Lung Cancer 87 (2015) 162–168

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Genetic variants in genes of tricarboxylic acid cycle key enzymes are associated with prognosis of patients with non-small cell lung cancer

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ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form
27 November 2014
Accepted 13 December 2014

Keywords:
Non-small cell lung cancer
Tricarboxylic acid cycle
Succinate dehydrogenase
Fumarate hydratase
Isocitrate dehydrogenase
Single nucleotide polymorphisms
Prognosis

ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) is characterized by poor prognosis and only a few molecular markers may be potentially used to predict the outcome. Metabolic reprogramming is a hallmark of cancer, including the alterations of tricarboxylic acid (TCA) cycle key enzymes. However, the significance of single nucleotide polymorphisms (SNPs) in genes encoding these key enzymes has not been investigated in NSCLC.
Patients and methods: In this study, we genotyped 18 potentially functional SNPs in 7 genes belonging to 3 TCA cycle enzyme families (SDH, FH and IDH) using Sequenom iPLEX genotyping system in a cohort of 500 NSCLC patients. Multivariate Cox proportional hazards model and Kaplan–Meier curve were used for the survival analysis.
Results: Our results showed that *SDHC* gene: SNP rs12064957, *IDH2* gene: SNP rs11540478 and *FH* gene: SNP rs1414493 were associated with overall survival (OS) and *SDHA* gene: SNP rs13173911, *IDH2* gene: SNP rs4932158 were associated with recurrence-free survival (RFS) of NSCLC patients. Unfavorable genotypes of these SNPs showed a significant cumulative effect on OS and RFS of NSCLC patients (both $P < 0.001$). Furthermore, survival tree analysis indicated that *FH*: rs1414493 was the primary risk factor contributing to OS of NSCLC patients and the *IDH2*: rs4932158 was the primary risk factor contributing to RFS of NSCLC patients.
Conclusion: Our data suggest that SNPs in TCA cycle key enzyme genes may serve as potential biomarkers to predict the outcomes of NSCLC. Further studies with different ethnicities are needed to validate our findings and generalize their clinical utility.

1. Introduction

Lung cancer is now the leading cause of cancer mortality for human, due to its high incidence, malignant behaviors and lack of effective treatment [1]. About 85% of lung cancer cases are non-small cell lung cancers (NSCLC), and there has been a significant increase in the incidence of NSCLC over the last two decades in China [2,3]. Although great improvement of treatment, the prognosis of NSCLC remains very poor. The 5-year survival rate of NSCLC patients is only about 15% in USA and 9% in developing countries [4]. To date, very few biomarkers have been identified for risk assessment or prognosis predication of NSCLC patients. Therefore, there is an urgent need for prognostic biomarkers to further improve the clinical management of patients with NSCLC.

The tricarboxylic acid (TCA) cycle, which occurs in mitochondria, is a core pathway for the metabolism of sugars, lipids, and amino acids [5]. Recently, the roles of mitochondrial alterations in cancer come to general attention with the discovery of mutations in mitochondrial TCA cycle core genes, which mainly include succinate dehydrogenase (*SDH*), fumarate hydratase (*FH*), and isocitrate dehydrogenase 1(*IDH1*) and *IDH2* [6]. SDH which consists

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http://dx.doi.org/10.1016/j.lungcan.2014.12.005
0169-5002/

of four subunits, i.e. SDHA, SDHB, SDHC, and SDHD, is an component enzyme of the mitochondrial complex II contributing to the generation of ATP by oxidative phosphorylation. Inherited or somatic mutations in genes encoding subunits B, C, or D of SDH are associated with the development of pheochromocytoma and paraganglioma [7–9]. More recently, *SDHB* mutations have also been identified to be associated with renal cell carcinoma and papillary thyroid cancer [10]. Also, the decreased expression of *SDHD* gene has been reported in gastric and colon carcinoma [11]. FH functions as a homotetramer to catalyze the TCA cycle process (the hydration of fumarate to malate) following SDH [12]. Homozygous null mutations in *FH* gene are associated with multiple uterine leiomyomatas and aggressive forms of renal cell cancer [13]. Recent evidences suggest that germline mutations in *FH* gene are also associated with developing risks of breast, bladder and testicular cancers [14,15]. The $NADP^+$-dependant enzymes IDH1 and IDH2 mainly contribute to the production α-ketoglutarate and NADPH in the mitochondria, and play a vital role in cellular defense against reactive oxygen species through the reduction of glutathione [16]. Heterozygous missense somatic mutations in *IDH1* and *IDH2* genes have been observed in gliomas, chondromas and acute myeloid leukemia (AML) [17–19].

Single-nucleotide polymorphisms (SNPs) are easily detectable genetic variants as they can be analyzed from blood samples [20]. Thus, SNPs are attractive molecular markers for translational studies. Several SNPs have been found to be capable of predicting survival of NSCLC [21–23]. Considering that the important role of TCA pathway core genes in cancer progression [24], we hypothesize that SNPs in these genes may alter gene expression and/or protein activity, and have an effect on the prognosis of NSCLC cancers. To test this hypothesis, we selected 18 functional SNPs in the *SDHA\B\C\D, IDH1, IDH2, FH* genes and evaluated their associations with survival in a Chinese cohort of 500 patients diagnosed with NSCLC.

2. Materials and methods

2.1. Study population

We initially enrolled 556 NSCLC patients from July 2009 to December 2011 at the Department of Thoracic Surgery, Tangdu Hospital affiliated to the Fourth Military Medical University (FMMU), Xi'an, China. In the present study, we excluded 56 patients, including 17 who had incomplete clinical information or contact lost during the follow-up; 13 who died within 2 months after surgery; 17 with unresectable metastatic tumors; and 9 with recurrence within 1 month after surgery. As a result, 500 NSCLC patients who received radical surgery were subjected to the genotyping assay and further analysis. Among them, 19 stage IV patients who had few malignant pleural effusions recognized at the time of surgery (defined as M1a stage) were included because they obtained radical surgical resection. Study subjects had no history of other cancers and they were newly diagnosed and confirmed by histopathology. Clinical data were obtained from medical records, and there were no age, gender, or cancer stage restrictions on recruitment. A standard follow-up was performed at 6-month intervals by a trained clinical specialist through on-site interview, direct calling, or medical chart review. The latest follow-up data in this analysis were obtained in December 2013. The median follow-up was 24.1 months (maximum follow-up time was 53.6 months). Blood sample (5 mL) from each individual was used for genomic DNA extraction using the E.Z.N.A. Blood DNA MidiKit (Omega Bio-Tek, Norcross, GA, USA) in the laboratory. Informed consents were obtained from each participant. The Ethic Committee of FMMU approved the study.

2.2. SNP selection and genotyping

Functional SNPs in the TCA pathway core genes were selected by an approach combining SNPs that were predicted in a web-based tool (available at http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) and SNPs that have been reported in related diseases. Only validated SNPs were selected, and SNPs with minor allele frequency <5% in Han Chinese population (CHB) were excluded. Potential functional SNPs were identified to meet the following criteria: (a) SNPs located in miRNA binding sites of 3′ untranslated region (UTR), SNPs in the transcription factor binding site of the 5′ flanking region (2000 bp upstream from the transcript start site), SNPs in splice sites or non-synonymous SNPs in exons. (b) SNPs were shown to be associated with disease according to the literature review [25]. If there were multiple potential functional SNPs within the same haplotype block (defined by the linkage coefficient $r^2 > 0.8$), only 1 SNP was included. Finally, we identified 18 SNPs including 2 SNPs in *SDHA* gene (rs13173911, transcription factor binding sites, TFBS; rs2864963, TFBS), 1 SNP in *SDHB* gene (rs3754509, TFBS), 3 SNPs in *SDHC* gene (rs12064957, rs4131826, TFBS; rs3935401, microRNA binding site), 3 SNPs in *SDHD* gene (rs10789859, rs544184, rs7121782, TFBS), 1 SNP in *IDH1* gene (rs12478635, TFBS), 5 SNPs in *IDH2* gene (rs11540478, splicing site; rs11632348, rs4283211, rs4932158, rs9708193, TFBS) and 3 SNPs in *FH* gene (rs12071124, rs1414493, rs7530270, TFBS). All selected SNPs were genotyped by iPLEX genotyping system (Sequenom, San Diego, CA, USA). Laboratory persons who conducted the genotyping assay were blinded to patient information. Internal quality controls and negative controls were used to ensure genotyping accuracy, strict quality control measures were implemented during genotyping with over 99.0% concordance with the main genotyping results. Strict quality controls were implemented in each assay during the genotyping and SNP with call rate >95% was included for further analysis.

2.3. Statistical and eQTL analysis

For each SNP, three genetic models (additive, dominant, and recessive) were selected for analysis and the model with the smallest *P* value was considered to be the best-fitting model. Two major endpoints were evaluated in this study: overall survival (OS) and recurrence-free survival (RFS). OS was defined as the time from diagnosis to death from any cause. RFS was defined as the time from diagnosis to the first date of recurrence. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by the Cox proportional hazards model after adjusting for age, gender, smoking status, tumor differentiation, clinical stage, histology and adjuvant treatment. Kaplan–Meier curve and log-rank test were used to assess the differences of survival. Statistical significance was set at a level of 0.05 and all analyses were done using the SPSS software package (version 19.0, SPSS, Inc.). In addition, the expression quantitative trait loci (eQTL) analysis was performed in two established databases (GteX: http://www.ncbi.nlm.nih.gov/gtex/GTEX2/gtex.cgi and seeQTL: http://gbrowse.csbio.unc.edu/cgi-bin/gb2/gbrowse/seeqtl/) to predict the relationship between the identified functional SNPs and gene expression.

3. Results

3.1. Distribution of patient characteristics and prognosis analysis

The study included 500 patients with resected NSCLC (Table 1). The median age at the time of diagnosis was 60 years (range, 27–86) for all patients. Among these patients, there were 268 (53.6%)