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Polymorphisms of peroxisome proliferator-activated receptors and survival of lung cancer and upper aero-digestive tract cancers

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ABSTRACT

Background: Peroxisome proliferator-activated receptors (PPARs) are transcriptional factors involved in several biological processes such as inflammation, cancer growth, progression and apoptosis that are important in lung and upper aero-digestive tract (UADT) cancer outcomes. Nonetheless, there are no published studies of the relationship between *PPARs* gene polymorphisms and survival of patients with lung cancer or UADT cancers.
Methods: 1212 cancer patients (611 lung, 303 oral, 100 pharyngeal, 90 laryngeal, and 108 esophageal) were followed for a median duration of 11 years. We genotyped three potentially functional single nucleotide polymorphisms (SNPs) using Taqman – rs3734254 of the gene *PPARD* and rs10865710 and rs1801282 of the gene *PPARG* – and investigated their associations with lung and UADT cancer survival using Cox regression. A semi-Bayesian shrinkage approach was used to reduce the potential for false positive findings when examining multiple associations.
Results: The variant homozygote CC (vs. TT) of *PPARD* rs3734254 was inversely associated with mortality of both lung cancer (adjusted hazard ratio [aHR] = 0.63, 95% confidence interval [CI] = 0.42, 0.96) and UADT cancers (aHR = 0.51, 95% CI = 0.27, 0.99). Use of the semi-Bayesian shrinkage approach yielded a posterior aHR for lung cancer of 0.66 (95% posterior limits = 0.44, 0.98) and a posterior aHR for UADT cancers of 0.58 (95% posterior limits = 0.33, 1.03).
Conclusion: Our findings suggest that lung-cancer patients with the CC variant of *PPARD* rs3734254 may have a survival advantage over lung-cancer patients with other gene variants.

1. Introduction

Lung cancer and upper aero-digestive tract (UADT) cancers are common cancers that are responsible for serious morbidity and mortality. According to Globocan 2012, lung cancer ranks third in incidence and first in mortality of cancers in the world; and third in incidence and first in mortality of cancers in the United States (U.S.) [1]. The overall 5-year survival of lung cancer is 16.6% in the

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U.S. in 2013 [2,3]. UADT cancers comprise the cancers of the airway and upper digestive tract, specifically, oral cavity, pharynx, larynx and esophagus, which are contiguous and commonly exposed to inhaled and sometimes swallowed substances. Collectively, UADT cancers rank fifth in incidence and fourth in mortality of cancers in the world and seventh in incidence and sixth in mortality of cancers in the U.S. in 2012 [1]. The overall 5-year survival is 61% for head and neck cancer (HNC) and 17.3% for esophagus cancer in the U.S. in 2013 [2]. Despite advances in understanding the pathogenesis of lung and UADT cancers, improvements in surgical procedures and the introduction of newer treatment regimens, survival, especially for lung and esophageal cancers has not changed much [2,4]. At the present time, stage, histology and treatment are thought to be related to cancer prognosis [5]; however there is only a limited amount of information available on genetic factors and cancer prognosis for both lung cancer and UADT cancers.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily [6–8]. Initially, PPARs are found to regulate genes that control glucose and lipid metabolism and are linked to type 2 diabetes mellitus risk [9,10]. Later it is discovered that they are involved in many other important biological functions, including early development, inflammation, cell differentiation, proliferation, and apoptosis [6,11].

Several studies on lung cancer illustrate that the activation of PPAR-γ could constrain cancer cell growth and oral administration of synthesized PPAR-γ ligand could inhibit lung cancer progression and metastases [12–14]. Compared to extensive research on PPAR-γ, studies on PPAR-δ are fewer and focus mostly on the digestive system, with inconsistent results [15–17]. Limited studies of non-small cell lung cancer (NSCLC) show that the function of PPAR-δ is different from that of PPAR-γ and that the former increases cancer proliferation [18].

Considering the cancer-related links with PPARs and the lack of epidemiologic studies examining their relation with lung- and UADT- cancer survival, we conducted this study to explore the association between PPARs gene polymorphisms and survival among patients with lung and UADT cancers.

2. Methods

2.1. Study population

The study population consisted of newly diagnosed cancer cases identified for the University of California at Los Angeles (UCLA) and University of Southern California (USC) population-based case-control study between 1999 and 2004 in Los Angeles County (LAC) [19]. These subjects included pathologically confirmed new cases of lung cancer or UADT cancers (oral cancer, pharyngeal cancer, laryngeal cancer and esophageal cancer) identified by the rapid ascertainment system of the LA County Cancer Registry under the Cancer Surveillance Program at USC [19]. Recurrent cancer cases were excluded from our study.

Recruitment occurred from 1999 to 2004. All participants were residents of LAC, aged 18–65 years old at the time of diagnosis and able to speak either English or Spanish or had a translator on site. Vital status was determined through linking the cases with the Social Security Death Index. The survival time was calculated as the interval between the date of diagnosis and the date of death, or the date of the last follow-up which was July 13th, 2012. The median follow-up time was 11.1 years in all cases, and 11.5 years in lung cancer cases and 10.8 years in UADT cancer cases, respectively.

Recruitment rates among eligible cancer patients were 39%, 54%, 45%, 42% and 35% for lung, oral, pharyngeal, laryngeal and esophageal cancer, respectively. A total of 1212 patients were included in our study. There were 611 patients with lung cancer, including 95 squamous carcinomas (SQC), 297 adenocarcinomas (ADC), 115 large cell carcinomas (LCC), 75 small cell lung cancers (SCLC) and 29 others. There were a total of 601 patients with UADT cancers, including 303 patients with oral cancer, 100 with pharyngeal cancer and 90 with laryngeal cancer; and 108 esophageal cancer patients. 497 UADT cancer patients were squamous carcinomas, 74 were esophageal adenocarcinomas, and 30 were other cell types.

2.2. Data collection

Trained interviewers used study specific standardized questionnaires to collect subject-reported data including age, gender, ethnicity, education level, smoking and alcohol drinking. Tobacco smokers were defined as those who smoked more than 100 cigarettes in their lifetimes. Alcohol drinkers were defined as those who drank at least one alcoholic drink per month for a period of at least six months. Cumulative level of smoking was measured by pack-years, which were calculated by summing packs per day times the number of years that a subject smoked that amount prior to the diagnosis of cancer. One pack-year is equivalent to smoking one pack per day for one year. Alcohol drinking was measured by the average number of drinks (including wine, beer or liquor) consumed per day. Interviews occurred within six months of diagnoses for 89% of cases.

Buccal cells were collected for DNA analysis by asking subjects to brush their buccal mucosa and rinse with mouthwash. Response rates for interviewed participants providing buccal cells were 89%, 68%, 88%, and 90% for lung, oral and pharyngeal, laryngeal and esophageal cancer cases, respectively.

All specimens were transported and stored at −70 °C in the Molecular Epidemiology Laboratory at UCLA, Fielding School of Public Health. DNA samples were isolated by using a modified phenol-chloroform assay [20]. We selected single nucleotide polymorphisms (SNPs) of which the minor allele frequencies (MAFs) in Caucasians were ≥5%; when the pairwise linkage disequilibrium (LD) r^2 was ≥0.8, we picked non-synonymous SNPs or SNPs located in regions regulating gene transcriptions, such as promoter areas from the National Center for Biotechnology Information SNP database. A total of one SNP in the gene *PPARD* (rs3734254) and four SNPs in the gene *PPARG* (rs10865710, rs1801282, rs3856806, and rs13306747) were selected. SNP genotyping was done using TaqMan (Applied Biosystems (ABI, Foster City, CA) 7900HT). Samples were first held at 92 °C for 10 min; then underwent 60 thermocycles of denaturing at 92 °C for 15 s and annealing at 62 °C for 80 s. After PCR amplification, end-point fluorescence was read using the ABI 7900HT Sequence Detection System, and genotypes were coded using the SDS 2.3 Allelic Discrimination Software. SNPs that did not meet the criteria of Hardy-Weinberg equilibrium (HWE) *p*-value ≥Bonferroni-adjusted *p*-value of 0.01 and a genotyping call rate ≥95% were excluded, leaving rs3734254, rs10865710 and rs1801282 in the analysis.

2.3. Statistical analysis

We first analyzed SNP genotypes (TT, TC, CC or CC, CG, GG) as dummy variables. These results were used to decide the appropriateness of the dominant or recessive model. Cox regression was used to estimate crude and adjusted hazard ratios (cHRs and aHRs) and their corresponding 95% confidence intervals (CIs). SNP genotypes were also treated as ordinal variables to be tested for p-trends. For lung cancer, we adjusted for age, gender, ethnicity, education level, and smoking; we also adjusted for cell differentiation and morphology that included lung SQC, lung ADC, LCC and SCLC. For UADT cancers, we adjusted for age, gender, ethnicity,