aimed to identify circulating metabolites as potential predictive biomarkers for overall survival of advanced-stage (III/IV) NSCLC patients treated with first-line platinum-based chemotherapy.

Materials and methods: Using two-stage study design, we performed global metabolomic profiling in blood of 220 advanced-stage NSCLC patients, including 110 with poor survival and 110 with good survival. Metabolomic profiling was conducted using Metabolon platform. The association of each metabolite with survival was assessed by Cox proportional hazard regression model with adjustment for covariates.

Results and conclusion: We found levels of 4 metabolites, caffeine, paraxanthine, stachydrine, and methyl glucopyranoside (alpha + beta), differed significantly between NSCLC patients with poor and good survival in both discovery and validation phases ($P < 0.05$). Interestingly, majority of the identified metabolites are involved in caffeine metabolism, and 2 metabolites are related to coffee intake. In fact, caffeine metabolism pathway was the only significant pathway identified which significantly differed between NSCLC patients with poor and good survival ($P = 1.48E-07$) in the pathway analysis. We also found 4 metabolites whose levels were significantly associated with good survival in both discovery and validation phases. Strong cumulative effects on overall survival were observed for these 4 metabolites. In conclusion, we identified a panel of metabolites including metabolites in caffeine metabolism pathway that may predict survival outcome in advanced-stage NSCLC patients. The identified small metabolites may be useful biomarker candidates to help identify patients who may benefit from platinum-based chemotherapy.

1. Introduction

Lung cancer is the one of the most common cancers, and the most common cause of cancer-related death in men and second most common in women [1]. The high mortality of lung cancer is mainly due to late diagnosis, resulting in poor response to clinical intervention [2]. Platinum-based chemotherapy is the standard treatment for advanced-stage (III/IV) NSCLC [3]. Although the prognosis is dismal, significant variations still exist. Unfortunately, current prognostic prediction tools, mainly built upon clinicopathological characteristics and proliferation markers, are not sufficiently accurate to predict which patients have virtually no benefit from the standard treatment and would, therefore, be candidates for alternative therapy, and which patients have high response to standard treatment such that more intensive therapy would not be required [4,5]. For example, downregulated survivin has been found to be significantly correlated with improved overall survival in NSCLC patients who received platinum-based therapy. However, the potential of survivin as a prognostic marker is variably reported depending on the lung cancer subtype and stage [4,6]. Large scale molecular characterization in tumor tissues may help, but somatic information for gene mutation and expression profiles are still rarely available in clinical practice. Therefore, there is an urgent need for novel, clinically useful prognostic biomarkers including noninvasive biomarkers for advanced-stage lung cancer.

Altered metabolic homeostasis plays important roles in the development of tumors [7]. Metabolites are sensitive to physiologic and pathologic stimuli, environmental exposures, pharmaceutical interventions, and have potential to serve as indicators of the overall

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physiological status, such as cancer. Additionally, metabolites are end products of intercellular pathways, and are much more stable compared to the upstream products, such as mRNAs and proteins [8,9]. Therefore, metabolomic profiling, which systematically quantify small-molecule metabolites and record unique chemical fingerprints, has emerged as an important tool to identify diagnostic and prognostic biomarkers in various biospecimens including serum, plasma, and urine. An increasing number of studies have utilized metabolomic profiling to identify biomarkers associated with early diagnostic, tumor characterization, prognosis, treatment response, and toxicity in various types of cancers [2,10–24]. In lung cancer, metabolomic studies have been performed in blood and urine samples to identify metabolite biomarker for lung cancer diagnosis [2,25–30], tumor characterization [31,32] and progression [2,27].

In our previous lung cancer study, we found that low levels of serum bilirubin were associated with increased risk of lung cancer incidence and mortality in male smokers [25]. To our knowledge, there has been no study to date examining the relation between circulating metabolomic profiles and clinical outcome in advanced-stage lung cancer patients. To fill the gap, we conducted a two-stage study to examine the role of blood metabolites in predicting overall survival among advanced-stage NSCLC patients who received platinum-based chemotherapy.

2. Material and methods

2.1. Study subjects

In this study, a total of 220 Caucasian advanced-stage (III/IV) NSCLC patients who received first-line platinum-based chemotherapy with or without radiotherapy were included. All study subjects are participants in an ongoing lung cancer case–control study at the University of Texas MD Anderson Cancer Center (Houston, TX). The study subjects were accrued from 1997 to 2009, and their median follow-up time are 14.0 months. Details of subject recruitment methods have been reported previously [25]. All study subjects completed an in-person interview using a structured questionnaire and donated 40 mL blood sample for molecular analysis. The blood samples were collected prior to any cancer treatment. Demographic characteristics, smoking history, family history of cancer, and exposure data were collected. Among these 220 patients, 110 had good survival (defined as survival > 18 months) and 110 had poor survival (defined as survival < 12 months). About 120 patients received chemotherapy only and 100 received chemoradiation. The poor and good survival groups were well-matched on age (+/−5 years), gender and mean blood sample collection time (+/−1 year). In current study, the study subjects were divided into discovery cohort (n = 110, 55 with good survival and 55 with poor survival), and validation cohort (n = 110, 55 with good survival and 55 with poor survival). Serum and plasma samples were used in discovery and validation phase, respectively. The clinical data for the cases were obtained from medical record review. The study protocol was approved by the Institutional Review Board of The University of Texas M. D. Anderson Cancer Center.

2.2. Metabolite profiling

Global metabolomic profiling was conducted with 200ul serum samples for discovery cohort and 200ul plasma samples for validation cohort at Metabolon Inc. (Durham, NC) as described previously [16]. Internal controls were used to control for experimental variability, which included controls for injection, processing, and alignment standards for quality assurance/quality control (QA/QC) procedures. All samples were kept at −80 °C until analyses were performed.

2.3. Pathway analysis

MetaboAnalyst 3.0 was used to identify the significant pathway of altered metabolites. This pathway analysis uses the high-quality KEGG metabolic pathways as the backend knowledge base, and integrates powerful pathway enrichment analysis and pathway topology analysis. First, by using the compound identifications (common names, HMDB IDs or KEGG IDs), 11 top differentially expressed metabolites in both discovery and validation phases were matched with the compounds contained in the pathway analysis. The metabolites which could not be matched were excluded from subsequent pathway analysis.

2.4. Statistical analysis

Stata (version 14.0; StataCorp, College Station, TX) was used for the statistical analysis. Differences in distribution of the host characteristics between poor survival and good survival were evaluated by Pearson $\chi 2$ test for categorical variables and Student *t*-test or Wilcoxon ranksum test for continuous variables. Only metabolites with detectable expression in at least 80% of samples were kept for analysis. There are 623 metabolites in the discovery data and 513 metabolites in the validation data which satisfied the criteria. A total of 454 metabolites overlap between the discovery and the validation phase were included in the final analysis. The difference between the poor survival and good survival were assessed by Wilcoxon rank sum test in both the discovery and validation phases and in the subgroup analysis for adenocarcinoma. In comparing the significant metabolites identified from the discovery and validation cohorts, we first chose 0.05 as P value threshold to identify significant metabolites. Thereafter, to further identify more candidate metabolites, we increased P value threshold for significance to 0.10 to find more significant metabolites and include them in the pathway analysis. The association of each metabolite with survival was also assessed by Cox proportional hazard regression model while adjusting by smoking status, stage, pathology, grade, performance status and treatment regimen (chemotherapy and/or radiotherapy). The risk score was defined as the weighted sum of metabolite where the weight is the beta coefficient. Additionally, for metabolites and risk score, we applied Kaplan-Meier analysis and the log-rank tests to evaluate the differences in survival on categorized metabolites/risk score subgroups.

3. Results

3.1. Characteristics of the study populations

Demographic and pathological characteristics of the patient cohort were shown in Table 1. Briefly, a total of 220 study subjects (discovery cohort: 110, validation cohort: 110) were included in the analysis. Among them, the discovery cohort included 55 patients with good survival and 55 with poor survival, and validation cohort included 55 patients with good survival and 55 with poor survival. In the discovery cohort, the poor and good survival groups were well-matched on age, gender, mean blood sample collection time, tumor grade, and tumor type. The poor survival group had more current smokers and fewer never smokers than the good survival group (P = 0.015). The poor survival group also had more patients with suboptimal performance status [2,3] than the good survival group (P = 0.001). In the validation cohort, the poor and good survival groups were well-matched on all selected characteristics except tumor stage. The poor survival group had greater percentage of patients with grade IV NSCLC than the good survival group (64% vs. 45%, respectively; P = 0.056), although the difference is only borderline significant.

3.2. Global metabolomic profiling of lung cancer

The basic study scheme was shown in Fig. 1. We performed the global metabolomic profiling for discovery cohort first, then for