Racial Diversity of Actionable Mutations in Non–Small Cell Lung Cancer

Aliccia Bollig-Fischer, PhD, Wei Chen, PhD, Shirish M. Gadgeel, MD, Angela S. Wenzlaff, MPH, Michele L. Cote, PhD, Ann G. Schwartz, PhD, MPH, and Gerold Bepler, MD, PhD

Introduction: Lung cancer is the leading cause of cancer-related deaths in the US. The reasons for higher incidence and poorer survival rates among black compared with white lung cancer patients have not been defined. We hypothesized that differential incidence of somatic cancer gene mutations may be a contributing factor. Previous genomic studies of non–small cell lung cancer (NSCLC) have not adequately represented black patients.

Methods: A matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry approach was used to analyze tumor DNA for 214 coding mutations in 26 cancer genes previously identified in NSCLC. The samples included NSCLC from 335 white patients and 137 black patients. For 299 of these, normal matched DNA was available and analyzed.

Results: *Epidermal growth factor receptor (EGFR)* exon 19 deletions were only detected in women cases, with increased odds for black women compared with white women (odds ratio = 3.914, 95% confidence interval: 1.014–15.099, $p = 0.048$). Beyond race, variations in mutation frequencies were seen by histology. DDR2 alterations, previously described as somatic mutations, were identified as constitutional variants.

Conclusions: This study is among the largest comparing somatic mutations in black and white patients. The results point to the molecular diversity of NSCLC and raise new questions as to the importance of inherited alleles. Genomic tumor testing will benefit both populations, although the mutation spectrum appears to vary by sex, race, and histology.

Key Words: Non–small cell lung cancer, African American, mutations, Oncogenes, Tumor suppressors.

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Lung cancers are a heterogeneous group of tumors traditionally categorized by histology, with the majority classified as non–small cell lung cancer (85%, NSCLC).[1] NSCLC is further subdivided into adenocarcinomas (~45%), squamous cell carcinomas (~23%), and large cell carcinomas (~3%), with other subtypes representing the remaining approximately 28%.[1] Large-scale gene mutation profiling is transforming our knowledge of the heterogeneity of NSCLC.[2] Genomic research now links specific oncogenes and recurring mutations to the disease phenotype and provides a rationale for use of molecularly targeted treatments that are improving lung cancer patients' outcomes.[2]

Black Americans are more likely to develop lung cancer and at an earlier age compared with white individuals despite lower rates of smoking in black adolescents.[3–7] Moreover, blacks with lung cancer show worse outcomes, including shorter overall survival and increased mortality, which persist when correcting for socioeconomic factors and unequal access to care.[7] These data suggest diversity in disease etiology across populations. The specifics of disease etiology govern the mutational processes giving rise to particular mutation patterns or signatures in cancer.[8] Along these lines, study by Alexandrov et al.[8] demonstrates that a specific mutation profile in lung cancers is attributable to smoking. The same study also reports distinct mutation signatures in lung cancers not yet linked to a cause. It is predicted that more causally linked cancer mutation signatures will emerge when both the numbers of specimens and diversity of patients under investigation increases. Thus, we hypothesized that the frequencies of specific mutations in NSCLC will vary between black and white populations. Previous studies have compared mutation frequencies according to race, but largely focused on a small subset of oncogenes—e.g., mutations in epidermal growth factor receptor (EGFR) and kirsten rat sarcoma viral oncogene homolog (KRAS)—and they yielded conflicting results.[9–13] More comprehensive research is required to thoroughly investigate cancer gene mutation differences and to derive insight as to diagnostics and individualized treatment modalities that provide patient benefit. Here, we present findings from a large-scale genomic study, with a large proportion of black patients, to examine whether the landscape of cancer relevant mutation frequencies varies according to race.

MATERIALS AND METHODS

Patients and Tissues

Biospecimen collection and outcomes data compilation were done according to the Helsinki Declaration and approved by the Wayne State University School of Medicine

Barbara Ann Karmanos Cancer Institute and Department of Oncology, Wayne State University, Detroit, MI.

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Author for Correspondence: Aliccia Bollig-Fischer, 4100 John R, Detroit, MI 48201. Email:bollig@karmanos.org

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Institutional Review Board. Fresh-frozen or formalin-fixed paraffin-embedded specimens were collected from patients who underwent a surgical resection for diagnosed or suspected lung cancer. Frozen specimen procurement procedures were implemented to reduce the resection to freezing time interval to less than 30 minutes. The overall procurement period ranged from 1985 to 2012 from five different case series. Frozen and archived FFPE specimens were reviewed to verify NSCLC diagnosis and to determine that tumor cell content was equal or greater than 70%. Only tumors pathologically confirmed to be NSCLC were included in the analysis. DNA from adjacent normal lung tissue was available and analyzed from 299 of the 472 cases. Demographic and clinical outcomes data collected included: the dates of birth, diagnosis, and last follow-up or death; sex, race, tumor histology, pathological, and clinical tumor stage (AJC staging manual, version 6.0) and self-reported smoking history (defined as life-time never smoker for those who had smoked <100 cigarettes, former smoker for those who had quit cigarette smoking for more than 1 year, and smoker for all others).

Genetic Analysis

Frozen NSCLC and normal tissue specimens (~100 mg) were pulverized using sterilized and frozen mortar and pestles and DNA was isolated using resin or phenol-based extraction methods. DNA was isolated from paraffin-embedded tissue using EZ1 Advanced magnetic bead technology, the EZ1 DNA de-paraffinization method and the EZ1 DNA Tissue kit (Qiagen, Valencia, CA). All sample DNA quantity and quality was assessed using a Nanodrop spectrophotometer and Quantifiler assay (Life Technologies, Carlsbad, CA), a real-time polymerase chain reaction-based approach for estimation of amplifiable DNA. A standard curve of known DNA concentrations was also analyzed for quantitation of each DNA sample.

Mutations were analyzed using the Sequenom MassARRAY System employing matrix-assisted laser desorption/ionization and time of flight mass spectrometry and the Sequenom LungCarta panel (Sequenom, San Diego, CA). The panel targets 214 sequence mutations in 26 oncogenes and tumor suppressors, which were previously identified in NSCLC.[14,15] The genes include: AKT1, ALK, BRAF, DDR2, EGFR, EPHA3, EPHA5, ERBB2, FGFR4, JAK2, KRAS, MAP2K1, STK11, MET, NOTCH1, NRAS, NRF2, NTRK1, NTRK2, NTRK3, PIK3CA, PTCH1, PTEN, PTPN11, PTPRD, and TP53. The coding mutation types include synonymous and nonsynonymous nonsense and missense point mutations, transversions and transitions, and short insertions and deletions(Supplementary Table 1, Supplementary Digital Content 1, http://links.lww.com/JTO/A748). The methodology starts with polymerase chain reaction of small DNA segments (<100 base pairs) encompassing the DNA mutation sites in a multiplex reaction. A follow-up extension reaction adds a single base to extension primers, the nature of the base pair added to a primer affects its mass and time-of-flight; thus, a wild-type and mutation sequence can be differentiated and quantified. The sensitivity of the approach allows for detection of a mutation that represents ≥10% of the sample. The assay precision and accuracy for both FFPE and frozen specimen DNA were validated for as little as 60 ng by us and others[16]; however, typically 480 ng of sample DNA was analyzed for this study.

Statistical Analysis

Descriptive statistics were provided for patients' demographic and clinical characteristics. Multivariable logistic regression was used to estimate the odds of having the genetic alteration, adjusted for potential confounders such as race (Black, reference: White), age (as continuous variable), sex (women, reference: men), tumor stage (II, III, or IV, reference: I), histology (squamous, other NSCLC, reference: adenocarcinoma), and smoking status (never, unknown, reference: ever). All statistical tests were traditional two sided at a significance level of 0.05. Given the nature of this exploratory study, *p* values were not adjusted for multiple testing. Single nucleotide polymorphism (SNP) allele frequency within each subgroup of race was compared with the National Institutes of Health Heart, Lung and Blood Institute Grand Opportunity Exome Sequencing 6500 Project database[17] using Fisher's exact test. The statistical software R 3.0.0 (The R foundation for statistical computing; http://www.r-project.org/foundation/) was used for all analyses.

RESULTS

Patient Characteristics

Descriptive statistics for the 472 NSCLC patients—137 black individuals and 335 white individuals—who contributed specimens to this study are listed in Table 1. The proportions for histology, sex, stage, and smoking history are given stratified by race; median age and pack years smoked by race are also indicated.

Frequency of Cancer Gene Mutations According to Race

An initial overview of the data from mass spectrometry analysis showed that for 180 of the 472 NSCLC specimens (38%) a mutation was detected (characteristics and mutations identified for each specimen are provided in the online Supplementary Data file, Supplementary Digital Content 2, http://links.lww.com/JTO/A749). Comparing frequencies for individual mutations across race showed no significant differences for point mutations; however, there was a higher frequency for the *EGFR* E746-T751>S deletion in exon 19 in tumor specimens from black patients compared with white patients ($p = 0.076$; Table 2). When the combined frequency of all variations of deletions detected at E746 (i.e., E746-T751 and E746-T750) were considered, we observed that these mutations occurred exclusively in women and more often in black women than in white women (odds ratio [OR]: 3.914; 95% confidence interval [CI]: 1.014–15.099; $p = 0.048$), after adjusting for age, stage, histology, race, and smoking status (Table 3). Across all mutation types, tumors from women were 80% more likely to carry at least one mutation than those from men after adjustment for smoking, age, race, stage, and histologic type (OR: 1.815; 95% CI: 1.200–2.747; $p = 0.005$)

